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THÈSE

présentée par :

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Copper transport and isotope fractionation in an agrosystem

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SUMMARY FOR THE WINE-GROWERS AND THE STAKEHOLDERS (IN FRENCH)

RESUME

Les **fongicides cupriques jouent aujourd'hui un rôle essentiel dans la viticulture conventionnelle et biologique**, la culture du houblon, de fruits et de pommes de terre et restent indispensables, en particulier en agriculture biologique, par manque d'alternatives efficaces. Depuis la fin du 19ème siècle, **le traitement des vignes par le cuivre a engendré une augmentation de sa teneur dans les sols viticoles** (souvent > 100 mg.kg^{-1}, alors que la moyenne mondiale des sols, sans ajouts de cuivre anthropique, est de 30 mg.kg^{-1}). Le Cu peut donc s'accumuler et atteindre des taux préoccupants dans les sols et, suite à des phénomènes de **transport par dérive, ruissellement et érosion, dans les eaux et les sédiments des écosystèmes aquatiques**. En Alsace, la viticulture représente la principale culture pérenne utilisatrice du Cu et la connaissance du cycle du Cu en contexte viticole demeure faible.

Ce travail de thèse vise à mieux comprendre et prédire le transport du cuivre en réponse aux variations climatiques et aux perturbations anthropiques (occupation du sol, épandage de fongicides cupriques) afin de (i) tracer l'accumulation et la distribution des fongicides cupriques dans des sols viticoles et leurs mobilisation via la lame ruisselante, (ii) étudier les processus de rétention et de relargage éventuel du cuivre dans un bassin d'orage, récepteur du ruissellement chargé en cuivre et (iii) évaluer les processus de transformation du cuivre dans des sédiments à long terme (phénomène dit de « vieillissement » ou aging en anglais). L'originalité de l'approche repose sur **un développement analytique de l'étude du fractionnement isotopique du cuivre, et l'application de ce développement à des expériences en laboratoire (mésocosmes) et de terrain (système en série bassin versant viticole-milieux humides artificiels)**.

Les résultats montrent que (i) durant 4 à 5 décennies d'exploitation des parcelles viticoles, **les sols en surface sont enrichis en cuivre de 9 à 28 fois par rapport au fond géochimique**, (ii) lors des évènements pluvieux, **~1% du cuivre appliqué (sous forme de fongicides cupriques) est mobilisé** pendant la saison, et la lame ruisselante transporte des teneur en cuivre jusqu'à 3 fois plus élevées que le seuil écologique recommandé pour le bon fonctionnement des écosystèmes aquatiques, (iii) les **zones tampons humide artificielle (bassin d'orage) récoltant les lames ruisselantes provenant de petits bassins versants viticoles peuvent retenir une grande partie du cuivre** et réduire ainsi la charge du cuivre dans les lames ruisselantes, et (iv) le **« vieillissement » du cuivre dans les sédiments des zones humides a tendance à réduire sa mobilité et sa disponibilité**.

ETUDE DU CUIVRE DANS LE VIGNOBLE ALSACIEN

Depuis la fin du 19ème siècle, le traitement des vignes par le cuivre a engendré une augmentation de sa teneur dans les sols viticoles. Celle-ci excède souvent 100 mg.kg^{-1}, alors que la moyenne mondiale des sols, sans ajouts de cuivre anthropique, est de 30 mg.kg^{-1}.[1] A ce jour, l'état moyen des teneurs en cuivre total des sols du vignoble alsacien demeure peu connu. Cependant, **des teneurs élevées peuvent avoir des effets toxiques vis-à-vis des organismes du sol, ce qui peut à la longue, réduire sa fertilité**.[2] Le cuivre s'accumule dans le sol via des processus de liaison à la matière organique et aux phases minérales et/ou par précipitation avec des carbonates présents dans la plupart des sols viticoles.[1] Cependant, les processus de rétention des fongicides cupriques (contenant du cuivre) peuvent varier selon le type de sol, ce qui a été jusqu'ici peu étudié dans les vignobles alsaciens.

Dans les vignobles, l'existence de sols nus ou faiblement enherbés et de pentes élevées (10-15%), favorisent l'érosion et le ruissellement ce qui augmente alors la mobilité du cuivre. Dans les lames ruisselantes, le cuivre, principalement transporté sous forme solide (lié aux matières en suspension), s'introduit en quantités importantes dans les écosystèmes aquatiques en aval.[3] Ces milieux aquatiques sont vulnérables, car à forte concentration, le cuivre peut modifier profondément leurs structures et leur fonctionnement.

Cependant, les lames ruisselantes provenant de bassins versant agricoles peuvent être interceptées par des zones tampons humides (bassin d'orage) avant d'être transférées vers des écosystèmes aquatiques en aval. Ces systèmes sont envisagés depuis peu comme une alternative opérationnelle et peu coûteuse au traitement des eaux de ruissellement chargées en pesticides (et notamment en cuivre) mais restent encore en phase d'évaluation. Des performances variables révèlent notamment des **lacunes dans la compréhension du devenir, du transfert et de la rétention du cuivre** au sein de ces milieux récepteurs connectés hydrologiquement aux bassins versants viticoles.

Dans ces milieux, le cuivre peut subir différentes transformations[4] en raison de processus :

- physiques, tels que la liaison aux sédiments et la sédimentation des matières solides
- chimiques, tels que la réduction (Cu(II)→Cu(I) et/ou Cu(0)) et la (co-)précipitation avec des phases minérales nouvellement formées (hydroxydes de fer, sulfures)
- et biologiques : prélèvement par la végétation et des microorganismes (algues, bactéries).

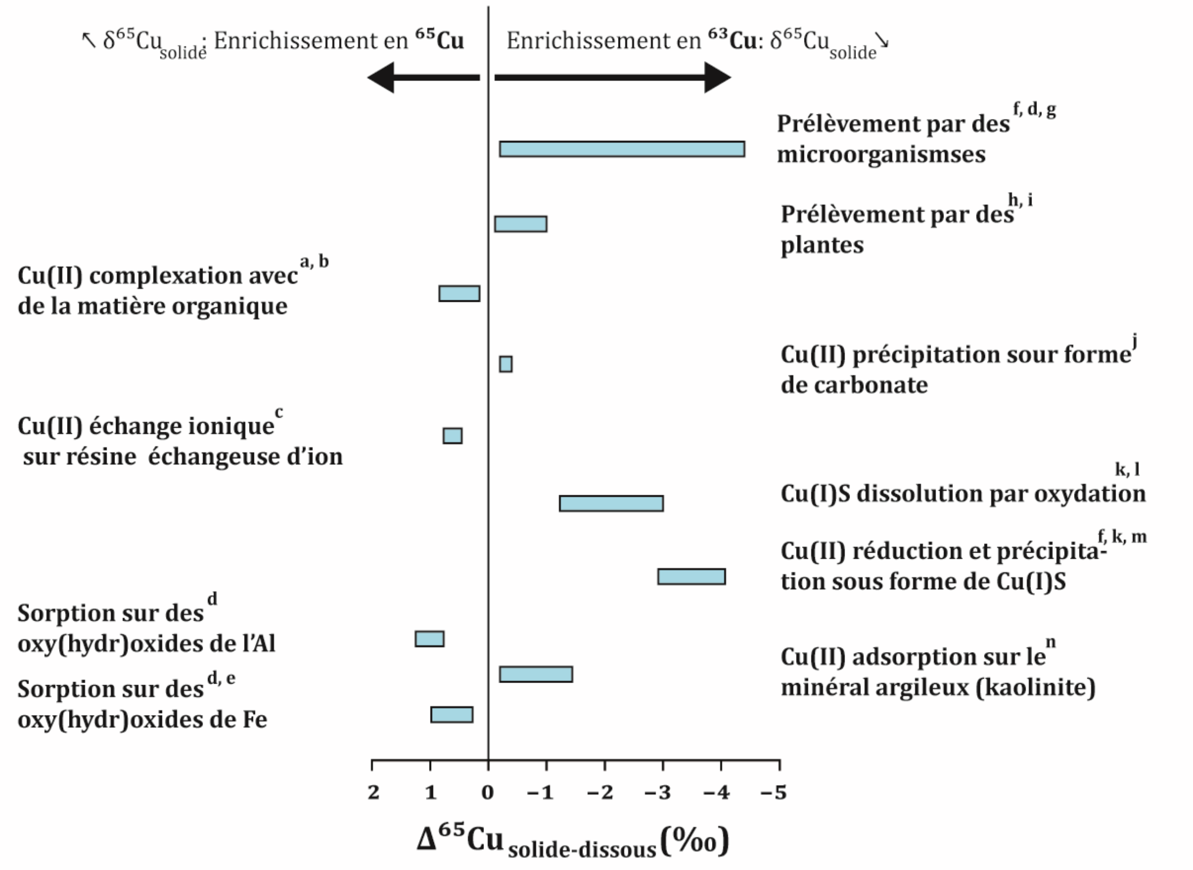
Cependant, en l'absence d'oxygène, condition prédominante dans les sédiments de ces milieux, certaines phases se liant avec le cuivre (comme les oxydes de fer) vont être dissoutes tandis que d'autres phases minérales, telles que les sulfures, vont se former et piéger ainsi du cuivre.[5,6] **Les changements des conditions ambiantes, notamment par des événements hydrologiques peuvent considérablement altérer la performance de ces systèmes vis-à-vis de la séquestration du cuivre et peuvent même devenir des sources secondaires de cuivre en le remobilisant.** Par conséquent, il est important d'étudier les processus contrôlant le devenir du cuivre dans les milieux humides tels que les bassins d'orage, en particulier en fonction des conditions hydrologiques et des changements de conditions ambiantes dans le milieu (teneur en oxygène, pH, etc.).

LES OUTILS ISOTOPIQUES : CAS DES ISOTOPES STABLES DU CUIVRE

En complément des outils traditionnels, tels que la mesure de concentration des métaux, les extractions chimiques (la liaison du cuivre aux différents constituants du sol) et les analyses minéralogiques, les isotopes stables des métaux peuvent nous renseigner sur les processus qui régissent leur comportement dans l'environnement.[7] L'analyse isotopique du cuivre (^{65}Cu/^{63}Cu) est désormais possible grâce au développement du spectromètre de masse à source à plasma et à multi-collection (MC-ICPMS). Ainsi, l'étude du rapport des isotopes stables du cuivre peut être adaptée au suivi du cycle du cuivre à l'échelle des agro-systèmes et des zones tampons humides afin de mieux comprendre les mécanismes régissant sa séquestration/mobilisation et son transport.

Les isotopes d'un élément donné contiennent le même nombre de protons et d'électrons, par contre, leur nombre de neutrons est différent et ainsi leur masse atomique varie. On distingue entre les isotopes stables et radioactifs. L'isotope carbone 14, ou ^{14}C est un isotope radioactif, et sa radioactivité décroît au fil du temps à un rythme régulier, ce qui permet de l'utiliser en tant que «chronomètre» pour estimer l'âge d'objets très variés : œuvres d'art, roches, fossiles, etc. En revanche, les isotopes stables ne se désintègrent pas et sont utilisés comme traceurs de processus biogéochimiques et/ou de source. **Le cuivre possède deux isotopes stables le ^{63}Cu et le ^{65}Cu (qui ont chacun 29 protons et 29 électrons, par contre possèdent 34 (^{63}Cu) et 36 neutrons (^{65}Cu) dans leur noyau),** dont l'abondance moyenne dans la nature est 69,17% (^{63}Cu) et 30,83% (^{65}Cu). L'inégalité de masse entraîne l'existence de différences dans leur comportement lors des réactions physico-chimiques et biologiques. Ceci entraîne une répartition différente des isotopes entre les réactifs et les produits lors des réactions produisant ainsi un fractionnement isotopique ($^{65}\text{Cu}/^{63}\text{Cu}_{\text{réactif}} \neq {}^{65}\text{Cu}/^{63}\text{Cu}_{\text{produit}}$).

Plusieurs études ont montré que la réduction/oxydation du cuivre produit un fractionnement distinct, que la liaison entre le cuivre et des hydroxydes métalliques, des argiles ou de la matière organique produit un fractionnement significatif, de même que la transformation biotique du cuivre (passage par des racines, translocation dans les plantes) (**Figure 1**).[8-11] **Notre hypothèse de travail est que mesurer le rapport isotopique du cuivre dans des échantillons environnementaux (tels que les sols, la lame ruisselante, la végétation) nous permettrait d'indentifier les processus principaux qui régissent le transport et la transformation du cuivre.**



a: Ryan et al., 2014, **b**: Bigalke et al., 2010, **c**: Maréchal et Albarède, 2002, **d**: Pokrovsky et al., 2008, **e**: Balistrieri et al, 2008, **f**: Zhu et al., 2002, **g**: Navarrete et al., 2011, **h**: Jouvin et al., 2012, **i**: Ryan et al., 2013, **j**: Maréchal et Sheppard, 2002 , **k**: Mathur et al., 2005, **l**: Kimball et al., 2009, **m**: Ehrlich et al., 2004, **n**: Li et al., 2015

Figure 1 : *Le fractionnement isotopique du cuivre lors des processus physico-chimiques et biologiques compilé de la littérature, exprimé en terme de* $\Delta^{65}Cu_{a\text{-}b} = \delta^{65}Cu_{solide} - \delta^{65}Cu_{dissous}$ *(la différence des rapports isotopiques du cuivre dans la phase solide et dissous).*

OBJECTIFS DE L'ETUDE

Plusieurs objectifs ont été formulés dans ce travail de thèse visant à mieux comprendre et prédire le transport du cuivre en réponse aux variations climatiques et aux perturbations anthropiques (occupation du sol, épandage de fongicides cupriques) :

- tracer l'accumulation et la distribution des fongicides cupriques dans des sols viticoles et leurs mobilisation via la lame ruisselante ;
- étudier les processus de rétention et de relargage éventuel du cuivre dans un bassin d'orage, récepteur du ruissellement chargé en cuivre;
- évaluer les processus de transformation du cuivre dans des sédiments à long terme (phénomène dit de « vieillissement » ou aging en anglais).

L'originalité de l'approche repose sur un développement analytique de l'étude du fractionnement isotopique du cuivre, et l'application de ce développement à des expériences en laboratoire (mésocosmes) et de terrain (système en série bassin versant viticole-milieux humides artificiels).

RÉSULTATS :

Accumulation du cuivre dans les sols viticoles du bassin du Hohrain (42 ha, Rouffach, France) et son transport dans la lame ruisselante

Les isotopes stables du cuivre (^{65}Cu/^{63}Cu, exprimé en δ^{65}Cu) ont été utilisés pour étudier les sources, les processus de rétention du cuivre anthropique (fongicides cupriques) dans les sols viticoles et son transport via le ruissellement à l'échelle d'un bassin versant viticole (Rouffach, Alsace, France).

- Le sol en surface du bassin versant viticole de Rouffach contient, en moyenne, 94 mg kg-1 de cuivre (prélèvements mensuels (avril-juillet 2011) sous les rangs de vigne tous les 15 m sur 3 transects nord-sud dans le bassin du Hohrain) en comparaison avec les sols locaux non viticoles qui se situent à 11,5 mg kg-1, soulignant l'enrichissement anthropique en cuivre de ces sols (Figure 2).

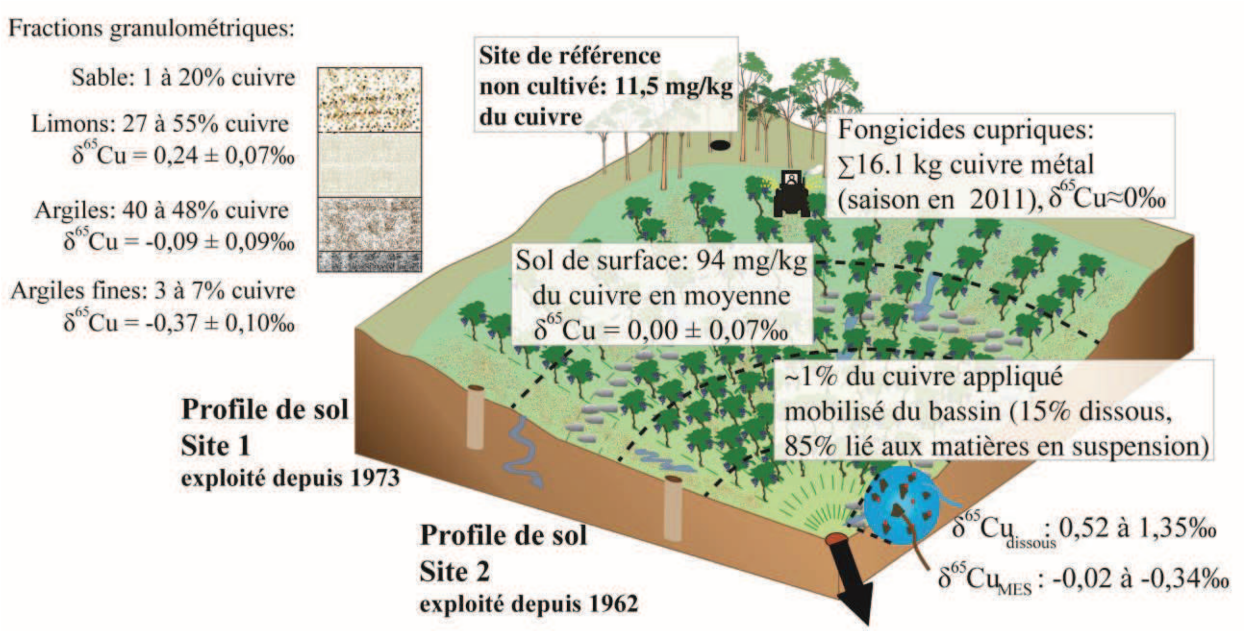


Figure 2 : *La répartition du cuivre dans les fractions granulométriques du sol en surface au points d'échantillonnage ('Site 1' et 'Site 2') et leur rapport isotopique, le bilan du cuivre au niveau du bassin versant du Hohrain (Rouffach, France) : le cuivre cumulé appliqué pendant la saison d'application en 2011 ainsi que le rapport isotopique moyen du cuivre dans les fongicides cupriques, la concentration en cuivre et son rapport isotopique dans sols en surface du bassin versant et la répartition du cuivre dans la lame ruisselante à l'exutoire du bassin versant ainsi que les rapports isotopique.*

- Les résultats, en accord avec les rapports isotopiques du cuivre, indiquent la prédominance du cuivre anthropique dans les deux parcelles étudiées jusqu'à ~60 cm au site 1 et dans tout le profil échantillonné au site 2 (exploitée depuis 11 ans) (Figure 3).
- Les $^{65}Cu/^{63}Cu$ dans les différentes fractions du sol (limons, argiles, fines argiles) (Figure 2) montrent que la rétention du cuivre anthropique dans le sol se fait par liaison sur des minéraux argileux (essentiellement dans les fractions fines du sol) et à la matière organique (présente dans toutes les fractions).
- Les lames ruisselantes transportent ~1% (~150 g) du cuivre appliqué (~16 kg de cuivre appliqué en cumulé sur la totalité du bassin versant) pendant la période d'étude (de Mai à Juillet en 2011) en majorité sous forme solide (Figure 2). Cela souligne l'importance de l'érosion des sols dans la mobilisation du cuivre. La signature isotopique ($^{65}Cu/^{63}Cu$) du cuivre lié aux solides en suspension dans la lame ruisselante correspond à celles dans les fractions argileuses (argiles et fines argiles), montrant ainsi leur mobilisation préférentielle dans les sols lors des évènements pluvieux (Figure 2).

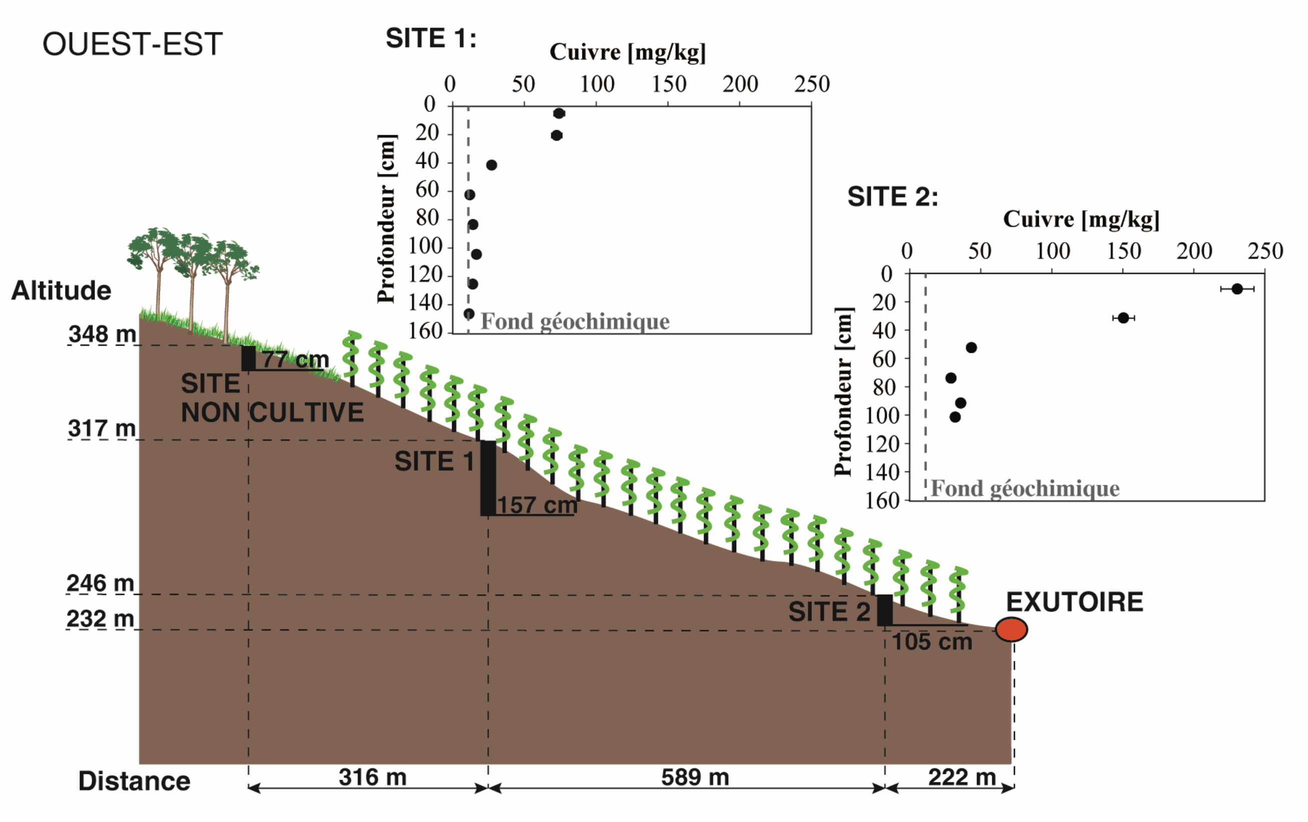


Figure 3 : Les concentrations en cuivre des sols viticoles en fonction de la profondeur aux sites d'échantillonnage ('Site 1' et 'Site 2') dans le bassin versant du Hohrain (coupe ouest-est), et la concentration en cuivre du sol local non cultivé ('fond géochimique du cuivre').

Transport et la transformation du cuivre dans un bassin d'orage à Rouffach

A Rouffach, un bassin d'orage planté (avec des roseaux communs) servant de zone tampons humide artificielle récoltant les lames ruisselantes du bassin versant du Horhain a été aménagé en site expérimental. Ce dispositif a permis de caractériser les processus naturels de transport et de transformation du cuivre, ainsi que le potentiel de ce système à réduire la charge du cuivre dans les eaux de ruissellement.

La charge (concentration multiplié par le volume) et les rapports isotopiques du cuivre ont été mesurés dans la phase dissoute, dans les solides en suspension (entrée, sortie, et l'intérieur du bassin d'orage) sur une base hebdomadaire et dans les sédiments ainsi que dans la végétation du bassin d'orage pendant une période d'applications des fongicides cupriques (Mai - Juillet 2011).

- Les résultats montrent que plus de 68% du cuivre dissous et plus de 92% du cuivre liés aux solides en suspensions transportés par la lame ruisselante ont été retenus dans le bassin d'orage (Figure 4) confirmant son potentiel de rétention.

- Le cuivre dissous est appauvri en isotope lourd (^{65}Cu) lors de son passage dans le bassin reflétant ainsi le fractionnement isotopique du cuivre ce qui semble indiquer l'adsorption du cuivre sur des hydroxydes d'aluminium (et de fer) et de la matière organique présents dans les sédiments.
- Les sédiments accumulent plus de 96% du cuivre pendant la période d'observation montrant que la végétation n'était pas un puits important de cuivre (Figure 4).
- En conditions de hauts débits transitoires dans le bassin lors des épisodes de pluie, le cuivre en sortie devient plus enrichi en isotope léger (^{63}Cu), suggérant la remobilisation du cuivre sous forme réduit (sulfure de Cu(I), Cu(0)) dans les sédiments du bassin. Cela indique que les bassins d'orages peuvent devenir des sources secondaires de cuivre.

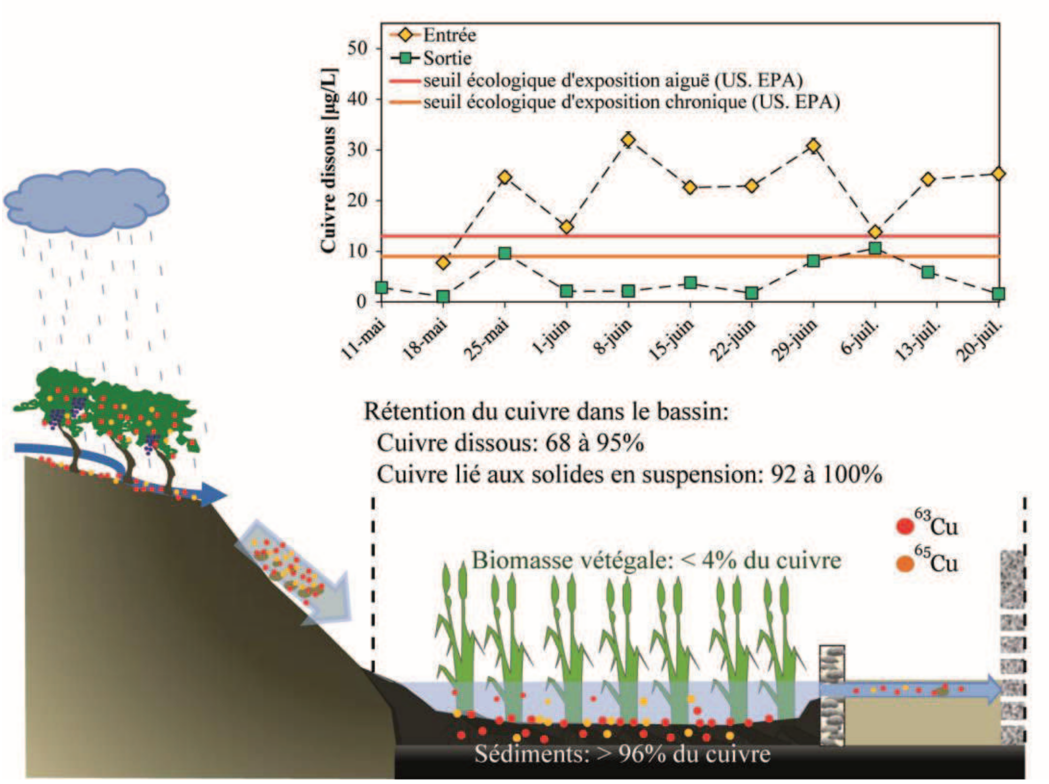


Figure 4 : *Rétention du cuivre, ainsi que sa répartition à l'intérieur du bassin d'orage à Rouffach (US. EPA : Agence Américaine de la Protection de l'Environnement).*

Evolution du cuivre dans les sédiments des zones humides

A l'aide d'une expérience en laboratoire (colonnes de Winogradsky), nous avons étudié les transformations que subit le cuivre dans les sédiments des milieux humides. Expérience fondamentale en écologie microbienne pour étudier les bactéries transformant le soufre et le fer,

les colonnes de Winogradsky sont des écosystèmes miniatures qui permettent d'amplifier les processus naturels dans les sédiments des milieux humides. Les sédiments du bassin d'orage à Rouffach ont servi pour construire les colonnes qui ont évolué en système fermé, et ont été échantillonnées au bout de 40, 80 et 520 jours.

- Les résultats ont montré que le cuivre était de plus en plus présent dans la phase résiduelle (le cuivre lié aux minéraux silicatés) ce qui indique le « vieillissement » du cuivre dans les sédiments et une réduction de sa mobilité avec le temps (Figure 5).
- La redistribution du cuivre dans les différentes fractions des sédiments en fonction de la profondeur due à l'activité microbienne a été mise en évidence. Cette redistribution induit un fractionnement isotopique, ce qui indiquerait la ré-oxydation des espèces de cuivre réduit en haut de colonne (en condition plus oxydante due à la présence d'algues), ainsi que sa mobilisation par la réduction des hydroxydes de fer en bas de colonne (en condition anaérobie, en l'absence d'oxygène).

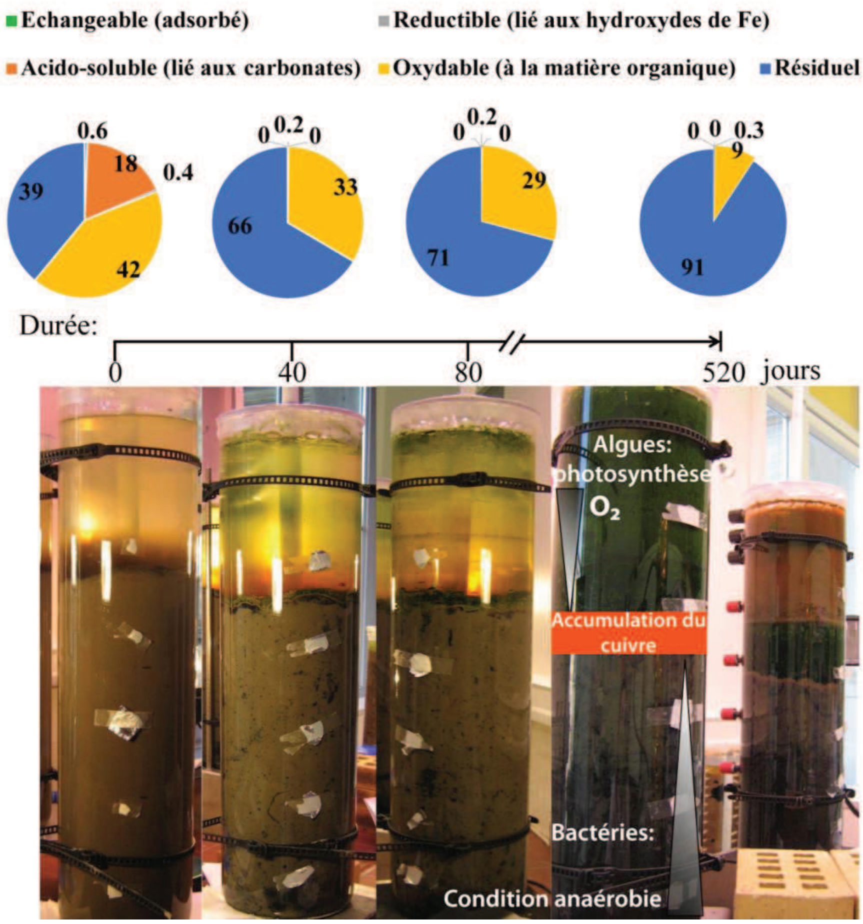


Figure 5: *Evolution des colonnes de Winogradsky et évolution du cuivre dans les sédiments mis en évidence par le changement de la répartition du cuivre (%) entre les différentes phases dans les sédiments (i.e., carbonates, hydroxydes de fer, matière organique, minéraux silicatés)*

IMPLICATIONS:

- Durant 4 à 5 décennies d'exploitation des parcelles viticoles, les sols en surface sont enrichis en cuivre de 9 à 28 fois par rapport au fond géochimique.
- Lors des évènements pluvieux, ~1% du cuivre appliqué (sous forme de fongicides cupriques) a été mobilisé pendant la saison, ce qui parait peu élevé. Cependant, la lame ruisselante transportait des concentrations en cuivre jusqu'à 3 fois plus élevées que le seuil écologique recommandé pour le bon fonctionnement des écosystèmes aquatiques.
- Les zones tampons humide artificielle (bassin d'orage) récoltant les lames ruisselantes provenant de petits bassins versants viticoles peuvent retenir une grande partie du cuivre et réduire ainsi la charge du cuivre dans les lames ruisselantes. Cependant, lors des hauts débits ces ouvrages peuvent relarguer du

cuivre préalablement accumulé dans leurs sédiments, et ainsi devenir des sources secondaires de cuivre.

- Le « vieillissement » du cuivre dans les sédiments des zones humides a tendance à réduire sa mobilité et sa disponibilité.

PUBLICATIONS

Babcsányi I., Chabaux F., Granet M., and Imfeld G., Using Isotopic Analysis of Copper to Assess Copper Transport and Partitioning in Wetlands, *Environmental Science and Technology,* **2014.**

Duplay, J.; Semhi, K.; Errais, E.; Imfeld, G.; Babcsanyi, I.; Perrone, T., Copper, zinc, lead and cadmium bioavailability and retention in vineyard soils (Rouffach, France): The impact of cultural practices. *Geoderma,* **2014.**

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I. Chapter: Introduction

This Chapter provides background information about the main subjects of the present PhD work, namely the problematic of copper (Cu) use in agriculture and assessing the fate of Cu in soils and wetlands based on Cu isotope fractionation. In the general introduction, the problematic of the use of Cu fungicides in a vineyard context and the approaches applied in the present work to trace the fungicide-derived Cu contamination is summarized. In the introduction, the relevance of studying the fate of anthropogenic Cu in the environment is discussed, with emphasis on Cu use in agriculture. The impacts of Cu contamination on soil and aquatic ecosystems and the biogeochemical processes affecting Cu in soils and in wetlands are then reviewed, while highlighting the main gaps of knowledge about Cu behavior in soils and wetlands. The theoretical background of stable isotope fractionation of chemical elements is then described, followed by the use of Cu stable isotopes in geological and environmental studies. Finally, we highlight the attempts that have been made so far to use Cu stable isotopes for tracing anthropogenic Cu in the environment, and present the research focus and the objectives of this PhD thesis.

1. General introduction

Copper (Cu) is a heavy metal that is omnipresent in the environment. Cu has been used by mankind since ~10 000 years and still today plays a vital a vital role in the industry and agriculture. Cu mining and the widespread use of Cu by humans caused the dispersion of this metal in the environment creating Cu contaminated sites, where elevated Cu levels can ultimately impair the ecological functioning of natural systems. Cu is an element, not a biodegradable compound, hence it accumulates in the environment creating areas contaminated with Cu that are hard to restore to natural conditions. The major challenges linked to Cu nowadays is to better understand the impact of the widespread Cu pollution that mainly affect soils while adopting strategies to limit its ecological impact on natural ecosystems.

Mine tailings and slags, smelters, fossil fuel burning in power plants, metal discarded in landfills, and agricultural use can release large amounts Cu into the environment that primarily impact soil environments.[1] Elevated Cu concentrations in soils can have toxic effects to soil biota, and may eventually reduce the fertility of soils.[2,3] The toxicity depends not only on Cu concentration, but also on the Cu speciation (i.e. complexed with organic matter, sorbed to clays, etc.). Therefore, we need to better understand Cu accumulation and distribution processes in soils, but also its mobilization by runoff that can transport Cu and eventually contaminate aquatic environments.

Several processes can control Cu behavior in the soil, in particular sorption to soil organic matter (SOM), to primary and secondary mineral phases (iron (Fe) and aluminum (Al) oxy(hydr)oxides, clay minerals) as well as co-precipitation with carbonates and hydroxides.[4] Most studies about Cu speciation in Cu-contaminated agricultural soils have shown that Cu was bound to SOM.[5-9] However, several other studies emphasized the role of carbonates in Cu retention in calcareous soils, which is often the case with agricultural soils.[10,11] Therefore, the processes that control Cu retention in agricultural soils still need to be elucidated.

Cu can be mobilized from soils by rainfall and transported by runoff in dissolved and particulate forms. Runoff can transport considerable amounts of Cu from Cu-contaminated land to receptor aquatic ecosystems and threaten their ecosystem functioning.[12] However, Cu mobilization by rainfall in Cu-contaminated sites has to be better understood with regards to the changing hydro-climatic conditions. Wetlands are interface environments between terrestrial and aquatic ecosystems, and can intercept Cu-contaminated runoff.

The transport of Cu in wetlands is controlled by the interplay of physical, chemical and biological processes.[13] Cu can be retained in wetlands by complexation with organic matter (in the sediments), by (co-)precipitation with mineral phases, plant uptake, as well as by oxidation-reduction processes.[14,15] By reducing conditions (i.e., in the absence of oxygen) that often prevail in wetland sediments, Cu(II) will be reduced in Cu(I) or Cu(0) via microbially mediated sulfate reduction that can sequester Cu in the sediments as Cu_xS_y, whereas the reductive dissolution of Cu-sorbing Fe(III) oxy(hydr)oxides can release Cu.[16-19] On the other hand, colloidal metallic Cu and CuS may be formed enhancing Cu mobility when oxic conditions change to reducing.[20] By changing hydrological conditions sediment re-suspension can occur and reduced Cu sulfides (Cu_xS_y) may be oxidized and Cu released into the dissolve phase.[21] Cu can thus be re-mobilized from wetland sediments and may not represent a permanent sink for Cu contaminants. Therefore, it is important to study processes governing Cu transport, partitioning and mobility in wetlands, particularly under variable hydrological and the changing redox conditions.

In addition to traditional tools, such as metal concentration measurements, chemical extractions of metals, mineralogical analysis, metal stable isotopes can potentially contribute to the understanding of processes that control metal behavior in the environment.[22] Cu stable isotopes ($^{65}Cu/^{63}Cu$) may be used to evaluate processes that govern the fate of anthropogenic Cu in the environment.[23,24] Stable isotopes are a potentially powerful tool as they are capable of integrating processes that control Cu partitioning in the environment across space and time, while traditional tools (listed above) provide only a point characterization of a system. The magnitude and the direction of the Cu isotope fractionation (i.e. the differentiated partitioning of isotopes between reactants and products during a physico-chemical reaction) can refer to the major processes controlling Cu, based on reference data on Cu isotope fractionation during physico-chemical and biological processes. The major processes governing Cu behavior, such as complexation with OM,[25] sorption to minerals,[26] precipitation,[27] oxidation-reduction,[28] and biological uptake[29] significantly fractionate Cu isotopes.

The aim of the present thesis is to evaluate if and how Cu stable isotopes may be applied to better understand Cu transport and transformation processes in agrosystems enriched in anthropogenic Cu. We performed our study in a system consisting of a vineyard catchment and a hydrologically connected stormwater wetland that intercepts the Cu-contaminated runoff. Our main hypothesis is that Cu stable isotopes coupled with more traditional approaches (e.g. mineralogical analysis, sequential extractions) can help to investigate Cu origin and behavior based on Cu isotope fractionation processes that may occur in Cu-contaminated soils and wetland sediments.

2. Cu: a major chemical element

Cu is a transition metal with an atomic mass of 63.546 g mol^{-1}. Cu has an electron configuration composed of 29 electrons, having one on the external orbital ($3d^{10}4s^1$). Indeed, the incomplete sub-shell confers to Cu special characteristics, such as remarkable electrical conductivity (1.67×10^{-8} Ωm).[30] Cu has two stable isotopes (^{63}Cu and ^{65}Cu), along with 26 other radioactive ones. Its melting point is at 1083°C and its density is 8960 kg $m^{3\ -1}$ (20°C).[31] Besides gold and silver, Cu is one of the first metals to be used by mankind, in particular by the discovery that it could be hardened with tin to form the alloy bronze that gave the name to a historical period, the Bronze Age. Its name is derived from the Latin "Cyprium" related to the island of Cyprus, where much of the Cu was mined in the Roman era.[30]

Cu in the form of native metal rarely occurs naturally, but the most important ores are the sulfides, oxides and carbonates. Cu has been identified in more than a hundred minerals in the 0, +I and +II oxidation states. The principal minerals of Cu are sulfides (such as chalcopyrite, bornite, chalcocite, covellite), (hydr)oxides (as cuprite, azurite, malachite), carbonates (as azurite, malachite) and silicates (as chrysocolla and dioptase).[32] Cu is obtained from these ores and minerals by smelting, leaching and electrolysis. The major Cu producing countries are Chile, China and Peru. Today, the most important use of Cu is in electrical equipment, building construction and infrastructure (wires, plumbing, etc.) and more recently for antimicrobial surfaces in healthcare.[30,33] The widespread use of Cu in industrial and agricultural production resulted in a significant modification of the Cu cycle at the global scale.

3. Global cycle of Cu and its anthropogenic modification

3.1. Stocks

Cu is present in all reservoirs of the Earth (Figure I-1). The most important Cu repositories are located in the Earth's core (2.4×10^{14} Gg), mantle (1.0×10^{14} Gg) and crust (9.5×10^{11} Gg).[1] In the crustal rock, Cu is present at an average concentration of 27 mg kg^{-1}.[1]

These endogenic reservoirs are followed by oceanic sediments (2.5×10^{10} Gg), soils (8.7×10^{9} Gg) and the men-used Cu stock (3.5×10^{5} Gg) (i.e. the global Cu stock in use by humans).[1] The average Cu concentration in soils is 30 mg kg^{-1}, where Cu originates only from the parent material.[34] It can be noticed that the global Cu reservoirs are largely dominated by solid forms of Cu (Figure I-1).

Cu stocks in freshwaters (6.3×10^{4} Gg) and oceans (1.9×10^{5} Gg) represent the following reservoirs in size. The mean dissolved Cu concentration in rivers is 1.19 µg L^{-1} (usually defined as <0.22 µm or <0.45µm) and in all uncontaminated freshwater systems is 3 µg L^{-1}, while in the oceans Cu is present at 0.13 µg L^{-1}.[1,2,35] The smallest natural Cu reservoirs are the natural biomass (that does not include the agricultural plant growth) (2.0×10^4 Gg) and the atmosphere.[1] The atmosphere contains only 2.6 Gg of Cu.

Fossil fuels constitute an important natural Cu reservoir (8.4×10^4 Gg), whose burning for energy production releases large amounts of Cu to soil and to the atmosphere.[1] Another anthropogenic Cu stock is the agricultural biomass (130 Gg) 90% of whom comes from natural soil and only 10% from fertilizers and pesticides.[1]

Evaluating the cycle of Cu at a global scale is still a challenging issue due to high heterogeneities in Cu distributions, as well as estimations of fluxes, in particularly those that are located beneath the earth's surface. Recently, a study suggested that underground exchange between continental and oceanic water bodies was largely underestimated in comparison to global river discharge to the oceans, in this case nutrient and metal fluxes at coastal areas may also be largely underestimated.[36]

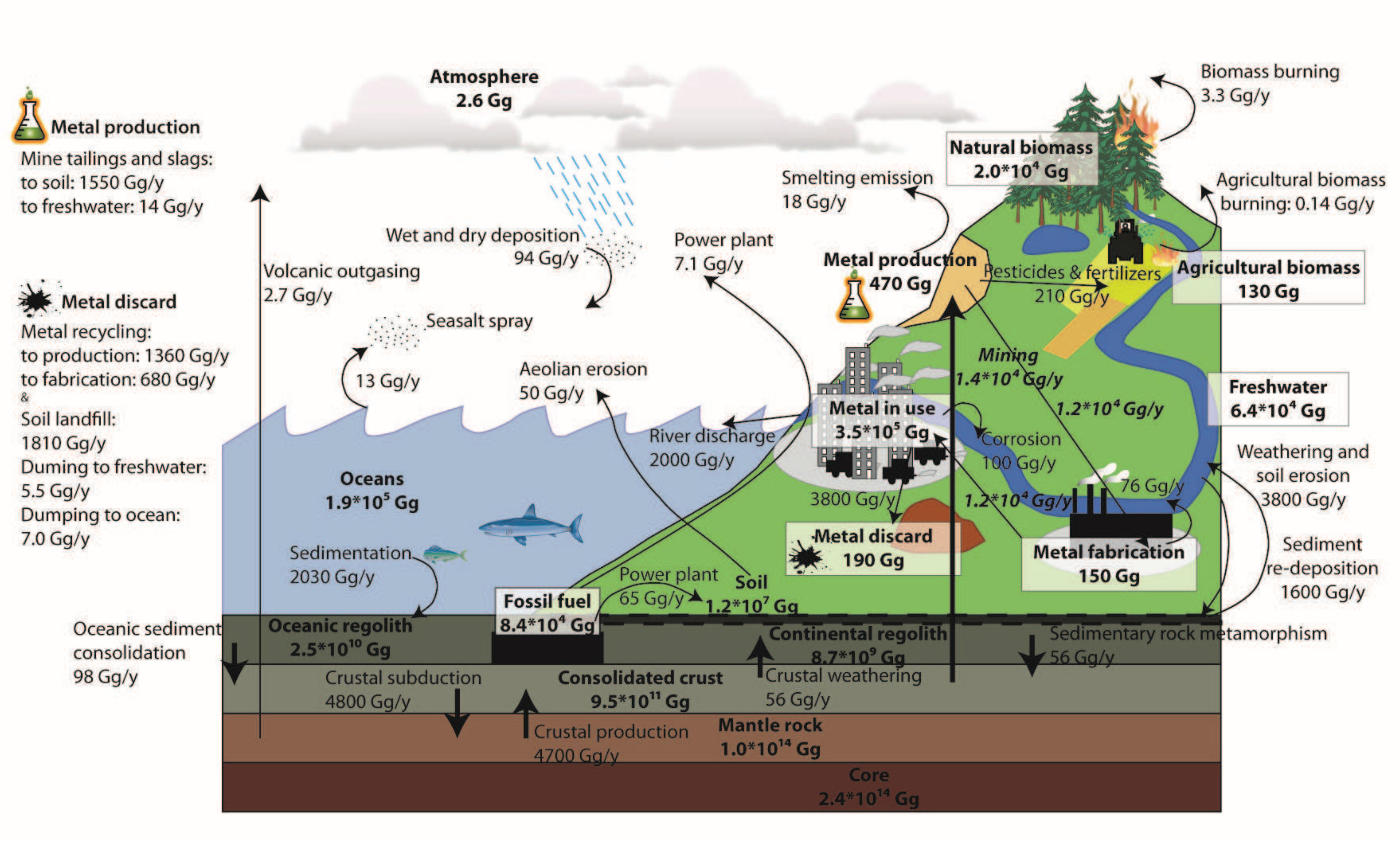


Figure I-1: *The global cycle of Cu (Figure based on data in Rauch and Graendel, 2007).*[1]

3.2. Flows and transformations

Although natural Cu reservoirs dominate, the anthropogenic Cu flows overrule natural ones (Figure I-1).[1] Overall, most Cu released from human activity impacts soils and surface freshwaters.

Although material flow between the Earth's core and the mantle is thought to be negligible, Cu flow between the mantle and crust is significant. Crustal subduction transports 4800 Gg Cu per year to the mantle, whereas crustal production transports almost the same amount of Cu back to the crust (Figure I-1).[1] However the highest Cu flow affecting the continental crust is anthropogenic and consists of extracting Cu from the Cu resources in the crust, which represents $1.4*10^4$ Gg year^{-1}.[1]

Different rock types that constitute the Earth's crust contain Cu commonly at trace levels at substitution sites in the mineral structures.[32] Cu occurs in many different minerals (160 Cu-bearing minerals have already been identified) the most abundant of which is chalcopyrite and it is economically the most significant.[37] Cu deposits can be formed by hydrothermal fluids containing significant amounts of Cu, such as porphyry Cu deposits, (i.e., formed by igneous intrusions in the upper crust), that yield about two-thirds of the world's Cu resources and sedimentary rocks that account for approximately one-fourth of world's identified Cu resources (Figure I-2).[37]

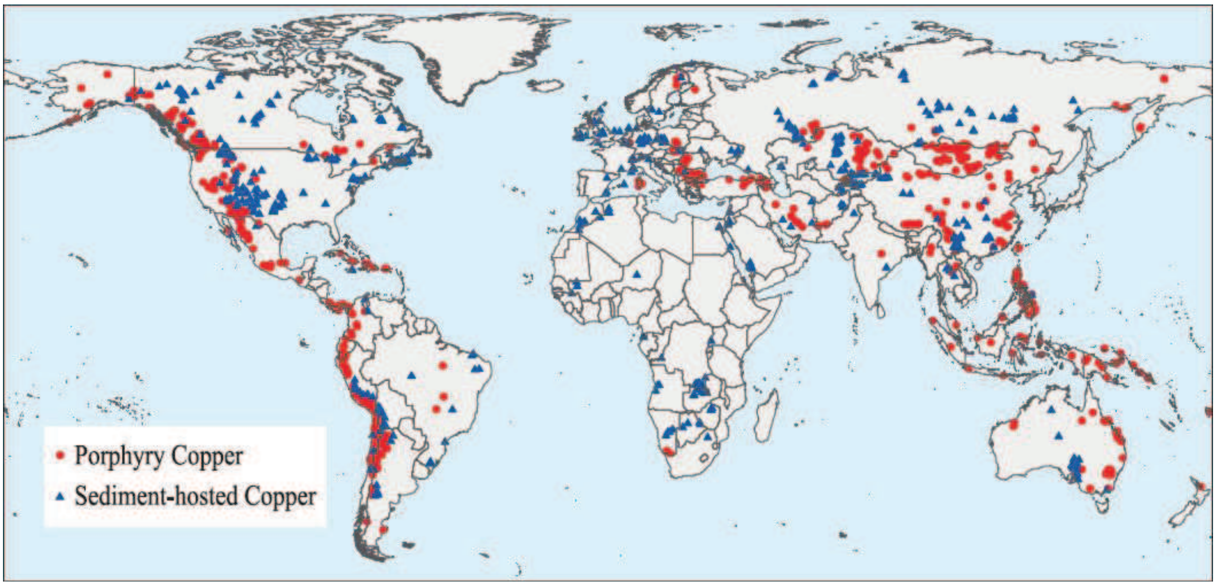


Figure I-2: *Distribution of known Cu deposits in 2008. Red dots indicate Cu associated with igneous intrusions (porphyry Cu deposits) and blue dots indicate Cu contained in sedimentary rocks (sediment-hosted Cu deposits). (Map prepared by the US Geological Survey)*[37]

The weathering of the crustal rock produces fluxes composed of the rock constituents and transports 56 Gg Cu year^{-1} to the soil at a global scale (Figure I-1).[1] Soils develop from the parent rocks by physical and chemical weathering processes and tend to reflect their chemical composition, though significant changes may occur due to the mobility of chemical elements in the soils.[32] Indeed the disintegration and the dissolution of primary minerals occur due to the thermodynamic instability of most primary minerals (formed under high pressure and temperature in depth) at the surface of the earth.[38] The dissolution of primary minerals will release Cu that can be affected by precipitation, adsorption, complexation with organic matter and biological uptake in the soil.[32] Besides weathering fluxes, rivers re-depose sediments (from soil erosion) to soils at a rate of 1600 Gg year^{-1}. Processes affecting the behavior of Cu in soils will be discussed in more detail in section **I.5**. The behavior of Cu during soil formation and the distribution of Cu along the soil depth profiles is still a common theme of research.[39]

Anthropogenic Cu fluxes into soils outweigh natural fluxes (Figure I-1). Mine tailings and slag deposits at Cu mines add 1550 Gg Cu year^{-1} to the soil Cu stock, while discarded Cu in soil landfills represent a flux of 1810 Gg Cu year^{-1}.[1] Around 210 Gg of Cu is applied yearly in agricultural production (as fertilizer or pesticide).[1] The large majority of Cu absorbed by the agricultural biomass is recycled to the soil (300 Gg year^{-1}).

The Cu load in rivers (3800 Gg Cu year^{-1}) mainly originates from soil weathering fluxes and soil erosion rather than from anthropogenic sources (such as mine drainage, discarded Cu dumping and corrosion of Cu equipment and infrastructure) (Figure I-1).[1] Cu in rivers is mostly in the particulate form, which is generally four to five orders of magnitude higher than the dissolved Cu concentration.[40,41] The relative distribution of elements between the dissolved and the suspended particulate matter (SPM) phases depends on the nature and concentration of mineral and organic ligands present in the dissolved phase and on the nature and amount of mineral and organic SPM as well as pH.[41,42] In rivers, the most important carrier phases of Cu are clay minerals, iron (Fe), aluminum (Al) and manganese (Mn) oxy(hydr)oxides and particulate organic matter originating from chemical soil weathering, biogenic processes and physical erosion.[41] SPM can also be formed *in situ* in rivers by precipitation of minerals (such as Mn oxides) or by the aggregation of colloidal matter to which Cu can be adsorbed.[41] Dissolved organic ligands, such as humic and fulvic acids as well as organic ligands produced by phytoplankton strongly complex with Cu which is the predominant form of dissolved Cu both in seawater and in freshwater.[2,43-45] Rivers transport 2000 Gg Cu year^{-1} to the oceans (Cu is partly re-deposed to land), which is suggested to be the major vector of Cu transport from the continents to the oceans.[1] In estuaries, the transient chemical conditions, in particularly the salinity induced flocculation (by the compensation of the negative overall charge of SPM surfaces by the positively charged dissolved ions) and settling of fine sediment particles and colloidal matter (mostly iron-humic acid colloids) can enhance Cu deposition to the sediments.[2,44,46] In aerobic sediment layers Cu is predominantly associated with organic matter, while in anaerobic sediments reduction of sulfate to sulfide leads to the precipitation of extremely insoluble Cu-sulfide or mixed metal sulfides through the co-precipitation of Cu with other metals.[43] Altogether 2030 Gg Cu is buried into the oceanic sediments yearly (Figure I-1).

The natural fluxes that transport Cu to the atmosphere are windblown dust mobilizing soil particles (50 Gg year^{-1}), seasalt spray (13 Gg year^{-1}) as well as forest fires (3.3 Gg year^{-1}).[1] Anthropogenic Cu fluxes are less important than natural ones and consist of releases from metal smelting (18 Gg year^{-1}) and Cu product fabrication (1.4 Gg year^{-1}), followed by fossil fuel burning for energy production (7.1 Gg year^{-1}), solid waste incineration (0.62 Gg year^{-1}) and biomass burning (0.14 Gg year^{-1}).[40] The airborne Cu exists essentially associated to oxidized anions, namely to carbonates, sulfates, hydroxides, and oxides and about 50% of the atmospheric Cu is water soluble and thus is affected by atmospheric washout. Atmospheric Cu is recycled back to the continents (60 Gg Cu year^{-1}) and oceans (34 Gg Cu year^{-1}) through dry and wet deposition.[43]

Overall, the most important Cu flows are anthropogenic and go from Cu extraction from the Earth's crust by mining, through Cu production (1.2×10^4 Gg year^{-1}) -by smelting- and transformation into products (1.2×10^4 Gg year^{-1}) towards the in-use Cu reservoir by humans. At the end of the cycle 3850 Gg Cu year^{-1} is discarded, 53% of which is recycled back to the production stage (1360 Gg year^{-1}) or the product fabrication (680 Gg year^{-1}) and the rest is buried in the soil in landfills with minor amounts released to surface waters from wastewater treatment plants (Figure I-1).[1] Anthropogenic Cu fluxes mainly affect soils creating a major problem of soil contamination by Cu. Even though only ~6% of all anthropogenic Cu inputs to the soil originate from Cu applications in agriculture,[1] large agricultural areas are impacted by this Cu use (~60 million ha dedicated to fruit production worldwide, FAO statistics).[47] Therefore, investigating the impact of Cu in agricultural areas is of growing interest due to challenges related to limiting Cu export off-site and soil remediation.[48]

4. Cu use in agriculture

Cu-related compounds are one of the oldest pesticides, mainly used in agriculture since the 1880s in Europe. At the turn of the 20th century, Cu products were used worldwide as the primary crop pesticide. Although the use of Cu compounds for agricultural purposes peaked in the 1930s, their proportions among all pesticides used decreased with the advent of synthetic pesticide molecules.[49] The world consumption of Cu sulfate (the most important Cu compound used in agriculture) is around 206,000 tons per year, and is principally used as a fungicide, but also for treating Cu-deficient soils.[50]

Cu is an essential nutrient for animals and plants, hence its use as a feed additive has become a common practice in particular in the poultry and swine industry.[49] In general total Cu in soils should exceed 4 to 6 mg kg^{-1} in mineral soils and 20 to 30 mg kg^{-1} in organic soils to avoid Cu deficiency in plants. Cu deficiency can be overcome by the addition of Cu fertilizers to the soil rather than by foliar application.[34] Farm manures, biosolids or composts also used as amendments to agricultural soils to improve soil fertility, can contain high amounts of Cu that can also represent a significant Cu input to agricultural soils.[32]

The principal agricultural use of Cu-related compounds is for plant protection against fungal diseases in orchards (e.g. apple, citrus, peach) and in vine-growing. Cu-based fungicides have been applied in European vineyards since the end of the 19th century. Cu and sulphur fungicides are still extensively used worldwide in the 21st century, representing more than 20% of the total amount of pesticides sold yearly (Figure I-3). Cu fungicides protect plants against fungal diseases such as the downy mildew, caused by *Plasmopara viticola*.[48] Cu fungicides include the Bordeaux mixture ($CuSO_4$ + $Ca(OH)_2$), Cu-oxide (Cu_2O), Cu-hydroxide ($Cu(OH)_2$) and Cu-oxychloride ($3Cu(OH)_2 \cdot CuCl_2$).[48] The protective effect of these Cu fungicides is expressed by the liberation of Cu(II) that destroys fungal spores by complexing with enzymes and blocking enzyme activity in such a way that causes a general disruption of cell metabolism and cell integrity.[51] Currently, no alternative treatments exist for controlling grapevine downy mildew in organic farming, even though some promising attempts have been recently made by using plant extracts of *Yucca schidigera*, *Salvia officinalis* and *Trichoderma harzianum*.[52]

Cu sprayed on the plant canopy will reach the soil by wash-off during rainfall, but can also be directly deposited to the soil. Because of the generally low Cu mobility in soils, Cu tends to accumulate in agricultural soils following repeated applications as Cu fungicides and Cu-containing biosolids. Several studies have been dedicated recently to the accumulation of Cu in agricultural soils and its transport to vulnerable aquatic receptors or to groundwater. There is a need to better understanding the environmental burden of agricultural applications of Cu and to recognize the risks associated with Cu-contaminated agricultural soils and ultimately aquatic ecosystems.[48,53]

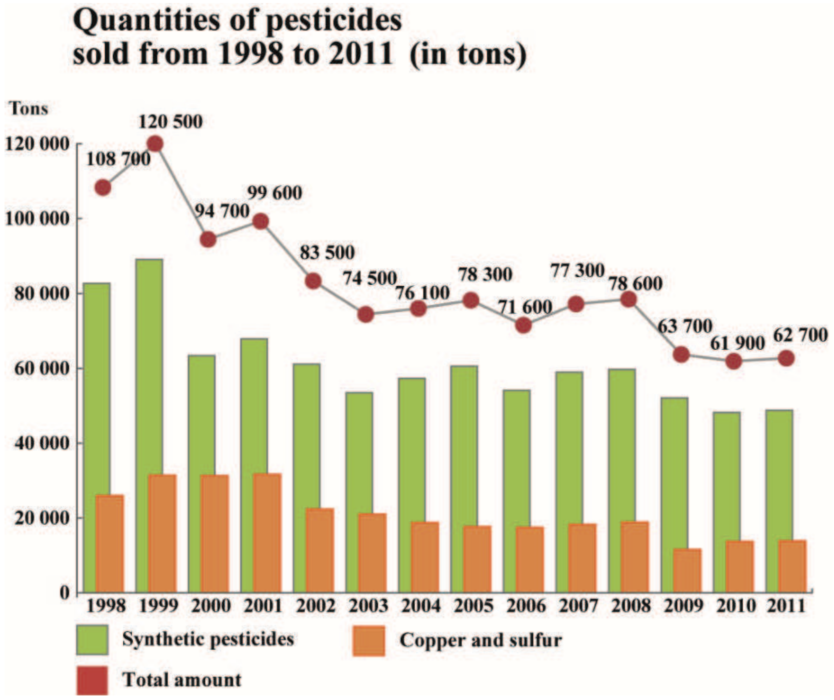


Figure I-3: *The evolution of the pesticide market at the global scale (Figure modified from UIPP Rapport d'Activité 2012).*

5. Cu contamination of agricultural soils: accumulation, mobility, transport, and ecotoxicology

5.1. Anthropogenic Cu accumulation in soils

Cu retention in soils can proceed through complexation with soil organic matter (SOM), sorption to Fe, Al, Mn oxy(hydr)oxides, (co-)precipitation with carbonates, as well as other minerals like hydroxides and to a smaller extent through sorption to clay minerals.[4,32] Sorption is the transfer of dissolved species from the liquid to a solid phase and includes adsorption by non-specific (i.e. ion exchange) or specific (i.e. chemisorption) mechanisms and precipitation. Adsorption can be limited in acidic conditions due to the competition of metal cations with the H^+ ions for the surface sorption sites of Fe/Al oxy(hydr)oxides, clays and organic matter.[54] Therefore, increasing the pH of the soil solution results in enhanced complexation and adsorption of soluble Cu.[34] It has been suggested that among all soil constituents SOM and oxy(hydr)oxides of Fe, Al and Mn largely control soil Cu behavior as they provide numerous sorption sites, i.e., hydroxyl groups on mineral surfaces and organic functional groups for the specific sorption of metals.[34] Specific sorption is based on inner-sphere binding of cations to OH-groups at the soil surfaces that are negatively charged at high pH.[4] A recent study suggested that at near neutral pH, SOM was the main soil constituent to adsorb Cu, while at acidic pH, oxides were the major Cu-sorbing phases.[55] Yet, sequential chemical extractions suggested that ~50% of Cu was SOM-bound in slightly acidic soils of Spanish vineyards as well as in Brazilian vineyards soils with slightly acidic to neutral pH.[56,57] X-ray absorption spectroscopy studies also converge to propose the prevailing role of SOM (or SOM coated mineral soil fractions) in controlling Cu speciation in contaminated agricultural soils.[6,8,58,59]

The respective role of different soil constituents in Cu binding to soil is still not completely understood and a matter of research. The soil texture also influences Cu distribution in the soil; the clay and silt fractions having much higher sorption capacity for Cu (i.e., specific surface) than coarse-grained materials, such as sands.[34] However, some studies suggested that Cu "hot spots" (i.e., Cu enriched sites) in contaminated soils were associated with particulate organic matter (i.e., plant residues) in the coarse-grained fractions underscoring the high affinity of Cu to organic matter.[9] Cu can be preferentially found in the coarse organic soil fractions associated with plant residues but can also accumulate in the fine soil fractions (< 5 µm) included in Fe oxy(hydr)oxides and clay-humus complexes even in calcareous soils.[60-62] Organo-clay complexes and humified SOM play an important role in Cu retention in the fine soil fractions.[63] The sorption capacity of clay minerals is generally related to their cation exchange capacity (montmorillonite > illite > kaolinite).[34] Some authors also emphasized the role of carbonates in Cu retention in calcareous soils, notably by precipitation of Cu as hydroxide or malachite.[10,11] SOM and carbonate precipitation may both control Cu retention in agricultural soils, however, the conditions by which one process dominates over the other is still not well understood.

Cu retention in soils is often described as a two-stage process with a rapid sorption of Cu to soil constituents followed by a slower process that renders Cu less mobile and bioavailable, which is referred to as "aging".[64,65] Processes of precipitation/nucleation and hydrolysis of Cu(II) followed by a micropore diffusion process and Cu occlusion within SOM may all govern the attenuation of available Cu in soils.[64,66] The aging of Cu can be long as in the case of Australian vineyards with acidic soils where fungicide-derived Cu stayed available (>50 wt%) for periods >10 years.[67] In particular in vineyards, Cu applications are usually combined with the use of other pesticides and fertilizers. Upon addition of phosphate fertilizers to soils, Cu sorption can be enhanced in acidic soils and decreased in calcareous ones due to pH changes.[68] Cu can act as a bridge between P and SOM in which case Cu binding to soil can be increased.[69] Cu has been found to limit the leaching of glyphosate (N-(phosphonomethyl)glycine)[70] herbicide in calcareous soils by forming ternary complexes (i.e. complexes involving three complex-forming species) between the soil matrix and the glyphosate.[71] In consequence, the interactions between co-occurring agricultural inputs in agricultural soils as well as the long-term transformations of Cu in contaminated soils influencing its mobility and bioavailability should be better understood.

5.2. Cu toxicity to soil biota

Cu is a micronutrient, essential for all living organisms, but its accumulation in soils can pose ecotoxicological concern and may eventually reduce biological activity in the soil. Cu toxicity is directly linked to its bioavailability. Cu bioavailability depends on pH, SOM content, clay content and soil texture.[34,72,73] Plants can also substantially change metal availability in their rhizosphere. Plants have protective mechanisms to reduce Cu bioavailability and thus toxicity, by excreting root exudates to solubilize Cu and remove excess Cu from soil and/or by changing pH and rendering Cu less bioavailable in soil, but also by physical adsorption of Cu onto cell walls in roots (avoiding biological uptake). [48,74-78] In alkaline calcareous soils, in which metals are less available, plants can excrete siderophores that are strong complexing organic ligands to acquire Fe and remedy for Fe deficiency, but these will also complex with Cu and enhance Cu bioavailability, and may eventually lead to toxic effects (i.e. leaf chlorosis).[32,76] Particular tissues accumulate Cu in living organisms. In plants, it is the root system, especially the fine roots that accumulate Cu. Some Cu also concentrates in the bark and in young shoots.[34] Excess Cu can provoke physiological responses in plants such as chlorophyll and carotenoid breakdown, increased membrane permeability and lipid peroxidation that cause symptoms like reduced plant growth, poor root system, leaf chlorosis, senescent leaves and necrotic stems.[34] Elevated Cu concentrations in soils can influence other organisms as well, such as earthworms that are important in maintaining soil quality and fertility. Elevated Cu concentrations (>33 mg kg^{-1}) can adversely affect earthworm populations depending on the soil properties (e.g., pH, OM content, clay content) that determine Cu bioavailability.[48,79] Microorganisms are also sensitive to soil contamination by Cu. Reduced colony number and microbial diversity can be a consequence of Cu accumulation in soils, hence altering nutrient decomposition rates (i.e. biodegradation) and transformation rates (e.g. nitrification, N_2 fixation).[2]

Cu accumulation in agricultural soils can lead to the long-term degradation of soil fertility due to potential toxicity. Therefore, assessing Cu accumulation processes in agricultural soils following repeated Cu applications and its distribution amongst soil constituents is a prerequisite for evaluating its impact on soil biota as well as its mobility from soil. Indeed, Cu contaminated soils are prone to release higher Cu quantities during rainfall that can reach and contaminate vulnerable aquatic ecosystems downstream.

5.3. Cu mobility in soils, transport by runoff and ecotoxicity to aquatic biota

In general higher soluble Cu concentrations are found in the soil solutions of Cu polluted soils compared to uncontaminated ones.[80] Therefore, higher mobilization of Cu can occur from polluted agricultural soils by rainfall that can reach groundwater and surface waters situated downstream to agricultural areas. The mobility of Cu depends not only on soil Cu concentration, but also on soil properties and other factors such as hydro-climatic conditions and the period of Cu aging in soils following Cu applications.[32,81] SOM has a dual role in the control of Cu behavior in soils: particulate organic matter adsorbs Cu that decreases Cu mobility, while the dissolved organic matter (DOM) increases the solubility of Cu through complexation.[48] Organic matter desorption from soil particles and the release of DOM-complexed Cu is suggested to govern Cu mobilization from soil.[82,83] This is underscored by further studies that show Cu in the dissolved phase of runoff (< 0.45 µm) is mainly associated to smaller organic-rich colloids and larger organo-mineral complexes (Fe, Mn oxy(hydr)oxides and clays).[84]

pH is a key parameter in Cu release from soil. Acidic soils are prone to higher Cu leaching, which can be reduced by amendments aimed at increasing soil pH.[85] In a Brazilian vineyard with acidic soil (pH~4) 6 to 34 times higher Cu concentrations were observed in the groundwater compared to background values.[86] However, Cu mobility can also be increased at alkaline pH (>~7.5) due to SOM mobilization.[87] Changing from neutral to basic conditions, the deprotonation of the hydroxyl groups at mineral surfaces decreases the positive net surface charge and organic molecules become more electronegative. Hence, mineral surfaces and SOM repel each other and thus enhance SOM desorption from mineral surfaces in soils.[88] Ionic strength is another parameter that substantially affects Cu mobilization from soil. Higher ionic strength of the washing solution results in a higher release of soil Cu.[89] Higher ionic strength can enhance the competition between Cu and other cations present in the solution for adsorption sites and can result in Cu desorption from the soil.[4,89]

Cu transported from agricultural land in runoff is predominantly associated with suspended particulate matter (SPM) mobilized by soil erosion.[90] Depending on soil types different Cu amounts can be transported off-site, notably Mediterranean stony red soils are prone to intense soil erosion and thus significant particulate Cu export.[91] The mobilization of Cu from vineyards results in the contamination of downstream rivers in which Cu accumulates in the sediments resulting in high sediment-bound Cu concentrations.[24,87] Overall, the mobilization of Cu should be better understood and Cu fluxes from agricultural areas to aquatic environments better constrained, as Cu can be highly toxic to aquatic organisms.

Aquatic organisms show high sensitivity to Cu causing both lethal and sub-lethal effects (i.e. altered growth, reproduction and behavior) at Cu concentration levels as low as 5 µg L^{-1} for crustaceans and salmonids.[2] Cu is also an algaecide causing cell wall damage and cell lysis along with inhibition of cell division in algae.[2,48] Cu toxicity in water depends on its chemical forms that determine its bioavailability to aquatic organisms.[92] Free ionic Cu is the most toxic form, whereas carbonate-Cu and organic-Cu complexes are less toxic being less bioavailable.[92,93] However, some Cu complexes with lipophilic organic ligands that are able to pass through cell membranes, thus resulting in toxic effects.[94] The main factors affecting Cu toxicity in aqueous environments include pH, redox potential, temperature, light intensity, salinity and water hardness by controlling the Cu chemical forms in water, thus its bioavailability, but also the physiology of organisms and thus their vulnerability.[2]

Cu can be mobilized from agricultural soils and can be transported by runoff to surface waters, where elevated Cu levels may change the composition and functioning of the aquatic ecosystem. Wetlands are at the interface between terrestrial and aquatic ecosystems and can intercept Cu contaminated runoff from agricultural land; hence assessing and predicting the behavior of Cu in wetlands may help improving our knowledge about the fate of Cu in agro-ecosystems and connected hydro-systems. Moreover the concomitant presence of soil and water confers to wetlands specific characteristics affecting the transformation of Cu.

6. Wetlands intercepting Cu contaminated runoff

6.1. Wetlands as biogeochemical hot spots in the landscape

"Wetlands are transitional lands between terrestrial and aquatic systems where the water table is usually at or near the surface or the land is covered by shallow water. Wetlands are defined by one or more of the following three attributes: 1) at least periodically, the land supports predominately hydrophytes; 2) the substrate is predominantly undrained hydric soil; 3) the substrate is nonsoil and is saturated with water or covered by shallow water at some time during the growing season of each year."[95] Even though wetlands cover only ~6 % of the land surface of Earth, they harbor a large biodiversity and are often referred to as the "kidneys of the Earth", since they have an important role in nutrient cycling, water supplies, flood regulation and waste treatment.[96] Artificial wetlands are constructed all over the world to take advantage of the water-treating capacity of wetlands in a cost-effective way.[97]

Biogeochemical hot spots are defined as "patches in the landscape that show disproportionately high reaction rates relative to the surrounding environment".[98] Wetlands are often characterized as biogeochemical hot spots owing to the presence of a water flow, that is a key factor in transporting nutrients and in creating conditions that enhance biogeochemical cycling rates.[98] Biogeochemical hot spots generally display high nutrient transformation rates attributed to physico-chemical and biological processes.[99] Nutrient transformations are largely produced by microbial-mediated redox reactions by which microorganisms oxidize organic or inorganic compounds (i.e. electron donors) coupled to the reduction of electron acceptors.[100] In wetland sediments due to water saturation and the resulting oxygen-depleted conditions, microorganisms will utilize alternative terminal electron acceptors (TEAs) to oxygen in the order of nitrate, Mn(IV), Fe(III), sulfate and carbon dioxide related to the energy gain from the oxidation-reduction reaction (i.e., sequential reduction chain).[100] A steep redox gradient can be formed in wetland sediments due to these microbial-mediated redox processes. However, some studies have shown that the sequential reduction chain (i.e., theoretical sequence of the usage of TEAs) does not always apply to wetlands due to high-scale heterogeneities.[101] Moreover, wetland plants create a well-oxygenated microenvironment in predominantly anoxic sediments (i.e. rhizosphere) by releasing oxygen through their root system.[15] Altogether, the environmental conditions (pH, temperature (T), hydrology, etc.), the presence and the availability of nutrients (both electron donors and acceptors) and also the competition between microorganisms will play an important role in the redox stratification of wetland systems and thus their nutrient transformation capacities.[102]

Redox processes control to a great extent the behavior of many trace elements, including Cu, therefore wetlands are also hot spots for Cu transformation processes.

6.2. Cu behavior in wetlands

The dissolved and SPM-bound Cu transported by water fluxes to wetlands can undergo sedimentation, sorption, precipitation, redox processes and plant uptake.[13] The extent to which these reactions occur depends on for instance the composition of the inflowing water, pH, T, plant species, hydrological regime and the type of solid medium.[13,103]

Cu is known to be mainly transported as bound to SPM.[104] Consequently, sedimentation of SPM-bound Cu is one of the principle processes in removal of heavy metals from Cu-contaminated water in wetlands.[13,103,105] Macrophytes, such as *Phragmites australis*, promote sedimentation by decreasing water flow rates.[54] Other parameters can promote sedimentation alos, for example the dimensions of wetlands, but also pH that promotes flocculation of fine particles (i.e., the attachment and settlement) and determines sedimentation efficiencies.[103]

Sorption processes greatly affect dissolved Cu entrapment in wetland sediments as in soils.[15] Living or dead organic matter accumulated in wetlands is an important sink for Cu via sorption and complexation processes.[14] In the aquatic environment, the surfaces of mineral particles are often covered with organic coatings (i.e., humic substances and biofilms) that can significantly enhance the sorption of metals to these particles..[106] It has been demonstrated that fungi and bacteria exuding organic compounds, especially exopolysaccharides (EPS) and proteins, can bind high amounts of metals to biofilms in sediments and biofilms associated with plants.[107] However, metals can be released from biofilm under changing hydrological conditions (i.e., flood events) and/or due to the degradation of the biofilm binding sites by microorganisms.[106]

Metals can also be bound to carbonates at high pH and high carbonate concentrations in wetland sediments.[15] However, sediment decalcification may occur under flooding conditions due to the increase in the CO_2 pressure in the sediment porewater during microbial decomposition of organic matter and may lead to the release of Cu sorbed to carbonates.[21] Cu can also absorb onto or co-precipitate with Fe(III) oxy(hydr)oxides deposited onto root surfaces (Fe plaques) of aquatic macrophytes or on sediment surfaces under aerobic conditions.[13,108] In suboxic conditions (dissolved oxygen concentrations < 0.16 mg L^{-1}), the mobility of sequestered metals can be altered through the dissimilatory reduction of Fe(III) into Fe(II) that causes the dissolution of Fe oxy(hydr)oxides.[109] However, Cu release may not be directly linked to the dissolution of Fe oxy(hydr)oxides, but rather to the pH increase accompanying reduction reactions. Upon pH increase the deprotonation of hydroxyl groups at mineral surfaces can lead to the detachment of the negatively charged OM (by electrostatic repulsion) and the release of associated metals.[88,110]

Under anaerobic conditions prevailing in the wetland sediments, microorganisms that utilize sulfate as electron acceptor for their heterotrophic metabolisms are often responsible for the mineralization of much of the sediment-bound OM. For instance, microbially-mediated sulfate reduction governs the sequestration of metals in the sediments by producing sulfides. Under reducing conditions Cu(II) is reduced to Cu(I) which combines with sulfides to precipitate as Cu_xS_y species in wetland sediments.[14,111] A recent study has revealed the formation of Cu(0) and Cu-sulfide colloids in soils upon changing conditions from oxic to anoxic and the enhanced mobility of these reduced Cu forms.[20] Cu(II) can also be reduced to Cu(I) and Cu(0) by the redox-active moieties of organic matter and also by Fe(II) under reducing conditions.[17,18] Under reducing conditions, the formation of Cu-sulfide can efficiently sequester Cu in wetland sediments, but may also result in its release due to colloidal Cu(0) and Cu_xS_y formation upon changing redox conditions.

Direct biological processes (i.e. metal uptake by plants) can also be a sink for metals in wetland systems.[108] However, plant metal uptake usually accounts for only a small proportion of total metal removal in wetlands.[13] Accumulation of Cu in macrophytes' biomass (i.e., *Typha augustifolia, Phragmites australis*) was usually estimated to be only about 1-2% of Cu removed in the wetland.[54,105] In contrast another study has found that plant uptake accounted for ~30% of the Cu removed in a constructed wetland planted by *Cyperus alternifolius*.[112] Besides direct uptake, plants can also alter metal mobility by excreting root exudates, regulating pH in the rhizosphere, releasing oxygen through their roots and by providing decaying organic matter to the system.[13] In their rhizosphere microorganisms can also alter the bioavailability of heavy metals through production of extracellular polymeric substances (EPS) that strongly complex metals.[113] However, Cu mobility may be enhanced and Cu can be released from wetlands through the formation of nanoparticulate Cu in the rhizosphere.[114] Common wetland plants (*Phragmites australis* and *Iris preudoacorus*) can transform Cu into metallic nanoparticles near roots in oxygenated rhizosphere as part of a defense mechanism against metal toxicity.[114] Biological uptake by algae is not expected to be an important Cu sink in aquatic environments due to its algaecide properties.

Cu behavior in wetland systems is driven by the interplay of physical, chemical and biological processes that eventually determine whether Cu will be entrapped or released from wetlands. Hydrological conditions may significantly alter Cu behavior in wetlands by changing the physic-chemical conditions and by sediment re-suspension that can ultimately lead to metal release. Therefore, additional studies are necessary to investigate the transport of Cu in wetland systems to better understand Cu mobility in variable hydrological and biogeochemical conditions that characterize wetlands.

7. Assessing Cu transport and transformation processes in soils and wetlands

Because of the complex behavior of Cu in the environment and the risks linked to Cu ecotoxicity in soils and natural water bodies, the assessment of the fate of anthropogenic Cu in agro-ecosystems is essential. Understanding the transport and transformation processes of Cu in agricultural soils and wetlands is a prerequisite for correctly evaluating the risks of agricultural Cu applications.

In this perspective several studies investigated Cu contaminated soils and connected water bodies (i.e., rivers and groundwater), and in particular Cu speciation in agricultural soils,[6,59] chemical Cu partitioning among soil constituents,[57,115] Cu distribution among particle-size soil fractions[61,62] as well as tracing Cu behavior based on Cu isotope fractionation.[23,24]

Cu speciation can be investigated by synchrotron-based X-ray absorption spectroscopy (XAS), which involves the characterization of Cu and its surrounding atoms at a molecular level. XAS techniques are powerful methods for measuring elemental distribution in soils as recent advances allowed for increased sensitivity to detect trace elements also. XAS spectra can be performed on samples using either bulk or microscopic spatial resolution.[8] XAS-based observations all concur that most Cu in agricultural soils is organically-bound or associated with mineral phases with organic coatings.[5,6,58,59] Cu in wetland sediments have not yet been investigated with XAS techniques. In contrast, floodplain soils under flooding conditions have been studied and the results have shown the gradual transformation of the predominantly SOM-bound Cu to precipitated Cu_xS_y species through the transient formation of metallic Cu(0) upon the establishment of reducing conditions.[16,20]

Due to the need for complex facilities for XAS techniques, several studies apply more available methods to study Cu partitioning in soils, such as chemical extractions. The total metal concentration in soils/sediments reflects the geological origins as well as the anthropogenic inputs, but do not indicate the mobility or bioavailability of the metals.[116] Evaluating Cu partitioning by extraction methods in soils can indicate the quantity of Cu that can be readily mobilized from soils by rainfall as well as its bioavailable fraction. Although a multitude of extraction methods exit, none of them is able to exclusively extract one specific Cu form from the soil (such as organically bound Cu).[117] While selective extractions are used to target one specific Cu species in the soils, such as mobile and bioavailable Cu, sequential extractions are applied to assess the partitioning of the whole Cu content in a soil amongst the operationally defined phases ranging from mobile to immobile forms (i.e., residual Cu). In sequential extractions the residue from a first extraction step is used as the material for a second extraction and so on through a defined number of stages.[116]

In a typical procedure, the first target species in soils is the loosely attached and water soluble metal at cation-exchange sites in the matrix, followed by a stepwise attack on the carbonate phase, iron and manganese oxy(hydr)oxides and organic matter.[116] Finally, more refractory soil components, such as the primary silicates, are dissolved. In the course of the extraction sequence, the strength of the solvent increases while its specificity decreases.[116] Overall, the sequential extractions improve our understanding of the processes in the soil/sediment that control the mobilization and retention of metals as well as to elucidate their transport mechanisms. However, sequential extractions often fail to quantify the distribution of Cu within the soil constituents due to poor specificity of the extractants. The specificity of extractants can also depend on the soil properties and may eventually lead to misinterpretation of the metal partitioning in contaminated soils/sediments. Therefore, these methods are a cost-effective way to assess metal partitioning in soils and sediments at contaminated sites, although the results should be carefully interpreted.

Assessing the distribution of metals in soils by particle-size fractionation may provide information about the metal-enriched soil fractions (such as clay particles), which can allow evaluating the mobilization of metals during erosion processes. A size sorting may occur while soil particles are detached from the soil by runoff water, as well as during transport. Size sorting may lead to the enrichment of clay particles in the eroded soil fractions.[118] Hence, metal accumulation in finer soil fractions displaying higher specific surface areas thereby leading to more off-site transport of metals by erosion. Cu distribution in soils is heterogeneous with Cu accumulating either in the coarser particulate OM or in clay soil fractions.[62] The distribution of Cu within the different soil particle-size fractions can thus be useful to better understand Cu accumulation in specific soils as well as to evaluate Cu mobilization by runoff.

More recently, the advance of multicollector ICP-MS allows for a more precise analysis of metal stable isotope compositions to detect the isotope fractionation processes in nature that produce small variations in the isotope ratios.[22] Using reference values in of Cu isotope fractionation during individual physico-chemical and biological processes, Cu isotope analysis may help to decipher the specific processes that govern Cu transport and transformation processes at contaminated sites. Metal contamination sources may also be identified if isotope ratios can be clearly distinguished between metal sources and fractionation during transport processes does not mask the isotope signatures of the sources. The basis and some applications of stable isotopes will be discussed in the following sections, as this approach has been selected for tracing anthropogenic Cu in an agricultural context in this Ph.D. work.

8. Stable isotopes of metals in environmental studies

Isotopes are atoms whose nuclei contain the same number of protons but a different number of neutrons. Isotopes can either be stable or unstable (i.e., radioactive). Isotopes are considered stable when their radioactive decay times are too long to be detected. Due to the differences in the mass of isotopes of a chemical element, their physico-chemical properties slightly differ. These differences are called isotope effects that lead to a considerable separation of the isotopes during reactions (i.e., isotope fractionation).[119]

Quantum mechanical effects are responsible for the differences in the physico-chemical properties of isotopes of an element. The energy of a molecule is restricted to certain discrete energy levels determined by the energy “pockets” of $1/2h\nu$, where h is the Planck’s constant and ν is the vibrational frequency of the atoms forming the molecule.[119] The molecule would possess a certain energy level above the minimum of the energy curve even at the absolute zero temperature, called the zero point energy (ZPE) (Figure I-4), at which molecules vibrate with their fundamental frequency. The vibrational frequency, which depends on the mass of the isotopes building up a molecule, is lower for molecules of heavy isotopes (lower ZPE) (Figure I-4). This means that the bonds formed by the heavy isotope are harder to break than bonds involving the lighter isotope. Thus, during a chemical reaction, light isotopes will in general react a little more readily.[120]

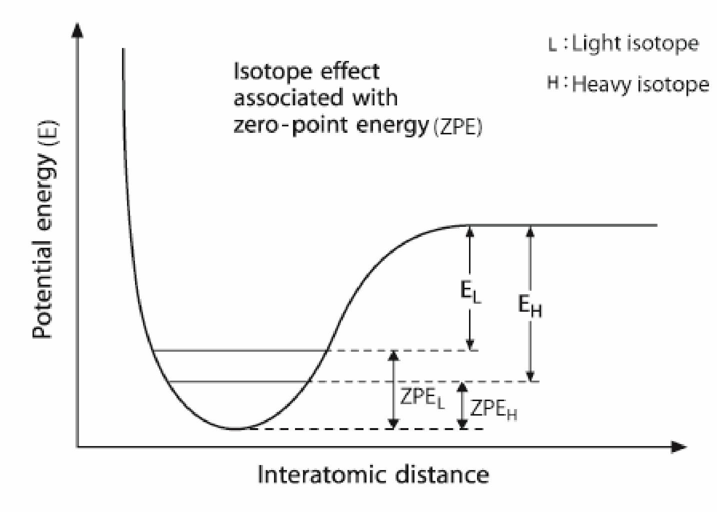


Figure I-4: *Schematic potential energy curve for the interaction of two atoms in a stable molecule (Figure modified from Hoefs, 2009).*

Based on these mass-dependent effects, we can distinguish between equilibrium and kinetic isotope effects. The kinetic isotope effect is produced when reaction is unidirectional in case of incomplete reactions or when the product is removed and will not continue to exchange with the reactants. Lighter isotopes are more reactive, therefore the product is enriched in the lighter isotopes. The equilibrium isotope effect is produced due to the mass dependence of equilibrium constants (K_{eq}) related to bond strength. Equilibrium isotope effects will tend to enrich heavier isotopes in the species with stiffest bonds (bond stiffness being greater for shorter and stronger chemical bonds).[121] The isotope fractionation between species or phases A and B are described by the fractionation factor $\alpha_{A\text{-}B}$:

$$\alpha_{A-B} = \frac{\mathrm{R_A}}{\mathrm{R_B}} \qquad (1)$$

where R_A and R_B are the isotope ratios of an element in species or phases A and B. Measured isotope ratios are expressed relative to a standard in per mil (‰):

$$\delta^{h/l}\mathrm{X} = \left(\frac{\mathrm{R}^{h/l}_{\mathrm{sample}}}{\mathrm{R}^{h/l}_{\mathrm{standard}}} - 1\right) \times 1000 \qquad (2)$$

where $R^{h/l}$ is the ratio of the heavier over the lighter isotopes of an element X in a sample and the standard. Therefore, in practice isotope fractionation between two reservoirs is more often expressed as the "Cap delta" Δ_{A-B} (‰):

$$\Delta_{A-B} = \delta^{h/l}X_A - \delta^{h/l}X_B \tag{3}$$

where $\delta^{h/l}X$ is the isotope ratio of an element X in reservoirs A and B. The two fractionation factors are related as:

$$1000 \times \ln\left(\alpha_{A-B}\right) \approx \Delta_{A-B} \tag{4}$$

This approximation can be made for most of the cases as α_{A-B} is close to 1 (generally in the order of 1.00x).

Probing the stable isotopic composition of metals in environmental matrices may help to infer which processes are governing the fate of metals in the environment. However, the extent and the direction of the isotope fractionations produced by different processes may overlap, in which case the information gained from isotopes can be limited. So far, variations in the isotopic composition of elements have been used to study climatic changes, biologically-driven reactions, weathering processes, and more recently to track the fate of both organic and inorganic contaminants in the environment. Within the broad spectrum of elements that exhibit measureable isotopic variations, metal stable isotopes are increasingly used in a wide range of fields; to study biological, geological, and environmental issues.[22,122,123]

8.1. Metal transport processes and tracing metal sources in the environment

Biogeochemical processes often lead to a significant isotope fractionation of metals in the environment. In general, the highest fractionation rates for metal isotopes were observed for oxidation-reduction processes.[22] For Fe isotopes microbial Fe(III) reduction produced the largest isotopically distinct Fe reservoirs on Earth. The temporal record of Fe isotope ratios in sedimentary rocks indicated redox-related changes in the Fe biogeochemical cycle over Earth's history, related to microbial metabolic processes.[124] Chromium (Cr) is a highly toxic metal when found in the soluble +6 oxidation state. Reduction of Cr(VI) to Cr(III) renders Cr immobile in the environment and less toxic. This reaction has been shown to produce a large isotope fractionation ($\Delta^{53/52}Cr_{Cr(III)-Cr(VI)} \approx$ -3 to -4‰) that enriches the precipitated Cr(III) in the lighter isotopes.[125] Hence, Cr isotopes can be used for evidence of Cr(VI) reduction and Cr(III) precipitation in groundwater which cannot be clearly identified by Cr concentration decrease due to dilution effects. Moreover, Cr isotope ratios in the groundwater also are used to estimate the rate of Cr(VI) reduction with the help of an isotope fractionation model (Rayleigh distillation) (Figure I-5).

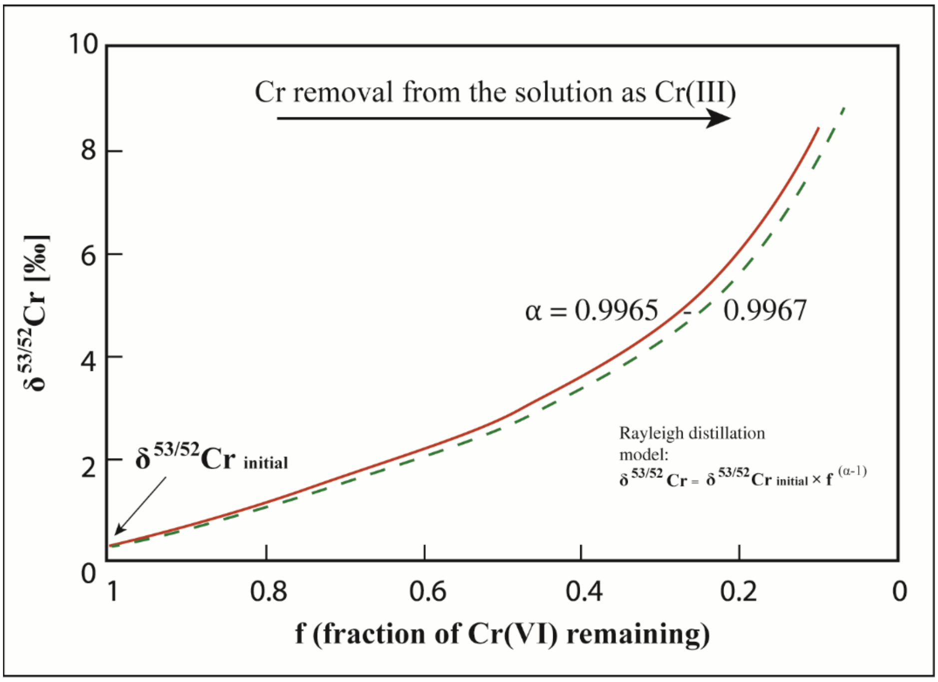


Figure I-5: *Cr(VI) reduction leads to the removal of Cr from groundwater as insoluble Cr(III). The Rayleigh distillation model fits the Cr isotope fractionation produced by the Cr reduction by different reducing agents (magnetite, sediment). (Figure modified from Ellis et al. 2002).*[95]

When isotope fractionation is negligible during transport, metal stable isotopes can fingerprint the contribution of metal sources in environmental samples for contrasting isotope signatures of sources. For example, zinc (Zn) stable isotopes have successfully been applied to distinguish between anthropogenic and geogenic Zn in smelter-impacted soils and to trace sources in river water (Figure I-6).[23,126-128] Using isotope ratios may help to determine the relative contribution of each source to the metal content of environmental samples using a mixing equation with as many end-members as the number of sources.

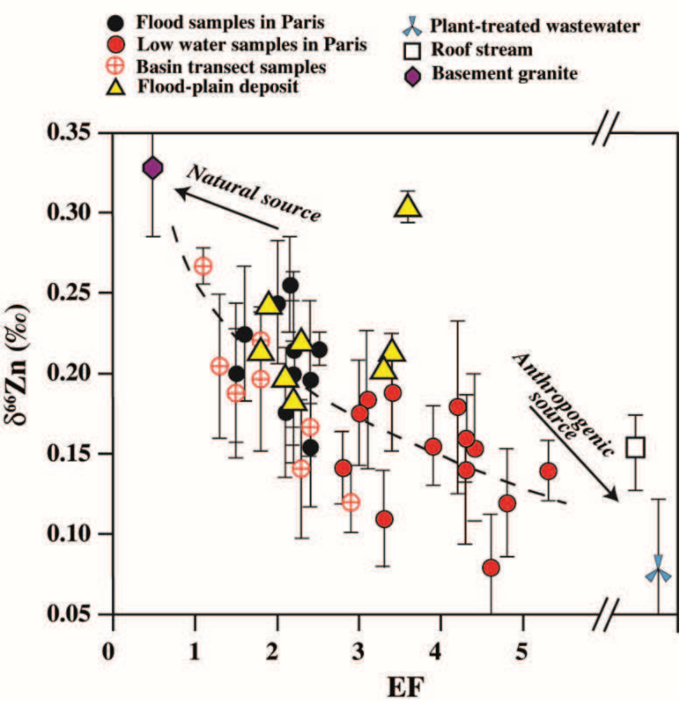


Figure I-6: *Zn isotope ratios versus Zn enrichment factors (EF) showing the relative contribution of the two end-members to the Zn load in the suspended sediments in the Seine river. The end-members are the natural geogenic Zn (granites) and the anthropogenic Zn from the wastewater treatment plant and the roof runoff. (Figure from Chen et al. 2009).*[92]

8.2. Cu isotope fractionation in the environment

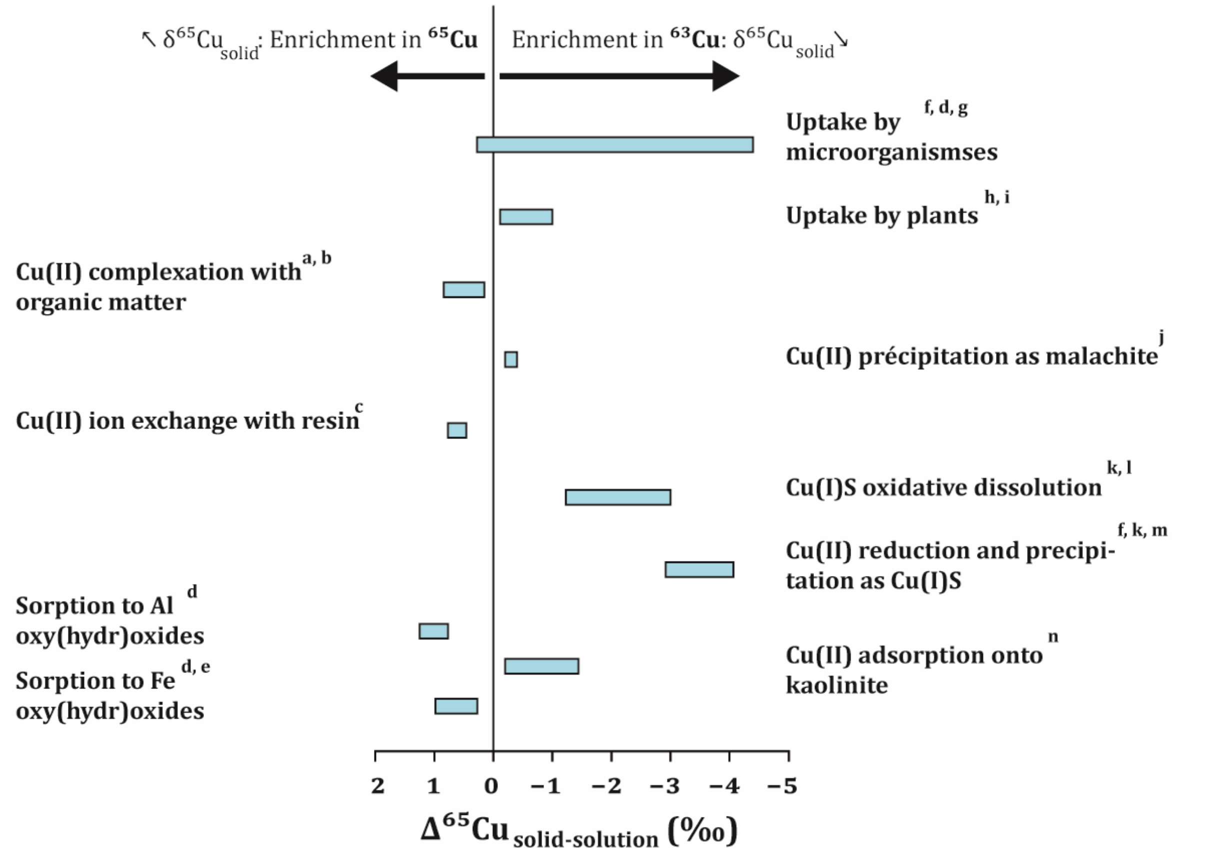
Cu has two stable isotopes: ^{63}Cu (69.17 %) and ^{65}Cu (30.83 %). The stable isotopic compositions are expressed as δ^{65}Cu (eq. 2) relative to the standard material: NIST 976. Cu isotope fractionation occurs during processes such as phase changes,[26,27,129,130] redox processes,[28] ion exchange,[131] complexation[132] and biological uptake[29] (Figure I-7).

Cu isotopes can be a useful tool in exploring and evaluating Cu ore deposits in diverse geological settings. The Cu isotopic compositions of geological materials were observed to vary up to ~16 ‰.[133] Redox-changes resulting in significant fractionation of Cu isotopes in ore deposits proved to help in interpreting the behavior of Cu during sedimentary mineralization processes,[134,135] as well as inferring the environmental conditions in which sedimentary Cu was formed.[136] Such fractionation of Cu isotopes during oxidation-reduction also helped to better understand Cu deposit formation in hydrothermal systems, even though tracing the origin of Cu in deposits was hindered by these redox-driven isotopic changes.[137]

Cu isotopes enabled studying the low-temperature aqueous geochemistry of Cu and elucidating metal migration and transport from terrestrial systems to the oceans. In rivers worldwide, Vance and co-workers[35] observed a significant difference in isotope ratios of the dissolved (Cu complexed with organic matter) and the particulate forms of Cu (Δ^{65}Cu$_{\text{dissolved-}}$

$_{\text{particulate}}$=0.66 ‰). Similar results were found in streams at historical mining sites in Spain and North-America with isotopically heavy Cu in the dissolved phase.[138] In boreal lakes, the isotopically heavy Cu was found to be homogeneous within different particle-size colloidal and dissolved fractions. The lack of a significant difference of Cu isotope distribution between different colloidal forms and truly dissolved phases was attributed to the similarity of the chemical structure and stability of the dominating Cu(II)-fulvic acid complexes of different size.[139] Overall, surface and ground-waters draining Cu deposits were found to be isotopically heavier relative to the Cu ores due to the preferential dissolution of ^{65}Cu during oxidative weathering of Cu sulfides.[133,140] However, this heavy Cu isotope signature in acid leachates can be masked in some cases when the presence of acidophilic bacteria (*A. ferrooxidans*) preferentially absorbs ^{65}Cu thus lowering the isotopic ratio of the aqueous phase.[141]

The most important isotope mass-fractionation is produced by oxidation-reduction processes that lead to the enrichment in ^{63}Cu of the Cu(I) phase by $\Delta^{65}Cu_{Cu(II)-Cu(I)}$=~3-4 ‰.[28,142] Cu has a high affinity for organic matter; therefore OM can largely determine Cu speciation and isotopic patterns. Cu complexation with dissolved and solid organic matter can induce a $\Delta^{65}Cu_{OM\text{-}complexed\text{-}Cu(II)}$ ranging from +0.14 to +0.84 ‰.[25,132] The magnitude of the isotope fraction was correlated with the stability of the Cu-organic ligand complex.[132] Cu speciation in solution can also influence the isotopic distribution patterns between different aqueous Cu species. Dissolved Cu carbonates and sulfates are expected to be isotopically heavier than free Cu by *ab initio* calculations.[143] The influence of solution speciation was further confirmed by the greater isotope fractionation induced by malachite precipitation from a chloride solution relative to a nitrate medium.[27] Cu sorption to mineral surfaces also fractionates Cu isotopes. The adsorption of Cu onto both amorphous and crystalline Fe/Al oxy(hydr)oxides produced a significant enrichment in the heavy isotope of the surface-adsorbed Cu ($\Delta^{65}Cu_{oxy(hydr)oxides\text{-}solution}$: 0.6 to 1.3 ‰).[26,144] In contrast, Cu adsorption onto the clay mineral kaolinite produced the enrichment of the kaolinite in the lighter Cu isotopes ($\Delta^{65}Cu_{kaolinite\text{-}solution}$: -1.46 to -0.17 ‰).[130] The difference in the Cu adsorption-induced fractionation may reside in the speciation of Cu that forms inner-sphere complexes with Cu-O on Fe/Al oxy(hydr)oxides, while it likely forms outer-sphere complexes with kaolinite.[130]



a: Ryan et al., 2014, **b**: Bigalke et al., 2010, **c**: Maréchal et Albarède, 2002, **d**: Pokrovsky et al., 2008, **e**: Balistrieri et al, 2008, **f**: Zhu et al., 2002, **g**: Navarrete et al., 2011, **h**: Jouvin et al., 2012, **i**: Ryan et al., 2013, **j**: Maréchal et Sheppard, 2002 , **k**: Mathur et al., 2005, **l**: Kimball et al., 2009, **m**: Ehrlich et al., 2004, **n**: Li et al., 2015

Figure I-7: *The range of Cu isotope fractionation produced by individual biogeochemical processes.*

Biologically-driven processes can also fractionate Cu isotopes. In the case of active uptake mechanisms by bacteria, biogenic Cu is isotopically lighter than the source by a $\Delta^{65}Cu_{biogenic\text{-}solution}$ of -0.2 to -4.4 ‰.[26,28,145] However, another study did not evidence significant isotope fractionation of incorporated Cu by some bacterial strains at circumneutral pH, while at acidic pH light isotopes were preferred.[26] Cu uptake by plants also shows preferential incorporation of isotopically lighter Cu. As the magnitude of Cu isotope fractionation varies according to species, Cu isotope fractionation helps to decipher Cu uptake mechanisms in plants. Strategy I plants seem to acquire Cu by reduction processes indicated by an enrichment of the whole plant in ^{63}Cu of ~1 ‰ (Figure I-8).[146] Strategy II plants display significantly smaller ^{63}Cu enrichment in their roots (of ~0.1 to 0.4 ‰), suggesting that complexation by phytosiderophores dominate Cu uptake.[29,146] Contrasting results were found for Cu isotope fractionation during Cu translocation within the plants. Although translocation mechanisms are still not fully understood, it was suggested that Cu(I) re-oxidation in roots and Cu(II) complexation by organic agents are probably involved in transporting Cu from roots to shoots.[146] A study of Cu isotope fractionation upon plant uptake in field conditions showed an opposite trend of isotope discrimination during plant uptake from soil (with $\Delta^{65}Cu_{plant\text{-}soil}$ of ~0.15 to ~0.5 ‰).[147] This underlines the importance of bioavailable Cu sub-pool in the soil that might have a very different isotope signature compared to the bulk soil.[147]

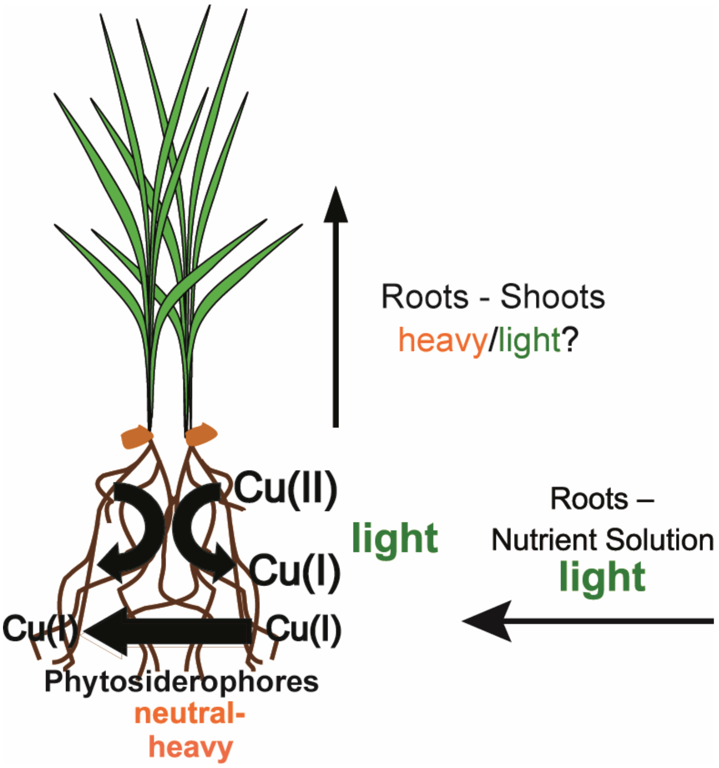


Figure I-8: *Cu isotopes to study Cu uptake and translocation mechanisms by plants (Figure modified from Jouvin et al., 2012).*[99]

How Cu isotope fractionated during soil weathering also has the potential to help better understand Cu biogeochemical behavior in soils. Contrasting behavior of Cu was evidenced by Cu isotopes in hydromorphic (soils influenced by water saturation) and oxic (well-aerated) soils. In hydromorphic soils $\delta^{65}Cu$ tended to increase (shift towards isotopically heavier Cu) with depth interpreted to be caused by redox-induced loss of isotopically lighter Cu to the soil solution or the vertical transport of organically-bound heavy Cu.[148] In oxic weathered soils, the opposite depth trend of $\delta^{65}Cu$ was observed attributed to not clearly identified weathering processes and/or plant cycling.[149] Fluctuating redox conditions were responsible for the variable $\delta^{65}Cu$ in the depth profile of a river floodplain soil.[150] These findings further highlight the importance of environmental conditions in Cu partitioning between dissolved and solid phases in common environments (i.e. soils, surface and ground-waters) for which Cu isotopes ratios may be used as a proxy.

8.3. Cu isotopes applied in contaminated sites

Cu isotopes were also used for tracing the fate of Cu as a contaminant in the environment. Cu isotopes were useful in tracing Cu transport in a smelter-impacted soil, but failed to distinguish between natural and smelter-derived Cu.[23] Historical contamination of lake sediments by Cu was highlighted by Cu isotopes ratios. Smelter-derived Cu deposition in a sediment profile significantly increased the δ^{65}Cu during the industrial era.[151] In a river system in France (Garonne) Cu isotopes failed to distinguish between the respective role of anthropogenic Cu contribution to the river Cu load and biogeochemical fractionation processes.[152] Markedly contrasting Cu isotope signatures of the different Cu sources and the lack of isotope fractionation during Cu transport and post-depositional processes are the main criteria for successfully using Cu isotopes in source tracing. Cu isotopes were recently applied as a tool to assess the fate of fungicide-derived Cu in a Mediterranean vineyard catchment. Anthropogenic Cu was suggested to be retained mainly in the organic soil fractions.[24]

Overall, based on the large spectrum of biogeochemical processes that cause isotope fractionation, Cu isotopes have a great potential to evaluate the transport and transformation processes of anthropogenic or geogenic Cu in the environment and decipher Cu uptake mechanisms by living organisms. Cu isotopes may therefore help to decipher the processes that govern the fate of anthropogenic Cu in an agro-ecosystem in which questions still remain open about the retention/mobilization processes of Cu (mainly applied as a fungicide). Cu stable isotopes may sign and contribute understanding the behavior of Cu in agricultural soils and connected wetland systems before it reaches and potentially contaminate aquatic ecosystems. The main goal of this Ph.D thesis was therefore to evaluate the contribution of Cu stable isotopes for tracing the fate of anthropogenic Cu in agro-ecosystems receiving anthropogenic Cu.

9. Research focus and objectives: Cu transport in vineyards

9.1. Cu in an agricultural context

Cu has been applied in European vineyards since the end of the 19th century. It can be presumed that higher than 8 kg ha^{-1} year^{-1} was applied in the beginning due to the lack of legal limits.[3,153] Currently, farmers apply Cu-based fungicides at typical rates of 1-2 kg ha^{-1} year^{-1} in Europe, 0-20 kg ha^{-1} year^{-1} in Australia and up to 65 kg ha^{-1} year^{-1} in Brazil.[3] While Cu is sprayed directly onto the vine canopy, spray drift and direct deposition onto the non-target soil (i.e., drift) cannot be avoided. Due to the drift and the wash-off from the vine plants, Cu reaches the ground and accumulates in vineyard top-soils resulting in Cu concentrations often exceeding the warning and critical limits for soils established in the EU respectively at 50 and 140 mg kg^{-1}.[48]

Most fungicide-derived Cu accumulates in surface soil horizons in which Cu concentrations often exceed 100 mg kg^{-1} .[3] The Cu concentrations in vineyard top-soils (O to 40 cm) greatly vary among winegrowing regions in France. Values reported in the literature range between 22–398 mg kg^{-1} in the French Mediterranean region, 17-417 mg kg^{-1} in the Beaujolais-Burgundy region, 40-243 mg kg^{-1} in Alsace and 5 to 523 mg kg^{-1} in the Bordeaux region.[48,53] Even higher values were reported for Spanish vineyards with Cu concentration reaching 666 mg kg^{-1} in Galicia, albeit the highest values were observed in Brazilian soils with Cu concentration exceeding 1000 mg kg^{-1}.

Elevated Cu concentrations in vineyards indicate the accumulation of fungicide-derived Cu in the soils. However, the processes by which the applied Cu fungicides are retained in the soils are still not completely understood. Still, the main concerns about fungicide-derived Cu accumulation in vineyards are adverse effects on soil ecology and fertility as well as Cu export by rainfall-runoff events that can reach aquatic ecosystems vulnerable to Cu pollution.[32,90,154] Wetlands hydrologically connected to vineyard areas can intercept Cu-contaminated runoff and may potentially be a means to reduce Cu transport from agricultural land to receptor aquatic ecosystems.[70] Therefore, the transport and transformation of Cu in wetlands needs to be studied with regards to the changing ambient conditions in wetlands due to variable hydrological patterns.

In this study we address the following questions:

- Which processes are responsible for the retention of Cu fungicides in the studied vineyard soils?
- How and to what extent is anthropogenic Cu mobilized and transported from the vineyard soils via runoff with respect to the hydro-climatic conditions (i.e., rainfall characteristics)?
- To which extent can anthropogenic Cu be retained in wetlands hydrologically connected to vineyard catchments?
- How the changing hydrological conditions impact the prevailing transport and transformation processes of Cu in wetlands and sediments?
- What are the long-term transformations of Cu in sediments at biogeochemical gradients?

9.2. Combining field-scale studies and laboratory experiments

Multi-scale investigations are complementary in the field of studying contaminant transport and transformation processes. While field-scale studies enable the integrative and *in situ* study of contaminant fate the overall biogeochemical functioning of ecosystems, laboratory experiments are used to study the factors and mechanisms controlling the behavior of contaminants.

When applying metal stable isotopes, interpreting field data cannot be done without the knowledge of the extent and the direction of isotope fractionation produced by individual processes that commonly govern Cu behavior in natural conditions.

For instance, Bigalke and co-workers studied the behavior of Cu in contrasted soils to get a better insight in the processes that affect the vertical distribution of Cu in soils.[149] They found that organic layers at the surface tended to be isotopically lighter compared to the mineral soils. They hypothesized that the biological recycling of Cu by the vegetation could be responsible for that. More recent studies at the laboratory-scale have confirmed that the vegetation takes up isotopically lighter Cu,[29,146] whose recycling to the soil by the decaying plant material can enrich the vegetation-impacted soil layers in lighter Cu.

Therefore, laboratory studies complement field observations to pinpoint the mechanisms controlling metal distribution in the environment.

In the present Ph.D. work, Cu isotopes were evaluated at three scales: i) at a vineyard catchment scale (42.7 ha), ii) at the scale of an engineered stormwater wetland (320 m^2) and iii) in a laboratory scale column experiment (63×15 cm). At the vineyard catchment scale Cu fluxes were tracked from the sources (Cu fungicides) through Cu accumulation in the vineyard soils and export off-site by rainfall-runoff. The stormwater wetland on the other hand can provide an insight into the reactive transport of Cu in dynamic environments and helps in quantifying ecosystem services of purifying Cu contaminated runoff. The column experiment was used to study transformations of the sediment-bound Cu at expanded biogeochemical gradients that commonly characterize wetland sediments. We hypothesize that the transport and transformation processes of anthropogenic Cu can be investigated both under field and laboratory conditions and the results compared with reference Cu isotope fractionations retrieved for individual biogeochemical processes in laboratory studies (Figure I-7).

Evaluating Cu stable isotopes to study Cu transport and transformation processes at the field scale is often not straightforward, hence additional characterization of the site and the samples are necessary to gain a better insight into Cu partitioning in natural systems.

Evaluating Cu isotope ratios in bulk soils often does not provide enough information about the processes that govern Cu behavior. Soils are a mixture of various primary and secondary mineral phases and SOM that may all affect Cu behavior and thus isotope fractionation in soils. A recent study carried out in a vineyard context, coupled Cu isotope ratio measurements with sequential chemical extractions of different Cu forms in polluted soils, as well SPM, and sediments in a river draining the vineyards. Combining several analytical methods may thus enable to understand the partitioning of fungicide-derived Cu in the studied vineyards.[24]

Here, we evaluated the Cu isotope partitioning in the different particle-size fractions of the vineyard soils, with special care to avoid causing isotope fractionation during sample treatments. Our objective was to gain insight into the specific retention/mobilization processes of fungicide-derived Cu in the studied soils. To complement isotopic measurement more traditional approaches have also been applied, such as:

- mineralogical analysis of soils/SPM/sediments,
- hydro-climatic data collection (i.e., rainfall patterns, runoff discharge at the outlet of the catchment),
- geochemical (i.e., chemical composition of soils/SPM/sediments, sequential Cu extractions),
- and hydrochemical analyses (i.e., dissolved anions and cations) in the water fluxes and in the liquid phase of bulk sediments.

9.3. Objectives of the thesis

The objectives of the Ph.D are formulated in the following at the different scales of investigation:

- **Catchment scale:** In the first part of the PhD, we used Cu stable isotopes to study Cu sources, retention processes in soils and transport in a vineyard catchment (Rouffach, Alsace, France). Our specific objectives at the catchment scales were i) to identify fungicide-derived Cu retention processes in the soils based on Cu isotope measurements, ii) to evaluate Cu export by rainfall-runoff events with the help of the δ^{65}Cu in the different particle-size soil fractions and in runoff-associated Cu (SPM, dissolved) collected at the outlet of the catchment, and iii) to evaluate the influence of Cu-fungicide applications and hydrological conditions on Cu mobilization by runoff.
- **Stormwater wetland:** Secondly, Cu stable isotopes were measured in different compartments of a stormwater wetland to investigate the behaviour of Cu. The stormwater

wetland collected Cu-contaminated runoff from the vineyard catchment (Rouffach, Alsace, France) during the period of Cu fungicide application (May-July). The specific objectives at the scale of the stormwater wetland were i) to elucidate fungicide-derived Cu retention processes based on Cu isotope fractionation and Cu mass balance (inlet-outlet), ii) to characterize Cu distribution among the major wetland compartments (i.e. the dissolved phase, the SPM, the bed sediments and the vegetation), and ii) to characterise sink and source functions of the wetland (for Cu) in the light of the changing biogeochemical and hydrological conditions.

- **Column experiment:** In the third part of the Ph.D. work, we set up Winogradsky columns to combine a fundamental experiment in the field of environmental microbiology (designed by the famous microbiologist Sergei Winogradsky at the end of the 19th century)[155] with Cu isotope analysis to study the (bio-)transformations of Cu in the wetland sediment. The sediments were sampled in the stormwater wetland collecting Cu-contaminated runoff from the vineyard catchment. The specific objective at the scale of the sediment column was to evaluate the behavior and the aging of Cu at biogeochemical gradients formed by microbially-mediated processes.

10. Thesis layout

This PhD includes 1 refereed publication (Chapter IV), 1 publication under revision (Chapter III) and 1 publication in preparation for which I am the primary author. One additional refereed publication for which I am a co-author is provided in the Annexes. The outline of the following chapters of the thesis is presented below and in the graphical outline of the PhD project.

Chapter II discusses the methodological development performed during the present thesis for precise measurement of Cu isotopes in diverse environmental samples from our study site (vineyard catchment, 42 ha, Rouffach, Alsace, France). The attempts for resolving difficulties of Cu extraction from particular samples are also presented in this chapter. The applied sequential extraction method for the assessment of the geochemical partitioning of Cu in sediments is also described.

Chapter III (under revision for Environmental Science and Technology) investigates the processes of the accumulation, the distribution and the mobilization of Cu fungicides in the vineyard soils (Rouffach, Alsace, France) based on geochemical, hydro-climatic and isotopic data. The catchment scale permitted notably to assess the impact of both the anthropogenic and hydro-climatic impact on the Cu transport by runoff. Combining sample pre-treatment, notably particle-size separation in Cu-enriched vineyard top-soils with Cu isotope analysis allowed for evaluating and setting up likely scenarios of anthropogenic Cu retention in soils. The identification of Cu sub-pools within the bulk soils also permitted to trace the sources of runoff-transported Cu.

In Chapter IV (published in Environmental Science and Technology, in 2014), we addressed the reactive transport of Cu in a stormwater wetland collecting the Cu-contaminated runoff from the above mentioned vineyard catchment (Rouffach). Combining hydrochemical analyses, Cu isotope analysis and mass balance approaches we described a possible scenario of dissolved Cu retention in the wetland sediments and *in situ* transformation. The performance of the wetland with regards to Cu entrapment was evaluated during variable hydrological and biogeochemical conditions

In Chapter V, the Cu transformation in the wetland sediments coming from the above mentioned stromwater wetland (Rouffach) was evaluated by setting up Winogradsky columns. The sediments evolved during a total of 520 days to allow for an establishment of biogeochemical gradients that are naturally present in lake and wetland sediments depleted in oxygen. Porewater extraction and the analysis of various redox-indicative species permitted to evidence the biogeochemical gradients with depth. Geochemical analysis of the sediments in the depth profile allowed for highlighting significant changes in the composition of major and trace elements, in particular Cu in the aged sediments. Sequential chemical extraction of Cu was applied to evaluate the changing geochemical partitioning of the sediment-bound Cu with temporal evolution of the sediments.

Chapter VI provides a synthetic summary and a discussion of the main findings of the different Chapters of this PhD work. Future experiments and research trends are suggested for the further evaluation of the isotopic tool in assessing the environmental fate of anthropogenic Cu. In particular we suggest to expand the current knowledge about i) Cu aging processes and isotope fractionation in soils upon aging, ii) Cu isotope fractionation of processes essential in a soil-plant-water system, iii) combining Cu isotope analysis with source-tracer isotope systems, and iv) combining Cu isotope analysis with more traditional isotope systems (C, H) for linking Cu fungicides and synthetic pesticides in agro-systems.

11. Graphical outline

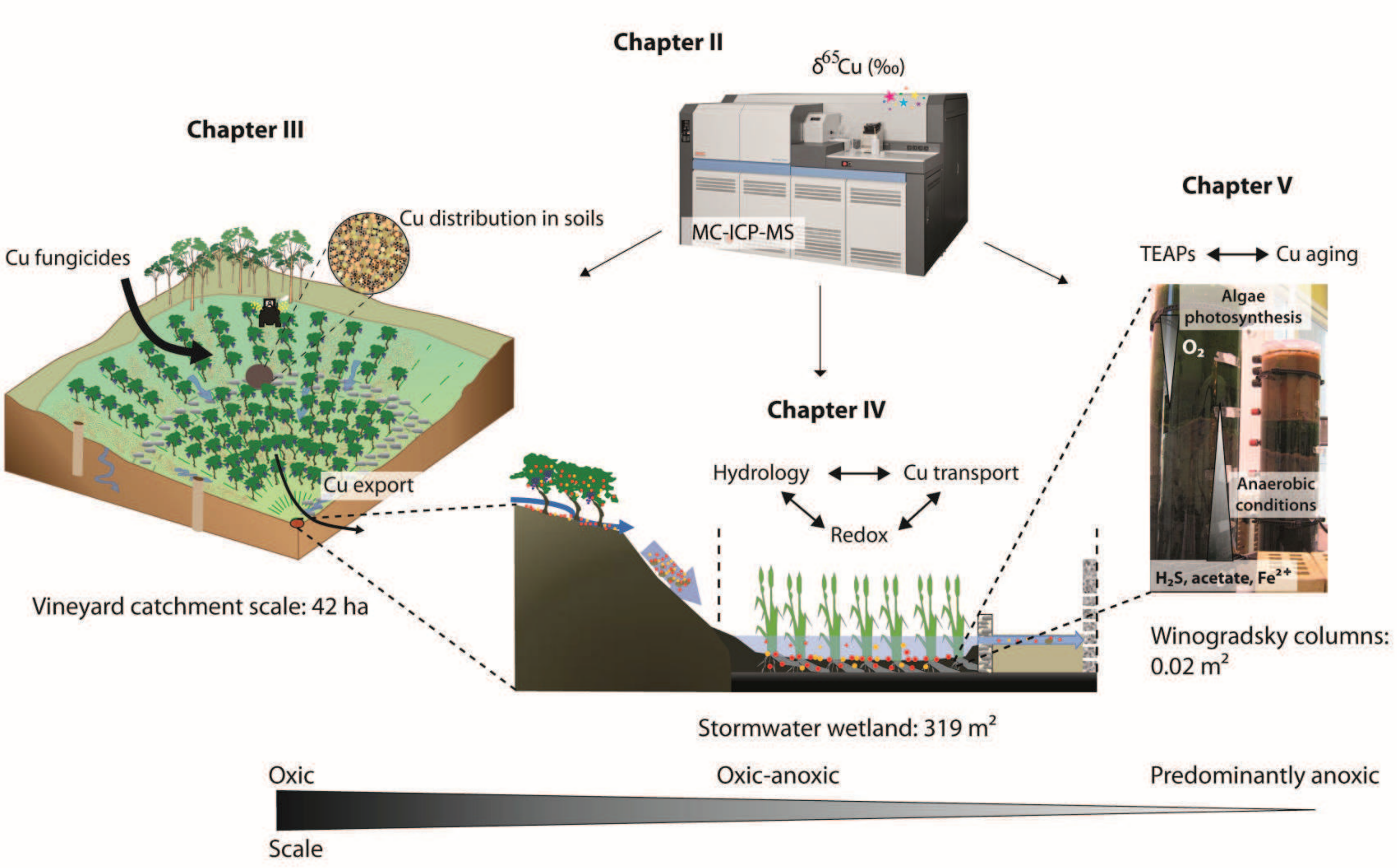


Figure I-9: *Graphical outline of the thesis.*

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II. Chapter: Methodology

This chapter presents in details the methods developed for Cu purification and isotope measurement. In particular, the adaptation of existing methods to treat different matrices (i.e., soils, sediments, water, plants) is described.

On the one hand, Cu isotopes as a tool to assess the transport and the transformation processes of Cu in environmental and geological studies require precise measurement of Cu isotope ratios in various types of matrices. A precision of ≤0.1‰ for the measurement of δ^{65}Cu (‰) is a prerequisite to evaluate Cu isotope fractionation upon natural processes. Such precision can be achieved today by the advent of a multicollector-ICP-MS technology. On the other hand, Maréchal et al.[1] developed a purification method to extract Cu from environmental samples at the late 1990's. Powerful purification is another prerequisite to reach precise measurement of Cu isotope ratios. From then, several authors have modified the original method of Cu extraction with higher efficiency (~100%) and purity. High efficiency and quality is fundamental to preclude any Cu isotope fractionation during the purification step while avoiding interferences with other elements during MC-ICP-MS analysis. Therefore, the optimization of the original protocol of Maréchal et al. was performed for the diverse environmental samples to achieve a good efficiency of the Cu purification that is explained here in details.

Overall, the Cu isotope analysis method was successfully adapted for most of our samples (soils/sediments, plants, water). However, some water samples (named below "critical samples") that had poor amounts of Cu compared to high concentrations of matrix elements (such as Ca) could not be satisfactorily purified with our method. An alternative Cu purification protocol and a Cu pre-concentration method in case of the "critical" samples, were applied but did not enable efficient Cu extraction.

Finally, the applied sequential chemical extraction method to quantify the geochemical partitioning of Cu in soil and sediment samples is described.

1. Sample treatment and Cu purification from environmental samples

The advent of the multicollector ICP-MS allowed the analysis of isotope ratios of heavier elements (>40 atomic mass unit) with a precision that is high enough to discern the natural variations in their stable isotope compositions.[2] However, for the precise analysis of the isotope ratios of Cu using MC-ICP-MS, Cu first needs to be entirely extracted from the matrix. The presence of other matrix elements (e.g.: Na, Ti) may bias the measured Cu isotope ratios by i) interfering with the measured Cu masses (spectral interferences), ii) decreasing the ionization efficiency of Cu in the plasma, and iii) inducing non-spectral interferences (changes in the instrumental mass bias).[3] The main criteria for determining the efficiency of the purification procedure are i) the recovery of Cu (must be ~100%) to preclude any Cu isotope fractionation during purification,[4] and ii) the high purity (i.e., devoid of matrix elements) of the extracted Cu to avoid matrix-induced interferences during Cu isotope analysis by MC-ICP-MS (Figure II-1).[5,6]

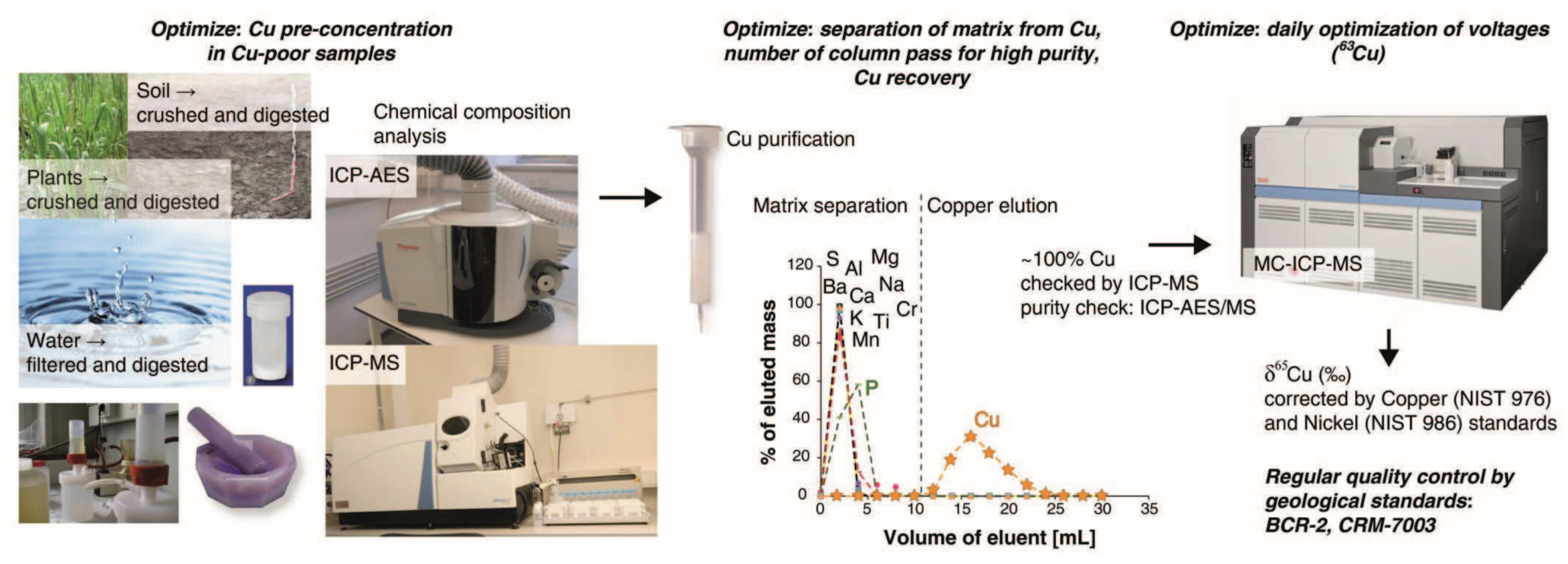

Figure II-1: *Schematic of the different steps of the methodology applied and adapted in this thesis to measure Cu isotope ratios in soil, sediment, vegetal, and water samples.*

The original Cu purification protocol was elaborated by Maréchal and co-workers.[1] This protocol is the most widely used in the literature; however, multiple adaptations were made by several authors in order to meet the mentioned requirements (i.e., ~100 Cu recovery and high purity) for precise isotope analysis of Cu in natural samples.[3,7-9] The purification method of Cu is based on ion-exchange chromatography exploiting the variable affinity of chemical elements to the exchange sites of a resin in a given media. The macroporous resin AGMP-1 (100–200 mesh, chloride form, Bio-Rad) is the most often applied for the purification of Cu as suggested in the original protocol of Maréchal et al. that allows the sequential separation of Cu, Fe and Zn.[1] The method consists of using this strongly basic anion-exchange resin to separate Cu from matrix elements in hydrochloric acid media (7 N HCl). This method has already been adapted to measure the Cu isotope ratios of environmental samples such as sediments[10-12], rocks[12,13], soils[14-16], stream waters[17,18] and also biological samples[19-22].

However, the separation of Cu from natural or contaminated water and soil samples is challenging, because numerous matrix elements are present in variable amounts that may impair the efficiency of Cu isolation by the original protocol.[8] Indeed, the elution of Cu may overlap with the other elements of matrix depending on the sample size and type. Such overlaps result in the incomplete separation of Cu from the sample matrix.[3] Another problem is the presence of co-eluted matrix elements following a single pass on the columns that necessitated modifications to the elution procedure for diverse sample matrices and, very often a second column pass.[3,15] Therefore, several authors have modified the purification method, notably by changing the acid normality of the hydrochloric solution (i.e. eluent),[8,9] by modifying the resin volume and the column geometry[7] or by adopting a sample pre-treatment for a preliminary elimination of much of the sample matrix.[23] Larner et al. have even developed a completely different method for the purification of Cu from biological samples that was based on the higher affinity of the Cu(I) to the anion exchange resin compared to Cu(II).[24] They efficiently eliminated the high Na amounts that typically characterize biological samples following a single column pass.

Therefore, the chemical purification procedure needs to be adapted for the different samples having contrasting matrices by optimizing i) the volumes of eluent for matrix elimination and Cu elution from the resin, ii) the resin volume to reduce blank contributions without exceeding the resin's ion-exchange capacity.[3,7,8] Finally, the adapted purification and Cu isotope analysis procedure needs to be validated by reference materials to control the accuracy and reproducibility of the method (Figure II-1).[9]

1.1. Purification method and optimization

The purification protocol of Maréchal et al.[1] was adapted by the stepwise change of the acid volumes (7 N HCl) for eluting the sample matrix and for total Cu elution (~100%) using different volumes of the anion-exchange resin bed depending on the sample type. The elution protocol was validated when it systematically allowed to recover ~100% of the Cu from the sample and when elements that may cause measurement interferences were significantly removed from the eluted Cu. The Cu purification protocol consists of the following steps: resin cleaning (I.), resin conditioning step (II.), sample loading (III.), matrix elution (IV.) and finally Cu elution (V.).

Sample chemical treatments and isotope analysis were carried out in a clean room with filtered air (class ISO 6), and chemical treatments were done under laminar flow hoods (class ISO 5) to minimize atmospheric contamination of the samples (i.e., high blank contribution). Suprapur reagents and acids double sub-boiled were used to further limit blank contributions. Labware was restricted to Savillex® PFA vials cleaned according to an in-house procedure: 24h bath in aqua regia, 24h bath in diluted HCl (v:v 1:5), 2 times 24h bath in MilliQ water (18.2 MΩ, Millipore), all steps were done on a hotplate at 120°C. Pipette tips, Bio-Rad chromatography columns, storage bottles, sample tubes and other labware were cleaned according to a "cold washing procedure" by soaking material overnight in diluted HCl (v:v 1:5) and two times in MilliQ water (24h).

Before the first use, the resin was processed to eliminate the finest resin particles by settling 7 to 10 times in water and throwing away the supernatant.[1] The resin is then packed into Poly-Prep chromatography columns (0.8 × 4 cm conical for resin bed) (Bio-Rad) for the column chemistry. Sample loading and the whole elution process was done in 7 N HCl + 0.001% H_2O_2. The hydrogen peroxide added in the hydrochloric acid served to keep the Cu in the +II oxidation state to avoid peak tailing throughout the ion-exchange chromatography process.[1] In our method, as in the method of Archer and Vance[7], a higher concentration of hydrogen peroxide (0.01%, instead of 0.001% in the original protocol) was added to the eluent to ensure Cu elution in a single peak, without any bubble formation in the resin bed (that might appear at higher H_2O_2 concentrations).

1.1.1. Cu purification from water samples

Four major steps are necessary to proceed the water samples collected from the field studies for Cu isotope analysis: i) separation of the dissolved phase (<0.45 µm or <0.22 µm) from the suspended particulate matter (SPM), ii) Cu pre-concentration in the sample (i.e., enrich Cu in the samples), iii) Cu extraction by a purification process, and iv) Cu isotope analysis (Figure II-1).[17,18,25]

Before routinely measuring Cu isotope ratios in water samples from the experimental site Rouffach (vineyard catchment, 42 ha, France), some tests were necessary to test the impact of: i) the filtration on Cu isotope analysis, ii) the pre-concentration of Cu, and iii) the procedure on the measurement of Cu isotope ratios.

1.1.1.1. Impact of filtration

Cu loss from the water sample may occur by sorption of Cu on the filter membranes during filtration,[26,27] likely leading to Cu isotope fractionation, and possible misinterpretation of the Cu isotope results.

Therefore, filtration tests were carried out to assess the impact of Cu sorption during water sample filtration (0.45 µm cellulose acetate membranes, Millipore) on the Cu isotope ratios. The effect of the Cu concentration was also tested with regards to the potential Cu losses on the membranes, as well as the effect of the variable Cu to matrix ratios in the environmental samples from Rouffach.

Filtration of the following solutions were investigated for:

i) synthetic Cu solutions (spiked with 50, 100 and 500 µg L^{-1}) prepared from a stock solution (1 g L^{-1}), and 18.2 MΩ water;
ii) pre-filtered water samples (<0.45 µm) from Rouffach in which Cu was spiked to obtain 70, 110 and 500 µg L^{-1} Cu;

The pH of all prepared solutions was adjusted to the average pH of water samples from Rouffach (pH ≈ 7.9), as pH is a key parameter in influencing sorption processes.[28]

An in-house filtration protocol was tested that had been previously established for the filtration of environmental water samples for isotope analyses of various elements (e.g. Ca, Sr, U). The protocol consists of i) cleaning the filter membrane by filtering ~150 mL of MilliQ water, ii) conditioning the membrane and the filtration assembly (PFA, Savillax) by filtering ~150 mL of sample, and iii) collecting the filtrate in pre-cleaned bottles. The filtrate that served for the conditioning of the membrane was systematically discarded. The Cu concentrations of all unfiltered and filtered solutions were determined by ICP-MS (Thermo Finnigan X-Series II, Thermo Scientific, Waltham, MA USA). The measurement uncertainty was 5% and an internal standard of rhodium (Rh) or indium (In) was applied during ICP-MS measurement to correct for matrix effects (i.e., the lowering of the ionization efficiency in the plasma in the presence of high matrix). Reference materials (SLRS-4, TM-24.3) were used for quality control of the Cu concentration measurements in each analytical session by comparing the measured Cu concentration to the certified value (± 5%). The Cu isotope analysis was done directly in the unfiltered and filtered Cu solutions, whereas the Cu isotope analysis was preceded by a Cu purification step in case of the natural samples from Rouffach to eliminate matrix elements.

The results showed a Cu recovery > 95% following the filtration steps; the yield was nearly 100% within the analytical error (± 5%) indicating that non-significant Cu loss occurred during the sample filtration (Table II-1).

	Cu recovery after filtration [%]	
Cu concentration [$\mu g\ L^{-1}$]	**Cu solutions**	**Samples from Rouffach spiked with Cu**
50	96.2	
100	97.5	
500	99.6	
70		96.4
110		99.1
500		103.0

Table II-1 : *Cu recovery following the filtration ($Cu_{filtered}/Cu_{unfiltered}*100$) of artificial Cu solutions and environmental water samples spiked with Cu at different Cu concentrations (50, 100, 500 $\mu g\ L^{-1}$ in Cu solutions and 70, 110, 500 $\mu g\ L^{-1}$ in spiked samples from Rouffach).*

The Cu isotope ratios expressed as $\delta^{65}Cu$ ($^{65}Cu/^{63}Cu$) (defined by eq. 3, see section 2.1) did not significantly differ between the unfiltered and the filtered samples (Figure II-2). The differences between the measured Cu isotope ratios of the corresponding unfiltered and filtered samples ranged from -0.08 to 0.02‰, and were within the analytical uncertainties of the Cu isotope measurements in water samples (± 0.1‰) (Figure II-2).

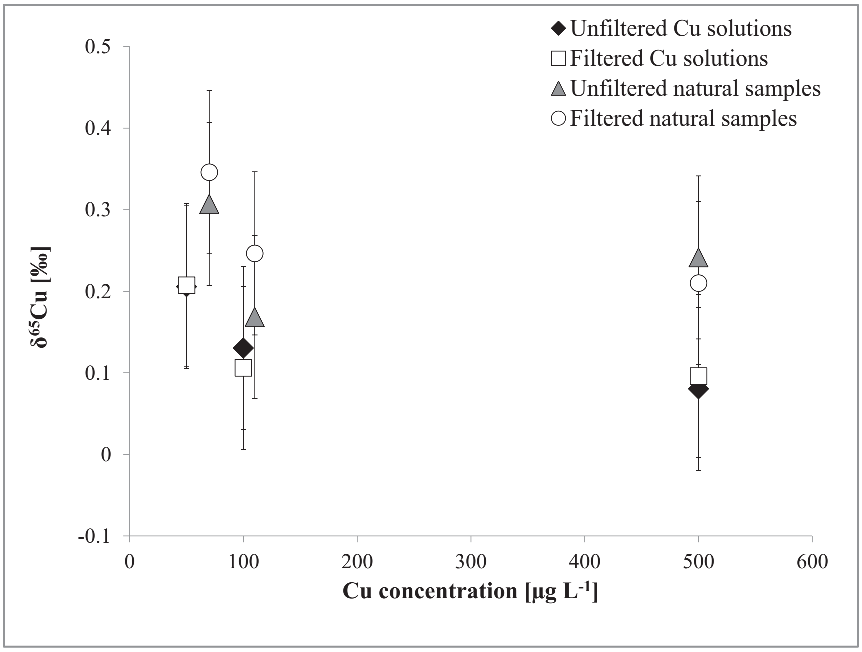

Figure II-2: *Cu isotope ratios of unfiltered and filtered Cu solutions and natural samples added with Cu at different concentrations.*

Consequently, the applied cellulose acetate Millipore membranes (0.45 µm) for the filtration of the water samples (at the investigated pH) did not significantly change the measured Cu isotope ratios. The filtration protocol was therefore adopted for all water samples (runoff, wetland water) collected during the field study carried out in Rouffach in 2011 (see Chapters III and IV).

1.1.1.2. Cu pre-concentration in water samples

Cu concentration in the natural samples from Rouffach ranged from 0.9 to 32.0 µg L^{-1}. Cu pre-concentration (i.e., enrichment of the Cu load in a given sample volume) was necessary for Cu isotope analysis to obtain ≥200 ng Cu. The amount of ≥200 ng Cu was necessary for precise Cu isotope analysis on the MC-ICP-MS and recovery check by ICP-MS following Cu purification. The most straightforward way to concentrate Cu from a water sample is to evaporate the required volume of water sample calculated based upon the initial total concentration of Cu as analyzed by standard ICP-MS.[8,25,29] However, the evaporation residue could not be directly dissolved in 1 mL HCl (7 N) for introduction into the chromatography column. An acid digestion was therefore performed according to an in-house digestion protocol adapted for water samples. Digestion following sample evaporation has been already applied in the case of the organic-rich river and lake waters by Ilina et al.[29] Acid digestion consisted of a successive treatment with i) 1 mL HNO_3 (~15 N) and 1mL HF (~22 N) at 70°C overnight, ii) 1 mL $HClO_4$ (~12 N) at 150°C during 24h, iii) 200 µL H_3BO_3 (saturated in H_2O), 2 mL 6 N HCl and 3 mL H_2O at 100°C for 3h and finally evaporation of all remaining acids at 150°C overnight. Digested samples were re-dissolved in 1 mL 7 N HCl+0.01 % H_2O_2 at least 2 times for the Cu purification step. In the case of some "critical" samples, i.e., the water samples characterized by a high matrix to Cu ratio (Ca/Cu > 10 000), this pre-concentration did not allow the good separation of Cu from the sample matrix by the Cu purification method applied for water samples (see section 1.2).

1.1.1.3. Impact of the single and the double purification

The detailed Cu purification protocol of water samples is summarized in Table II-2. The exact volumes of the eluent (7 N HCl + 0.01% H_2O_2) used throughout the ion-exchange chromatography for the matrix elution and Cu recovery were determined by an elution calibration. Cu recovery was systematically determined by analyzing an aliquot of the sample by ICP-MS before and after column chromatography.

Sample type	**Resin volume**	**Resin wash**	**Conditioning**	**Sample load**	**Matrix elution**	**Cu elution**
			7 N HCl + 0.01% H_2O_2			
Water	1.6 mL	3×7 mL 0.5 N HNO_3 alternating with 7 mL H_2O, 1×7 mL 7 N HCl, 1×7 mL H_2O	6 mL	1 mL	4×1 mL	26.5 mL

Table II-2 : *The Cu purification steps elaborated for water samples.*

The reproducibility of Cu isotope measurements following a single purification (samples passing once through the column chromatography) was evaluated by repeating the processing of a water sample from Rouffach. The 2 standard deviations of the δ^{65}Cu were 0.27‰ (n = 5), which is more than twice as higher as the values found in the literature (~0.1‰).[3,11,13,30] This higher uncertainty may be due to the presence of co-eluted matrix elements in the extracted Cu that create interferences during the measurement of the Cu isotope ratios (Table II-3). Matrix elements in the purified Cu were measured by ICP-AES (iCAP™ 7200, Thermo Scientific). In order to eliminate all matrix elements from the Cu cut, samples were re-purified once again using the same purification protocol (Table II-2). The reproducibility of Cu isotope measurements following the double purification of samples (repeating 2 times the Cu purification step) was evaluated by the repeated processing of 3 different water samples from Rouffach (having different matrix to Cu ratios). The 2 sigma value of the δ^{65}Cu was ≤0.06‰ (n = 3) and the listed matrix elements were all under the detection limits in the purified Cu. Based on this result, a double purification was adopted for all environmental samples from Rouffach.

Duplicate samples	Cu	Al	Ca	Co	Fe	Mg	Na	Si	Zn
	[μg L^{-1}]								
Inlet 22/06 (A) 1st purification	230	23	132	5	72	21	68	165	88
Inlet 22/06 (B) 1st purification	243	133	109	24	65	20	60	149	132

***Table II-3 :** Co-eluted matrix elements detected in the extracted Cu following a single purification step compared to ~230 μg L^{-1} Cu evaluated on duplicated water samples (Inlet 22/06 Rouffach), all these co-eluted matrix elements were below the detection limits following the 2nd purification of Cu*

Besides achieving good Cu recovery (~100%) and a high purity Cu, another quality aspect to reach for precise Cu isotope analysis is the blank contribution (of the digestion and the purification). The blank contribution accounts for all the Cu added to the samples during the samples treatment, i.e, by atmospheric contamination, by the addition of acids, by Cu desorption from the wall of the containers, etc. Although all the efforts made to reduce Cu addition to the samples (i.e., samples are processed in laboratory with filtered air, acids are distilled, labware is acid-cleaned) some Cu from external sources may still be loaded and processed.

Therefore the total Cu mass in the sample was compared to the total procedural blank (digestion, purification) to evaluate blank contribution. The blank contributions are monitored by adding to each sample series a blank sample that is processed in parallel to the environmental samples. The total procedural blank (including the acid digestion and the double purification) was 1.4 ± 1.2 ng Cu (n=3) (in which the purification step contributed to 0.8 ± 1.1 ng Cu, n=9). The bias caused by the blank contribution is estimated to ~0.06‰ (1.4 ng), considering the range of Cu isotopic variations observed in the nature (~-4.5 to ~+8‰)[31] and the typical δ^{65}Cu measured in the samples from Rouffach (~+1‰). This bias is smaller than the measurement uncertainty of Cu isotope analysis determined for the two geological reference materials (≤ 0.08‰) used in this study (see later).

1.1.2. Cu purification from solid samples (soil, sediment, SPM)

Solids in our study include soil, sediment and suspended particulate matter (SPM) samples. Cu elution during the purification step can vary depending on the sample matrix.[3] Therefore, the processing of solids by chemical purification on the resin AGMP1 was re-calibrated using higher resin volumes to increase the resin exchange capacity. The acid digestion protocol used for dissolving solid samples was also checked for Cu recovery on geological standards (BCR-2, USGS, CRM-7003 Analytika, Prague).

1.1.2.1. Digestion of solid samples

Sample purification is preceded by a sample preparation including drying (60°C, 48h), powdering and dissolution of the solid sample. The powdering of solid samples is done using an agate disk mill. The protocol consists of i) cleaning the agate material by powdering quartz in it and cleaning with detergent and tap water and rinsing with 18.2 MΩ water and ethanol (normapur, Merck) prior to drying, ii) conditioning the disk mill and the sieve by powdering and sieveing ~5 g sample (discarded) and iii) powdering the sample by ~5 g portions. The powdered samples are sieved at <100 µm.

Then, 50 mg powdered sample was weighed into Savillax vessels and digested by the successive addition of HF-HNO_3 acid (v:v 4:1) at 70°C, $HClO_4$ acid at 150°C and a mixture of HCl (3N) and H_3BO_3 acids at 110°C. At the end samples were evaporated to dryness at 150°C and re-dissolved twice in 7 N HCl for Cu concentration analysis by ICP-MS. The geological standards BCR-2 and CRM-7003 were used to quantify the Cu recovery of the acid digestion, which was ≥97%.

1.1.2.2. Cu purification calibration from solid samples

The column chromatography was recalibrated for the soil and sediment samples. The resin volume was first increased to 2 mL, instead of 1.6 mL for the water samples. A soil sample from Rouffach has been chosen for the calibration of the chemical purification (*n*=1). During the elution every 2 mL cut were collected and analyzed by ICP-MS for Cu and ICP-AES for matrix elements.

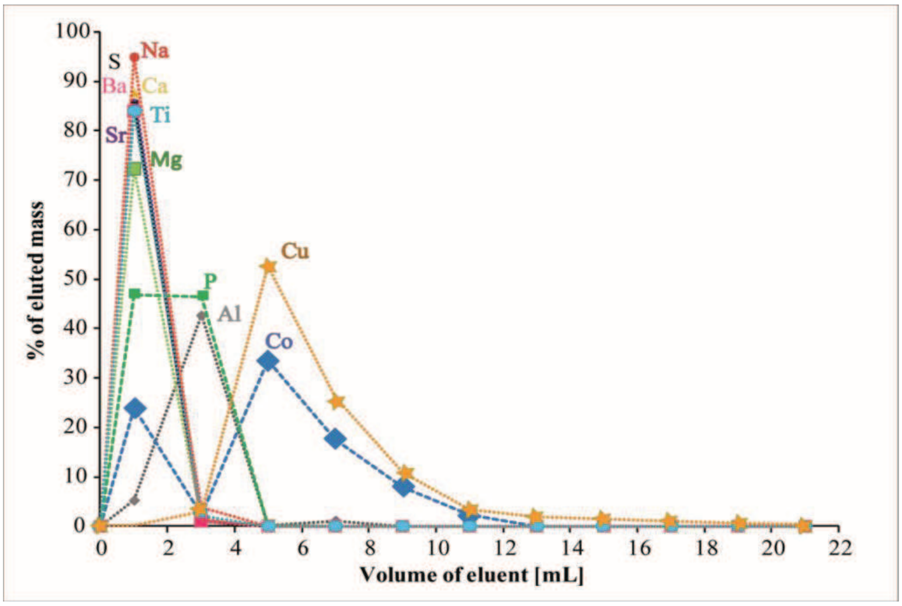

Figure II-3: *Elemental elution from a soil sample (50 mg) using 2 mL resin AGPM1.*

Results show that a 2 mL resin failed to separate Cu from the matrix elements due to a premature elution of Cu (5% broke through in the first 4 mL of the matrix removal) (Figure II-3). Chapman et al. found that the premature elution of Cu varied with matrix type and was most pronounced for samples with higher P and Fe concentrations due to the displacement of Cu from the exchange sites of the resin.[3] The results emphasize that a higher resin volume is needed to remove matrix components while retaining Cu for the subsequent elution step. Therefore a second tuning of the ion-chromatography has been carried out on 3 mL resin.

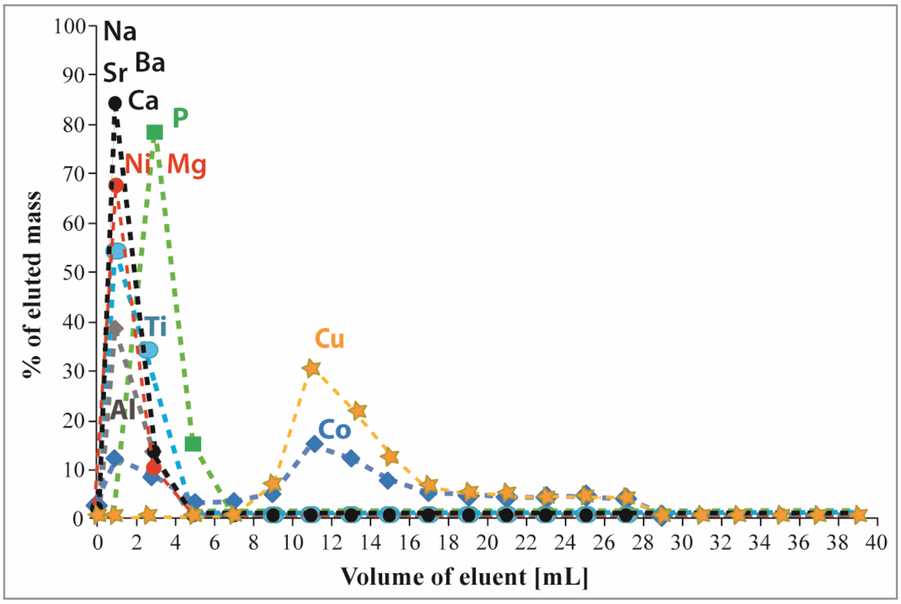

***Figure II-4:** Elution discretization of a soil sample (50 mg) using 3 mL resin AGPM1.*

Figure II-4 shows that 3 mL resin allowed to separate Cu from the matrix components with the exception of Co. Separation of Cu from Co remains challenging, as shown previously, because the close distribution coefficients for the two species between the resin and hydrochloric media (7 N).[11,13] However, Co is not expected to significantly interfere with the Cu isotope measurements.[11] In a recent study Co was efficiently eliminated from the Cu fraction with the method of Maréchal et al.[1] by increasing the acid molarity to 8.[9]

In order to ensure a high purity Cu, a second chemical purification step was performed using 2 mL resin. Reducing the matrix charge following the first purification step allowed to decrease the resin volume in the second purification step to spare time and decrease the acid volumes, and thus the blank contributions. The Table II-4 describes the two-step purification protocol for treating soil samples from the Rouffach site, and can probably be extended to other sites and solid samples.

Sample type	Resin volume	Resin wash	Conditioning	Sample load	Matrix elution	Cu elution
			7 N HCl + 0.01% H_2O_2			
Solids 1st purification	3 mL	3×10 mL 0.5 N HNO_3 alternating with 10 mL H_2O, 1×9 mL 7 N HCl, 1×9 mL H_2O	10 mL	1 mL	5/6×1 mL	45 mL
Solids 2nd purification	2 mL	3×7 mL 0.5 N HNO_3 alternating with 7 mL H_2O, 1×7 mL 7 N HCl, 1×7 mL H_2O	8 mL	1 mL	4×1 mL + 2×2 mL	26 mL

Table II-4 : *The Cu purification protocol elaborated for soil, sediment and SPM samples from Rouffach.*

Each purification step was followed by a fast digestion of samples in 1 mL HNO_3 to mineralize the organic matter leached from the AGMP1 resin. Purified samples were then dissolved in 0.5 N HNO3, from which an aliquot was taken for Cu recovery check by ICP-MS. Purity control of the Cu extracts was done by ICP-AES analysis or by performing a broad mass scan by ICP-MS (from ^{7}Li to ^{238}U) during Cu analysis.

1.1.3. Cu purification from vegetal samples

The protocol of Maréchal et al. has also been used to treat plant samples.[19,20,22] Plants need first to be washed, dried and powdered prior to digestion and Cu purification. Here the wetland plants (*Phragmites australis,* Cav.) were separated into below-ground (rhizome and roots) and above-ground (stem and leaves) sub-samples and were washed for 5 minutes in 18.2 MΩ water (Millipore), dried at 60°C and powdered with an agate disk mill (see in the section 1.1.2.1.) prior to acid digestion. Again, the acid digestion protocol of the plant samples was checked for Cu recovery by a standard vegetal material (NIST SRM 1570a). Then, the Cu purification protocol also required slight modifications compared to that established for water and soil samples due to the different matrices typically found in vegetal samples (i.e., high Na concentrations).[24]

1.1.3.1. Digestion and recovery check

Acid digestion of the plants was done on 200 mg powdered sample. A cold digestion (at ~22°C) was first applied by adding 4 mL HNO_3 (~15 N) and 5 mL H_2O_2 (30%) overnight to avoid sample loss by intense CO_2 formation. 2 extra mL of H_2O_2 were then added and left react 48h for the degradation of the most reactive organic matter. After degasing, the acid mixture was heated on a hotplate at 70°C for 2 more days. By further addition of 0.5 mL H_2O_2 no more visible reaction could be observed, therefore acids were evaporated at 70°C. Then a mixture of HCl and HNO_3 (v:v 4:3) was added to the Savillax and heated at temperatures gradually increasing from 100°C to 150°C during 2 weeks. Finally a treatment with HF and HNO_3 was done at 70°C to destroy the siliceous parts of the plants. At the end 0.5 mL H_3BO_3 was added to dissolve the fluorides. The Cu recovery following the plant digestion was quantified using the spinach leaves reference material (NIST SRM 1570a) by ICP-MS and was 99.4%.

1.1.3.2. Purification calibration

Cu purification was tuned for the plant samples by using 2 mL resin. Plant samples were re-dissolved in 7 N HCl + 0.01% H_2O_2 to yield ~200 to 500 ppb Cu concentrations. Two samples were used for the Cu elution discretization (1 above-ground and 1 below-ground sample).

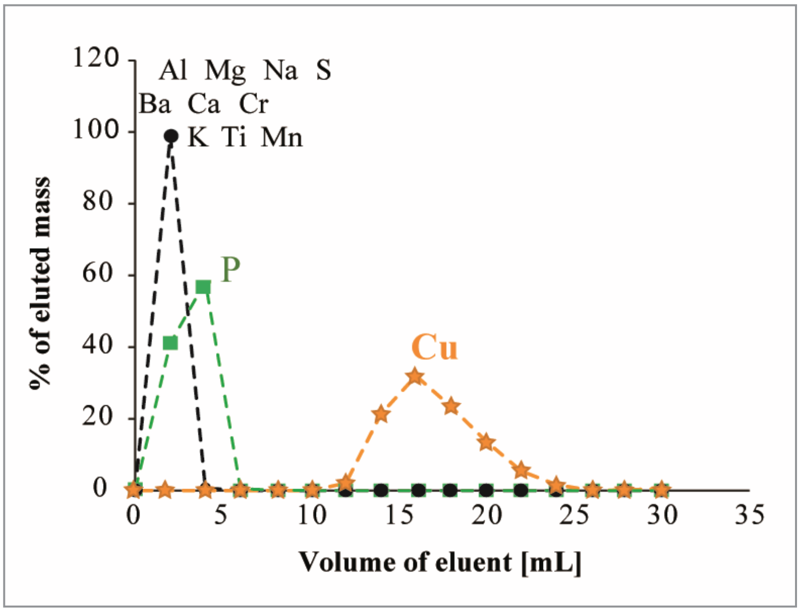

Figure II-5: *Cu elution curve of the plant samples using 2 mL AGMP1.*

Results of the matrix component and Cu analysis show a good separation of Cu from the matrix elements using a 2 mL resin volume (Figure II-5). The Cu elution curves were identical in the 2 samples. For a higher purity of Cu a double purification protocol was adopted for the plant samples as well by performing twice the same purification protocol. The final purification protocol is summarized in Table II-5. Blank contribution of the acid digestion and double purification amount to 5.4 ng Cu (*n* = 1) which is ≤0.7% of the total Cu contained in the digested plant samples.

Sample type	**Resin volume**	**Resin wash**	**Conditioning**	**Sample load**	**Matrix elution**	**Cu elution**
			7 N HCl + 0.01% H_2O_2			
Plant samples	2 mL	3×7 mL 0.5 N HNO_3 alternating with 7 mL H_2O, 1×7 mL 7 N HCl, 1×7 mL H_2O	8 mL	1 mL	4×1 mL + 2×2 mL	26 mL

Table II-5 : *The Cu purification protocol elaborated for plant samples from Rouffach.*

1.1.4. Method validation with geological standards

Two geological reference materials (BCR-2 and CRM-7003) were used to validate the purification and isotope measurement protocol developed in this thesis. Cu isotope analysis of geological standard CRM-7003 yielded 0.18 ± 0.07‰ (*n*=23, 2SD), including 3 separately digested samples that were separately purified 9 times and successfully measured 23 times by MC-ICP-MS. Bigalke et al. obtained for the same geological standard 0.22 ± 0.05‰ *(n*=5, 2SD), which corresponds to our measured value within uncertainties.[15] The other geological standard BCR-2 was digested 2 times, undergone by chemical purification in 3 different series and was measured by MC-ICP-MS 6 times. The obtained δ^{65}Cu was 0.22 ± 0.08‰ (*n*=6, 2SD). Two other studies have published Cu isotopic values for the BCR-2 standard that are respectively 0.21 ± 0.11‰ (*n*=5, 2SD)[15] and 0.22 ± 0.04‰ (*n*=8, 2SD)[9].

Hence, the δ^{65}Cu value obtained following our protocol is in agreement with the values published elsewhere, thus validating our Cu isotope analysis method. The measurement uncertainty given for our isotopic data for the entire procedure (digestion, purification and isotope analysis) is ±0.08‰ for the isotopic data included in the 2nd and 3rd chapters of the thesis and ±0.1‰ for those in the 1st chapter. A higher measurement uncertainty for the isotopic data presented in the 1st part of the PhD is explained by less replicates of the geological standards (*n*=3) for evaluating the external reproducibility when the corresponding study was published.

1.2. "Critical" samples

In order to have enough quantity of Cu (around 200 ng) for Cu isotope analysis, water samples were concentrated by evaporating the required volume of the sample at 100 °C as mentioned before. The residue left after evaporation contained organic and inorganic substances that could not be directly dissolved in 1 ml of 7 N HCl + 0.001 % H_2O_2. In consequence, samples could not be directly introduced on the resin for further chemical purification of Cu. Therefore, a preliminary digestion of the residues was necessary. In some cases however, when Cu was present in small concentration (1-2 µg L^{-1}) in a Ca-, Mg- charged matrix (Ca/Cu > 10 000), some white precipitates were formed in the solution, which could not be dissolved in 1 ml acid. In this case, precipitates were centrifuged out (Juan B4 centrifuge, Thermo Electron), but a bad recovery of Cu (<90 %) after the anion-exchange chromatography was systematically obtained. The poor Cu recovery following Cu purification hindered the Cu isotope analysis of the water samples with large matrix elements to Cu ratio (typically those sampled at the outlet of the stormwater wetland of Rouffach). A series of tests was therefore carried out to resolve the problem of Cu recovery of these samples.

1.2.1. Early Cu elution from "critical" samples

In order to preclude an eventual shift in the Cu elution curve due to the higher matrix to Cu ratios in these "critical" water samples (i.e., Ca/Cu concentration ratios higher than 10 000), the Cu elution was discretized on 1.6 mL resin as matrix-related effects on the yield of the Cu purification have already been observed.[3] The impact of the sample pre-concentration rate on the Cu recovery was tested as well as the Cu recovery following acid digestion of the evaporated samples.

A Cu purification according to the protocol modified from Maréchal et al. for water samples was performed on the same wetland water sample concentrated x100 and x130. Each 2 mL cuts were collected and analyzed by ICP-MS for Cu to evaluate possible shift of the Cu elution curve and by ICP-AES for major matrix components.

Sample	Concentration factor	Mass of concentrated Cu [ng]	Cu recovery after digestion	Cu recovery after purification (all cuts included)
Wetland water 11/05/2011	×100	128	103%	105%
	×130	166	105%	98%

Table II-6 : *Cu recoveries after sample acid digestion and column chemistry (in all cuts collected) as impacted by the pre-concentration of the water sample.*

The good Cu recoveries after sample digestion show that despite the formation of white precipitates Cu remains in solution and could be centrifuged out of the precipitates (as suggested by other authors as well) (Table II-6).[1] The eluted Cu recovery was around 100% (all collected cuts summed up). However, the discretized elution curve shows that early elution of Cu prevented Cu separation from the matrix components.

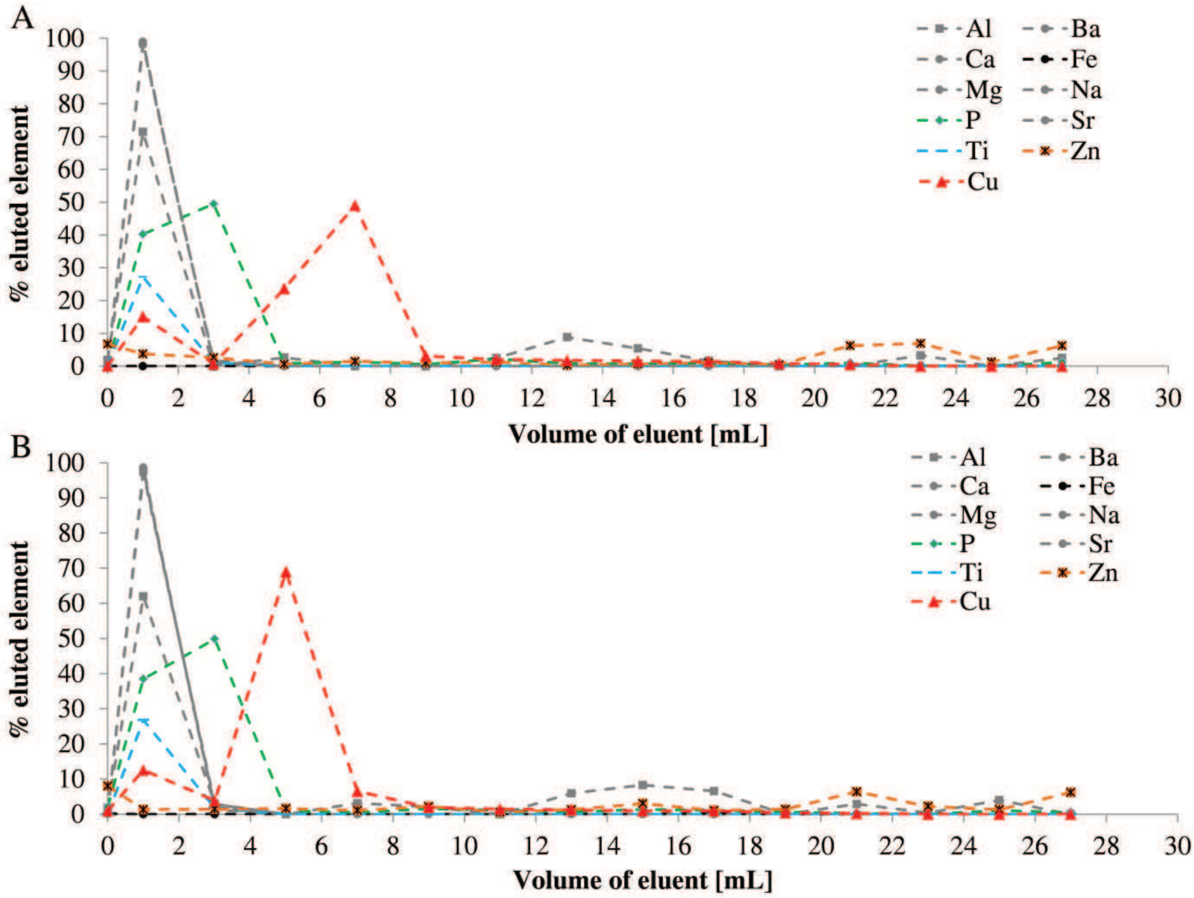

Figure II-6: *Elution of Cu and matrix components of the selected "critical" wetland water sample concentrated 100 times (**A**) and 130 times (**B**).*

Around 15% of Cu was not retained by the resin (in the first 2 mL fraction), suggesting that Cu could not efficiently access to the exchange sites. This might be due to the preferential access of Ca, Mg in the exchange sites of the matrix, since Ca and Mg were present in high concentrations in the samples (i.e., Ca/Cu > 10 000) (Figure II-6). Most of the matrix elements were eliminated in the first 4 mL of 7 N HCl.

These results show that in water samples with high matrix (especially Ca) to Cu ratios (i.e., Ca/Cu>10 000), Cu could not be efficiently separated from matrix elements with this method. According to our observations we could determine a Ca (most important matrix component in the water samples from Rouffach) to Cu ratio threshold above which the Cu purification could not be achieved with the method described. This threshold for the Ca/Cu ratio is around 10 000 – 15 000 (Figure II-7), thereby compromising the treatment of 12 out of a total of 32 water samples from Rouffach. Therefore, further attempts were made to improve the Cu purification protocol for the "critical" samples that consisted of modifying the column chromatography and the pre-concentration step.

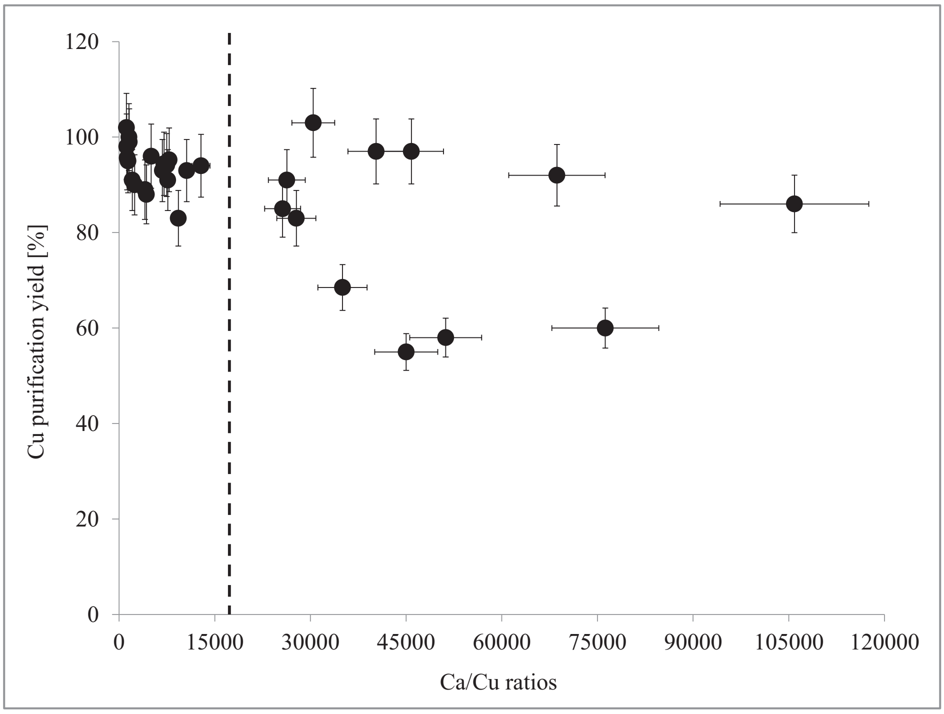

Figure II-7: *The Cu purification yield performed on the water samples from Rouffach as a function of the Ca to Cu concentration ratios.*

1.2.2. Other purification tests

A more recent separation procedure was also tested for treating the “critical” samples. The protocol of Larner et al.[24] aimed at resolving the difficulties of the protocol of Maréchal et al.[1], such as the overlap between the elution curves of Cu and the matrix components resulting in the poor purity of Cu following a single column chromatography and Cu loss during the purification. The major differences of the Larner’s protocol consist in utilizing the higher affinity of the reduced Cu (1+) to the anion-exchange resins that should enable a more efficient Cu purification by column chromatography. The reduction of Cu is done by the addition of ascorbic acid (L-AA). The detailed purification protocol is described in Table II-7.

Process	Medium	Volume [mL]	Cu valency	Harvested fractions for analysis
Resin loading	AG1-X8, 200-400 mesh	2		
Cleaning	0.1 N HNO_3, H_2O	10, 2		
Conditioning	2 N HCl	10		
Equilibration	0.01 N HCl-0.02 N L-AA	4*2		
Sample loading	0.01 N HCl-0.02 N L-AA	1	+I	Collected
Matrix elution	0.01 N HCl-0.02 N L-AA	30	+I	3*10 mL
	6 N HCl	5	+I, +II	2*2 mL, 1 mL
Cu oxidation	6 N HCl-0.01% H_2O_2	2	+II	Collected
Cu elution	0.1 N HNO_3-0.01% H_2O_2	15 (+40)	+II	3*5 mL, +6*5 mL

Table II-7 : *The detailed purification protocol of Larner et al. using 2 mL AG1-X8 resin.*

A “critical” water sample (Wetland water 11/05*100 (128 ng Cu)) was tested. Sample load was collected separately. The matrix elution was collected in 3 fractions of 10 mL of eluent, 2 fractions of 2 mL and a final 1 mL. Cu oxidation step was gathered separately and finally the elution of Cu was discretized into 3 fractions of 5 mL, in addition 6 fractions of 5 mL acid eluent were also collected to ensure the complete recovery of Cu.

Cu separates were evaporated to dryness, re-dissolved in 1 mL HNO_3 (~15 N) and refluxed at 160 °C for 3h to destroy remaining ascorbic acid. Then elution cuts were re-dissolved in 0.5 N HNO_3 for analysis by ICP-MS.

Results of the novel purification procedure revealed an early breakthrough of Cu (5%) in the sample load and the matrix elution steps, therefore the resin volume was augmented to 3 mL Tests were also carried out with a “critical” water (Wetland water 11/05*130 (166.4 ng Cu)) using both AGMP1 and AG1-X8 resins.

	AGMP1 resin		AG1-X8 resin	
	Cu mass [ng]	% of total Cu	Cu mass [ng]	% of total Cu
Sample load	0.0		0.8	9%
Matrix elution	14.8	9%	13.3	
	0.0		0.5	
	0.0		0.0	
	0.0		0.0	
	0.0		0.0	
	0.0		0.0	
Cu oxidation: 6 N HCl	0.0		0.2	74%
	1.2	88%	0.9	
	0.2		0.1	
Cu oxidation: 6 N HCl+H_2O_2	71.8		0.0	
Cu elution	72.2		121.4	
	0.3		0.0	
	0.1		0.0	
	0.1		0.0	
	0.1		0.0	
	0.1		0.0	
	0.2		0.0	
	0.2		0.5	

Table II-8 : *The results of the discretization of the purification protocol of Larner et al. using AGMP1 and AG1-X8 resins (3 mL) showing the early breakthrough of Cu during matrix elution and the poor Cu recovery.*

An early elution of Cu still prevailed on the 3 mL resin with a higher rate (9%) due to the higher pre-concentration rate of Cu (x130, instead of x100 during the preliminary tests). Further tests of the new purification protocol using 3 mL AGPM1 resin were performed on the geological standards (BCR-2 and CRM-7003).

Results revealed poor Cu recovery for the BCR-2 standard (84%), but good recovery was observed for CRM-7003 (106%). However, the ICP-AES analysis of the purified Cu revealed the presence of significant quantities of co-eluted elements (Ti, S, Fe, P, Al, see Figure II-9) that underscores the insufficient purification of Cu from the sample matrix.

	Al [ng]	Co [ng]	Cu [ng]	Fe [ng]	P [ng]	S [mg]	Ti [mg]
CRM-7003	-	-	330.8	3169	5008	8	44
BCR-2	5062	801	307.7	16316	6253	8	94

Table II-9 : *The co-eluted matrix elements found in the purified Cu from the geological standards (BCR-2, CRM-7003).*

Overall, the insufficient Cu recovery yields found when purifying “critical” samples and the presence of co-eluted matrix elements in the purified Cu prevented from adopting this purification method for treating and measuring “critical” samples from Rouffach.

Another strategy for the treatment of the “critical” samples was to pre-concentrate Cu while eliminating interfering matrix components. In fact, the major drawback of Cu pre-concentration by evaporation is the concomitant concentration of the matrix components in the samples that is supposed to cause the poor Cu yields of the purification step.

1.2.3. Co-precipitation tests (with $KMnO_4$ and $Mg(OH)_2$)

Two co-precipitation methods were tested to pre-concentrate Cu in the “critical” samples. The first method has been previously applied for the Cu pre-concentration in seawater samples for Cu isotope analysis.[23] It consists of the co-precipitation of Cu with another element (Mg) whose precipitation is dependent on the pH. The second method was developed for the pre-concentration by co-precipitation of radium (Ra) onto manganese dioxide.[32] This pre-concentration step permits an efficient separation of Ra from major elements including, Ca that also poses purification problems for Cu.

1.2.3.1. $Mg(OH)_2$ co-precipitation

The original co-precipitation protocol consisted of adding 15 mL 4.5 M NH_4OH for 1 L of seawater sample and leaving for 3 hours for the formation of precipitates. Then the supernatant was separated from the precipitate by centrifugation. The precipitate was washed twice with 18.2 MΩ milliQ water. The procedure was repeated twice with the collected supernatant by adding respectively 30 mL of 4.5 M NH_4OH for the first and 10 mL for the second step. The precipitates were combined and re-dissolved twice in 7 N HCl + 0.01% H_2O_2 for Cu purification. In seawater the Ca/Mg ratios are around 1/4, whereas in the “critical” samples from Rouffach it ranges from 3/1 to 8/1. Therefore a special care should be taken to assure Cu co-precipitation with $Mg(OH)_2$ without the precipitation of $Ca(OH)_2$. However, based on calculations it has been shown that the maximum pH that can be reached by the addition of 4.5 M NH_4OH is 11.95, which is less than 12.50 (the pH where the precipitation of $Ca(OH)_2$ is expected to take place, based on its solubility).

Our protocol for testing the above described method consisted of re-dissolving in 16 mL 0.05 N HNO_3 (pH=1.3) of the acid digested water sample (23/03/2011 wetland water from Rouffach) and completing to 50 mL with 18.2 MΩ milliQ water. In order to reach the OH^- concentration of $6*10^{-3}$ mol L^{-1} for the saturation of the solute on with regards to $Mg(OH)_2$, 70 µL of 4.5 M NH_4OH is added in the solution of 50 mL (which is ten times less than the volume used by Bermin et al. 15 mL for 1L of seawater).

During the tests first 70 µL 4.5 M NH_4OH was added to the samples by 10 µL increments and controlling the pH of the sample, until reaching the pH of 10.5 (as the sample was re-dissolved in an acid, some of the added OH- was expected to be consumed for neutralizing the acidity). Then, the solution was left for 3 hours to allow the formation of precipitates. However, no visible precipitate formation took place in the samples. Therefore we continued the addition of 10 µL increments of 4.5 M NH_4OH until reaching a total of 700 µL 4.5 M NH_4OH added. Altogether, some $Mg(OH)_2$ precipitates were formed in the samples (evidenced by visible observations) but the amount might not have been sufficient to scavenge Cu from the sample. Therefore, the testing was finished at this stage and another co-precipitation method was tested for the pre-concentration of Cu.

1.2.3.2. MnO_2 co-precipitation

Since the $Mg(OH)_2$ co-precipitation method failed to concentrate Cu in water samples from Rouffach, we tested a second method based on the formation of MnO_2 precipitates by the addition of both reactants necessary for the precipitate formation.[32] Moreover, Cu has great affinity to adsorb onto Mn-oxides.[28]

The original protocol consisted of adding 100 µL of 0.5 M KMnO4 to 0.4 to 1.8 g coral powder sample dissolved in 1 to 2 mL 6 N HCl and 200 mL H_2O following organic matter digestion. Then the pH is adjusted to 8-9 by the addition of 1 M NaOH and 200 µL of 0.5 M $MnCl.4H_2O$.[32] This causes MnO_2 to appear as a dark brown precipitate following the chemical reaction that is expected to scavenge the Cu from the solution by co-precipitation and sorption:

$$2MnO_4 + 3Mn^{2+} + 2H_2O \rightarrow 5MnO_2 + 4H^+$$

For the tests an acid digested water sample (23/03/2011 wetland water from Rouffach) was prepared as mentioned above. 100 µL 0.5 N $KMnO_4$, 200 µL 0.5 N $MnCl_2$ and 200 µL 25 % NH_4OH were subsequently added to the sample. Following the addition of the ammonium solution the pH was ~7. The precipitates were let form by shaking the solution by end over end mixing for 1 hour. The precipitates were then centrifuged out at 4000 rpm during 20 min and the supernatant was analyzed by ICP-MS to evaluate the efficiency of the co-precipitation of Cu with MnO_2.

30% of Cu was still present in the supernatant following the centrifugation step, which underscored that the precipitates did not collect the total Cu amount in the sample. 100% of Ca remained in solution that indicated that Ca could be efficiently separated from the concentrated elements. Cu purification tests on the re-dissolved precipitates indicated a good Cu recovery (~ 100%) meaning that the presence of Mn did not lower the yield of Cu purification.

In order to increase the Cu pre-concentration by the MnO_2 co-precipitation, 3 more tests were performed, consisting in i) increasing the time of the shaking to enhance contact time between the precipitates and Cu, ii) increasing the pH of the solution to enhance Cu sorption to the MnO_2 precipitates, and iii) increasing the amount of the precipitates (Table II-10). The precipitates were centrifuged out and the supernatant analyzed by ICP-MS for Cu as described above.

Test 1	**Test 2**	**Test 3**
Formation of the same amount of MnO_2 precipitates: • 100 µL 0.5 N $KMnO_4$, • 200 µL 0.5 N $MnCl_2$, • 300 µL 25% NH_4OH (addition of 100 µL more ammonium to enhance Cu sorption by increasing the pH)	Formation of 50 % more MnO_2 precipitates: • 150 µL 0.5 N $KMnO_4$, • 300 µL 0.5 N $MnCl_2$, • 400 µL 25% NH_4OH (addition by increments of 100 µL and check pH to stay below pH 10 to avoid Ca precipitation)	Formation of 100 % more MnO_2 precipitates: • 200 µL 0.5 N $KMnO_4$, • 400 µL 0.5 N $MnCl_2$, • 600 µL 25% NH_4OH (addition by increments of 100 µL and check pH to stay below pH 10 to avoid Ca precipitation)
Increase the contact time for 8 hours (end over end shaking)	Increase the contact time for 8 hours (end over end shaking)	Increase the contact time for 8 hours (end over end shaking)

***Table II-10 :** The table summarizes the three tests that aimed to increase the efficiency of the Cu pre-concentration by co-precipitation with MnO_2.*

During the test 1, pH measurements revealed that altogether 1 mL of 25% NH_4OH was needed to increase the pH to 8 - 9.

	Test 1	**Test 2**	**Test 3**
Cu [µg L^{-1}]	0.65	0.60	0.60
% of total Cu remaining in the supernatant	15	15	15

***Table II-11 :** The results of the Cu concentration analysis by ICP-MS in the supernatants from the three tests of Cu pre-concentration by co-precipitation with MnO_2.*

Cu analysis showed the presence of 0.65 to 0.60 µg L^{-1} Cu in the supernatant that accounted for 15% of the total Cu in the water sample (Table II-11). These results were promising as Cu remaining in the supernatant was twice as low as Cu remaining after the first co-precipitation tests.

The co-precipitation was repeated in the supernatants collected from the 3 tests to collect 100% of the Cu from the water samples. The same amount of reactants were applied as during the 3 tests (slightly more 1.5 mL 25% NH_4OH was added to increase the pH to 8-9) (Table II-10).

	Test 1	Test 2	Test 3
Cu [µg L^{-1}]	0.60	0.50	0.50

***Table II-12 :** The results of the Cu concentration analysis by ICP-MS in the supernatants following the repeated co-precipitation with MnO_2 (as described in Table II-10).*

Following the repeated co-precipitation, ICP-MS analysis revealed the presence of 0.6 to 0.5 µg L^{-1} Cu in the supernatants (Table II-12). This means that no additional Cu could be harvested from the supernatants.

The potential contamination by the addition of the reactants cannot explain the persistence of 0.5 µg L^{-1} Cu in the supernatants. The blank contribution was evaluated by the addition of the same amounts of reactants to MilliQ water, and following the retrieval of the precipitates by centrifugation, the supernatant was analyzed by ICP-MS. 0.09 µg L^{-1} Cu was measured in the blank supernatant (MilliQ water) that cannot explain the 0.5 µg L^{-1} Cu remaining in the supernatant following the repeated co-precipitation tests in the water samples.

The constant Cu concentration in the supernatants after the 1st and the 2nd co-precipitation steps might be due to the competition between Cu and other matrix elements for the binding sites on the formed MnO_2 precipitates. The persistent organic matter in the sample following the acid digestion may also complex Cu in the solution and may prevent Cu from adhering to the surface of the precipitates.

Due to a lack of time no further pre-concentration methods were tested for the successful treatment of the “critical” water samples from Rouffach. In the future, other pre-concentration methods will be tested, in particular a solvent extraction method developed for Cu isotope analysis in seawater samples that are characterized by high matrix (Mg, Ca, Na, etc.) to Cu concentration ratios.[33]

2. Cu isotope measurements with MC-ICP-MS

Cu isotope measurements were done on a Neptune MC-ICP-MS (Thermo Scientific, Waltham, MA, USA). MC-ICP-MS combines an inductively coupled plasma (ICP) source, an energy filter (ESA), a magnetic sector analyzer, and multiple collectors for the measurement of ions (Faraday cups) (Figure II-8). The purified samples are introduced by free aspiration into a nebulizer, where liquid is transformed into an aerosol. The ions are then produced in the ICP ion source, in which the hot temperature Ar plasma (~6000 K) tears off electrons creating positively charged ions. The ions are entrained through the sampler and skimmer cones that constitute the plasma interface, characterized by high temperature and pressure gradients. A vacuum (~1 mbar) is produced between the sampler and skimmer cones to extract the ions behind the skimmer cone. Much of the sample is lost between the two cones as ions undergo supersonic expansion while accelerated across an electrical potential gradient (up to 10 KV) between the two cones. The ions are then focused into a beam via a series of slits and electrostatically charged plates. Due to the large ion energy spread in the ion beam produced in a plasma energy focusing is necessary. This is performed by an energy filter (electric sector analyzer, ESA), which results in a consistent energy spectrum in the ion beam. Then the ion beam passes through a magnetic field where the ions are separated on the basis of their mass to charge ratio. This double-focusing system produces flat-topped peaks, allowing for precise isotope ratio analysis.

These mass-resolved beams are then collected by an array of Faraday cups where the ions reaching the collectors are converted into voltage. Isotope ratios are calculated by comparing voltages from the different collectors. The advantages of this technique are that the electric sector energy filter provides flat-topped peaks, while the simultaneous collection of the different mass-resolved beams precludes imprecision due to the fluctuation of the plasma energy and the intensity of the ion beams produced. Moreover, the bent geometry of double-focusing keeps the noise level low.[34,35]

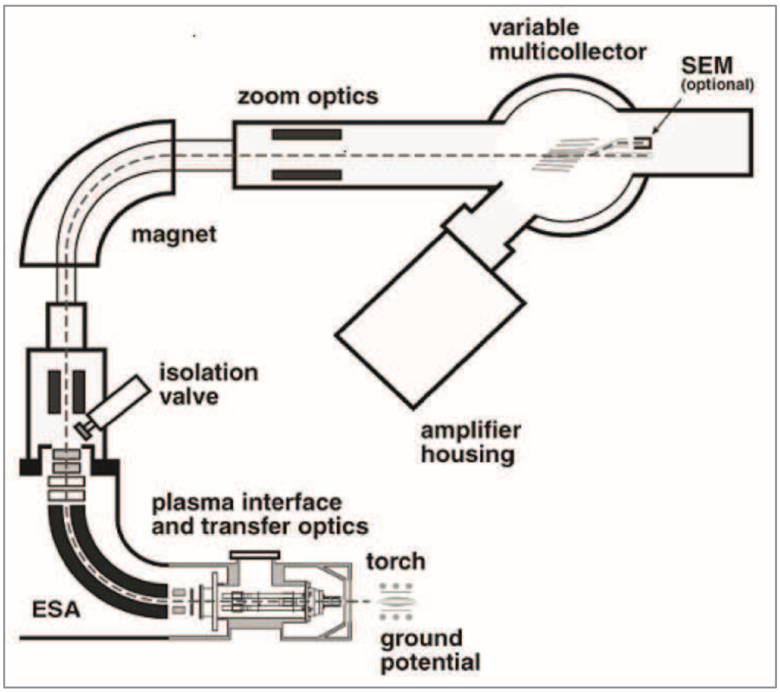

Figure II-8: *The schematic image of a double focusing multicollector ICP-MS (ESA: Electric sector analyzer (Figure from Goldstein and Stirling, 2003).*[36]

Due to the so-called space charge effects, the measurement of isotopes ratios is biased as the heavier isotopes are transmitted through the instrument with less efficiency. This phenomenon is the instrumental mass bias that needs to be corrected.[35]

2.1. Cu isotope analysis method

Cu is introduced to the ion source (ICP) through a micro-concentric PFA nebulizer (50 µL min^{-1}) in a 0.5 N HNO_3 medium following the double purification. In the beginning measurements were done in "dry plasma mode", attained using a desolvating system (Apex HF, ESI, NE, USA). After 2 years of Cu isotope measurements, high instabilities were observed in the instrumental mass bias that resulted in incorrect standard values. Therefore, a cyclonic glass spray chamber was installed instead of the Apex, which was used for the Cu isotope analysis of soil samples from the catchment study (Chapter III) and the sediment samples from the Winogradsky experiment (Chapter V). The quality of the isotope measurements was regularly checked by measuring a geological standard within each analytical session.

The Cu isotope measurements were done in a low resolution mode. A single measurement consisted of a block of 40 cycles of 8 s integrations, except for the blank measurement. Automatic sampler (CETAC ASX110-FR, Teledyne CETAC, NE, USA) was used for passing the standards and the samples as well as the blanks before each sample and standard. The gain of the pre-amplifier of each Faraday-cup was calibrated daily with respect to that of the axial collector. Following a plasma stabilization warm-up period of 30 min to 1 h, the peaks of the measured isotopes were centered daily and Faraday cups were repositioned if necessary to get centered peaks on all the collectors used simultaneously. Some parameters of the inlet system were also tuned before each measurement sessions, such as the torch position, sample gas and auxiliary gas flows, in order to reach the highest sensitivity and a stable ion beam (Table II-13). Between samples and standards, the system was rinsed during 8-10 minutes with 0.5 N HNO_3 to reduce memory effects and cross-contaminations. The baselines were corrected by analyzing the acid blank (10 cycles of 8 s integrations) before each analysis for an on-peak-zero baseline correction. A sample size of 100 µg L^{-1} Cu was analyzed for isotope ratios, and the ion beam intensity for ^{63}Cu in a 100 µg L^{-1} solution varied from 5 to 6 V in different analytical sessions when using Apex and from 1.5 to 2.2 V in case of the spray chamber. Each sample was measured at least twice. Sampler and skimmer cones were regularly cleaned in a diluted HNO_3 acid bath using a sonicator and rinsed with MilliQ water.

Introduction system:	**Apex**	**Cyclonic glass spray chamber**
Mass resolution (m/Δm) :	LR (300)	
Cones :	Ni sampler, Ni skimmer	
Cool gas [L min^{-1}]:	15	
Auxiliary gas [L min^{-1}]:	~1.00	
Sample gas [L min^{-1}]:	~1.00	
X position of the torch [mm]:	0.9 - 1.95	0.8 - 1.3
Y position of the torch [mm]:	~-3	~-2.9
Z position of the torch[mm]:	-2.2 - 1.3	-1.3 - 0.2
RF power [W]:	1200	
^{63}Cu ion beam intensity [V]:	5 - 6	1.5 - 2.2

Table II-13 : *The typical inlet parameters of the MC-ICP-MS tuned daily (except for the cool gas and the RF power) and some other operating parameters.*

When measuring isotopes by a multicollector ICP-MS, the mass discrimination effect induced by the instrument has to be corrected. This is the process by which the heavy isotopes of the same element are transmitted with a better efficiency, which introduces a bias in the isotope ratio analysis. In fact, space charges effects are assumed to have the strongest influence on the instrumental mass discrimination. After the positively charged ion beam leaves the skimmer cone, the mutual repulsion of ions limits the total number of ions transmitted by the ion optics. The light ions are deflected more extensively than heavy ions that remain preferably in the central ion beam resulting in better transmission efficiency.[35] Among the applied mass discrimination correction functions, the exponential function was chosen the most suitable for Cu isotope analysis.[1]

A correction factor, β has to be applied to each measured isotope ratio. A combination of sample-standard bracketing and external normalization was used for correcting instrumental mass bias. Nickel standard (NIST 986) was used for calculating β with the exponential function.[1] Indeed, each sample was spiked with the Ni standard to obtain a concentration of 200 µg L^{-1} before isotope analysis (external normalization). When the glass spray chamber was adopted the Ni concentration was increased to 800 µg L^{-1} to get a higher intensity ion beam on the monitored ^{62}Ni and ^{60}Ni. Standard bracketing consisted of passing a mixture of Cu (NIST 976) and Ni (NIST 986) standards between each sample. The Ni was chosen for mass bias corrections of Cu isotope ratios, because Ni isotopes are of the same mass range (58-62 amu) as Cu isotopes (63, 65 amu) and it has been shown that there was an excellent correlation between ln(^{63}Cu/^{65}Cu) and ln(^{60}Ni/^{62}Ni), meaning that instrumental mass discrimination is the same for the two elements.[37] Mass bias corrections and δ^{65}Cu values were calculated according to the equations (1 to 3).

$$R^{i}_{real} = R^{i}_{measured} \times \left(\frac{m^{i}_{1}}{m^{i}_{2}}\right)^{\beta^{i}} \tag{1}$$

$$\beta^{i} = \frac{\ln \times \left(R^{i}_{real}/R^{i}_{measured}\right)}{\ln \times \left(m^{i}_{1}/m^{i}_{2}\right)} \tag{2}$$

where R represents the real or measured isotope ratios of an element i and m_1 and m_2 denote the precise masses of the two measured isotopes of i and β the mass bias correction factor. The certified isotope ratios of the standards are $^{62}Ni/^{60}Ni$ (NIST 986): 0.1386 and $^{65}Cu/^{63}Cu$ (NIST 976): 0.4456.

The $\delta^{65}Cu$ values were calculated by dividing the β corrected Cu isotope ratios by the mean of the adjacent standards:

$$\delta^{65}\mathrm{Cu} = \left[\left(\frac{^{65}\mathrm{Cu}/^{63}\mathrm{Cu\,sample},_{\beta-\mathrm{corr}}}{\left(^{65}\mathrm{Cu}/^{63}\mathrm{Cu\ standard},_{\beta-\mathrm{corr,before}} + {}^{65}\mathrm{Cu}/^{63}\mathrm{Cu\ standard},_{\beta-\mathrm{corr,after}}\right)/2}\right) - 1\right] * 1000 \tag{3}$$

where $^{65}Cu/^{63}Cu$ are the β corrected Cu isotope ratios of a sample and the adjacent standards.

2.2. Interferences on $^{65}Cu/^{63}Cu$ measurements

The two hindrances for precise measurements of Cu isotopes ratios are the instrumental mass bias and the spectral interferences. The mass bias correction has already been discussed. Here we present some analytical work that aimed at testing interferences of some matrix elements on the measurement of Cu isotope ratios. The spectroscopic interferences are due to three main types: the isobaric ions (e.g.: ^{58}Ni and ^{58}Fe), multiply charged ions (e.g.: ^{130}Ba (II+) and ^{65}Cu (I+), intense adjacent signals, and polyatomic ions (e.g.: $^{47}Ti^{16}O$ and ^{63}Cu).[34]

We prepared solutions of a mixture of the Cu and Ni standards and spiked these solutions with variable concentrations of the matrix elements that have been detected in the purified samples following a single purification: Ca, Mg, Ti, Na, Si, Ba, Co, Zn, and a mixture of Ca-Si-Na. The tests were carried out in dry plasma mode using the Apex desolvator.

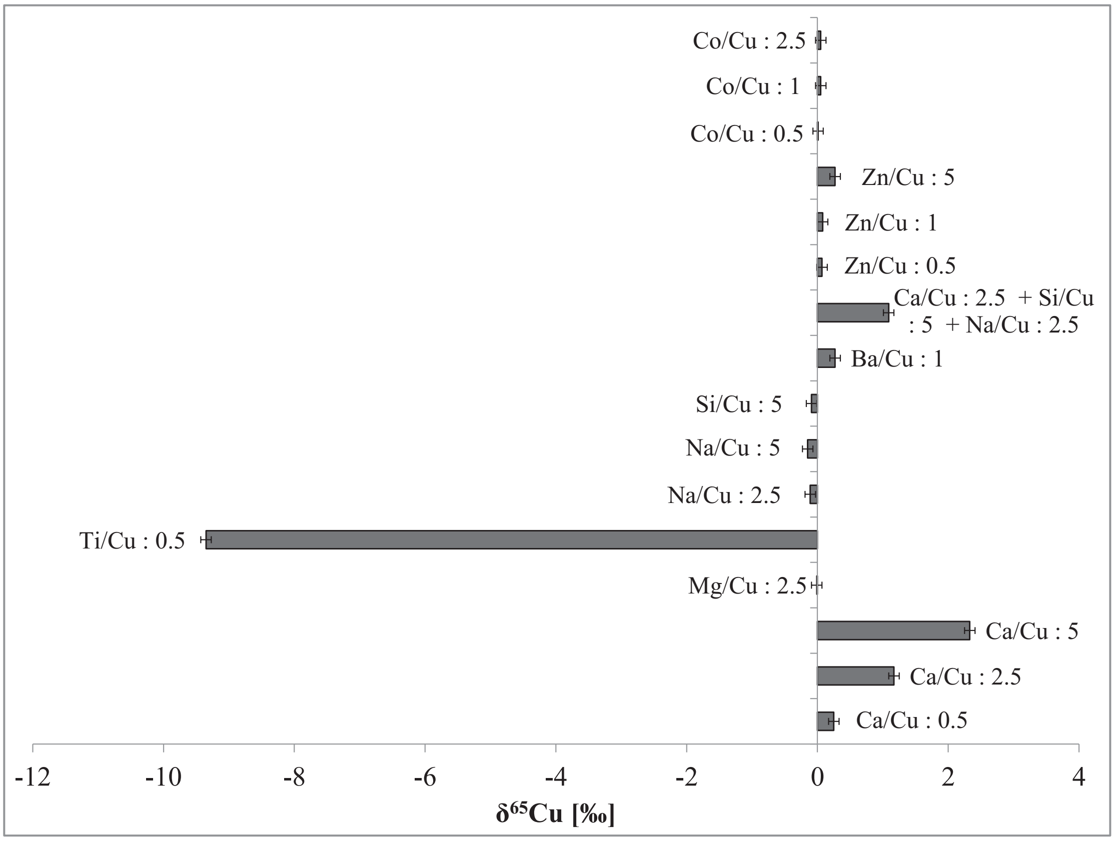

Figure II-9: *Test of the spectral interferences caused by the spiking of the Cu standard with matrix elements that might be present in purified samples following a single purification.*

The tests revealed that by the above described operating conditions, the isotopic shift caused by the presence of the selected elements was significant in the case of Ti, Ca, Na, Ba and Zn (Figure II-9).

The bias caused by the presence of Ba can be attributed to the formation of the double-charged Ba(II+) species in the plasma that causes interference on the ^{65}Cu mass likely explaining the shift towards positive δ^{65}Cu values.[5] The influence of Na on the measured δ^{65}Cu is likely due to the interference of the ^{23}Na^{40}Ar species on the ^{63}Cu mass.[9] As for the interferences caused by the Ti, they are mostly due to the formation of polyatomic species in the plasma, the most important of which will interfere with ^{63}Cu (^{47}Ti^{16}O), ^{62}Ni (^{46}Ti^{16}O) and ^{65}Cu (^{49}Ti^{16}O).[5] In our case, the interferences caused by Ti on the ^{63}Cu mass overrule those on the ^{65}Cu, shifting the δ^{65}Cu towards positive values. Bigalke et al.[16] found a similar shift towards negative δ^{65}Cu values, however, two other studies have found shift towards heavy isotopic compositions due to Ti.[9,13] The differences can be due to different instruments and operating conditions applied and the non-spectral effects (i.e. mass bias effects) that also significantly influence the measured Cu isotopic compositions.[38] The Ca induced isotopic shift is somewhat surprising as we would expect a shift towards lighter isotopic values due to the formation of ^{44}Ca^{16}O that interfere with the ^{60}Ni mass. There is no known spectral interference from Zn on the Cu masses, and the measured ^{60}Ni and ^{62}Ni. Therefore, the isotopic shift induced by the presence of Ca and Zn can be attributed to non-spectral interferences, i.e. changes in the mass biases.

To overcome the matrix interferences on the δ^{65}Cu measurements, sufficient Cu purification should be reached through careful calibration of the column chromatography and adopting a double-purification.

3. Sequential extractions

For the purpose of elucidating the geochemical partitioning of Cu in the sediment layers of the Winogradsky columns (Chapter V), we applied a modified protocol from Tessier et al.[39,40] The principle of sequential extraction is to apply extracting reagents that specifically target different forms of Cu present in soil/sediment samples (Table II-14). The extracted phases are named exchangeable, acid-soluble, reducible, oxidable and the residual fraction.[39] These fractions are extracted respectively with magnesium chloride ($MgCl_2$ at pH=7), acetic acid and sodium acetate adjusted to a pH of 5, ammonium hydroxide hydrochloride ($NH_4OH.HCl$) in 25% acetic acid adjusted to a pH of 3.5, hydrogen peroxide (H_2O_2) with subsequent desorption of Cu by ammonium acetate adjusted to a pH of 2, and finally the residual fraction is dissolved in a mixture of HNO_3 (10 mL), HCl (10 mL) and HF (10 mL). The extracting reagents are successively applied to 1 g of soil or wet sediment sample in quantities of 25 –30 ml. Each dissolving step comprises reaction times up to 16 hours in an end-over-end shaker. After each extraction step, the samples are centrifuged at 4000 rpm for half an hour. The supernatant is removed after centrifugation and the residue washed with 20 mL of MilliQ water during 5 min in an end-over-end shaker. The first 3 steps are done at ambient temperature, the peroxide treatment is done in a heated ultrasonic bath (85°C), while the residual fraction is dissolved in the acids on a hotplate at 100 to 200°C.

Operationally defined fractions	Targeted Cu forms	Extracting reagents
Exchangeable	Weakly sorbed Cu	$MgCl_2$
Acid soluble	Cu attached to carbonates and specifically sorbed Cu	Acetic acid
Reducible fraction	Cu bound to Mn and Fe oxy(hydr)oxides	$NH_4OH.HCl$
Oxidable fraction	Cu bound to organic matter	H_2O_2, Ammonium acetate
Residual fraction	Cu occluded in primary minerals such as clay minerals and phyllosilicates	HNO_3, HCl, HF

Table II-14 : *Operationally defined fractions and applied extracting reagents for sequential extraction of Cu.*

The extracted solutions were analyzed with regard to Cu and other trace metals as well as major components such as calcium, magnesium, iron, manganese, phosphorous, sulfur and silicon with ICP-AES by preparing the calibrating standards in the extracting reagent solutions to minimize matrix effects.

3.1. Reproducibility of sequential extraction

The extracted Cu amounts during the course of the extractions may vary due to sample heterogeneity and the slightly differing conditions by which the extractions are done, as well as the variable sample properties (carbonate content, OM content, etc.).[41] Therefore, we evaluated the reproducibility of the extraction of the different fractions of Cu types based on triplicate soil and a sediment samples from Rouffach. Standard deviations did not exceed 8% (2sd) for all extracted Cu fractions in the soil samples (Table II-15). In the sediment, standard deviations are generally higher but stay below 10% for the acid soluble, reducible, oxidable and residual fractions (Table II-15). The highest deviation is denoted for the exchangeable fraction (100%); however, the Cu amounts extracted in the exchangeable fraction were very small (<0.01 µg g^{-1}; data not shown). The reproducibility of the extracted Cu fractions was comparable to that published by Duplay et al.[40] for the same extraction procedure in soil samples (5%), evaluated on duplicated samples. The dissolution of the whole sample was not achieved in either of the samples. For the soil triplicates 0.104 g of soil remained in average undissolved after the residual extraction step, representing 10.4% of the 1 g of the treated soil. The average mass of the final residue of the sediment was 0.062 g. Summing up all Cu extracted during the whole procedure, the cumulated value (107%) corresponded to the total Cu concentration in the soil considering the measurement uncertainties. However, in case of the sediment samples the whole amount of Cu extracted corresponded to ~77% of the total sediment-bound Cu. A higher uncertainty can be assumed for the Cu recovery estimations in case of the sediments due to the higher sample heterogeneities, in particular the variable humidity present in the 1 g of wet sediments aliquot. Overall, results suggest that nearly one third of Cu may not be extracted from the sediments.

	Reproducibility (2sd) [%]				
Samples	**Exchangeable**	**Acid-soluble**	**Reducible**	**Oxidable**	**Residual**
Soil sample from Rouffach	5.7	1.4	7.7	5.3	3.5
Sediment sample from Rouffach	100	9.5	8.3	1.3	8.2

Table II-15 : *Reproducibility of the sequential extraction procedure with regards to Cu as evaluated on a triplicate of a soil and sediment samples from Rouffach (n=3), the 2 standard deviations of the triplicates are expressed as % of the extracted Cu amounts in the different steps.*

3.2. Selectivity of the extracting reagents

Even if, all the extracting procedures are operationally defined, they provide useful information about the geochemical partitioning of metals in geological materials. The specificity of the extracting reagents used during the course of the sequential extractions procedure with regards to the targeted phases can be evaluated by the analysis of some characteristic elements (such as Ca, Si and Fe) in the extracted fractions.

The analysis of Ca in the extracted fractions shows that most of the calcium content in the sample is extracted by the application of acetic acid (95% and 94%) which means that almost all of the carbonates were successfully extracted with acetic acid.

Samples	**Si [mg L^{-1}]**					
	Acid soluble	**Washing**	**Reducible**	**Washing**	**Oxidable**	**Washing**
Sediment	278	13	59	115	201	22
Soil	230	6	90	2391	277	43

Table II-16 : *The average Si concentrations detected in the extracted fractions in the sediment and soil samples from Rouffach (n=3)*

The Si analysis in the extracted fractions shows that some Si has been extracted from the acid-soluble, reducible and the oxidable fractions (Table II-16). These results suggest that the extracting reagents may have partially attacked the silicates in the soils and the sediments. For instance, acetic acid may also have attacked Fe-bearing silicates, such as chlorite, a clay mineral present in the soils and sediments from Rouffach.[42] Acetic acid might thus not be selective for the carbonate phases, and partly dissolve other soil or sediment minerals.

The results of Fe extractions showed that most Fe was present in the residual fractions (>85%), whereas ~15% in the acid-soluble fractions. These results suggest that Fe oxide phases were not extracted in the reducible fraction. Indeed, it has been suggested that the applied hydroxylamine extracting reagent for the reducible fraction can hardly extract crystalline Fe oxy(hydr)oxides.[41] Even if no Fe was found in the reducible fractions, Fe oxides may still be present in the soils and sediments extracted in the residual fractions. This implies that the Cu amounts extracted in the reducible fraction may not account for the totality of the Fe oxides-bound Cu.

Overall, the reproducibility of the sequential extraction procedure is better than 10% for the extracted Cu fractions in both soil and sediment samples. However, the incomplete dissolution of the soil and sediment material may preclude the complete extraction of Cu from soils and sediments. The unextracted Cu amounts may be added to the residual Cu fractions to estimate the partitioning of the sediment-bound Cu. The poor extraction efficiency of Fe oxides using hydroxylamine hydrochloride, in particular the crystalline oxides, may lead to the underestimation of Cu bound to Fe oxides.[42]

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III. Chapter: Copper stable isotopes for evaluating copper in soils and runoff in vineyards

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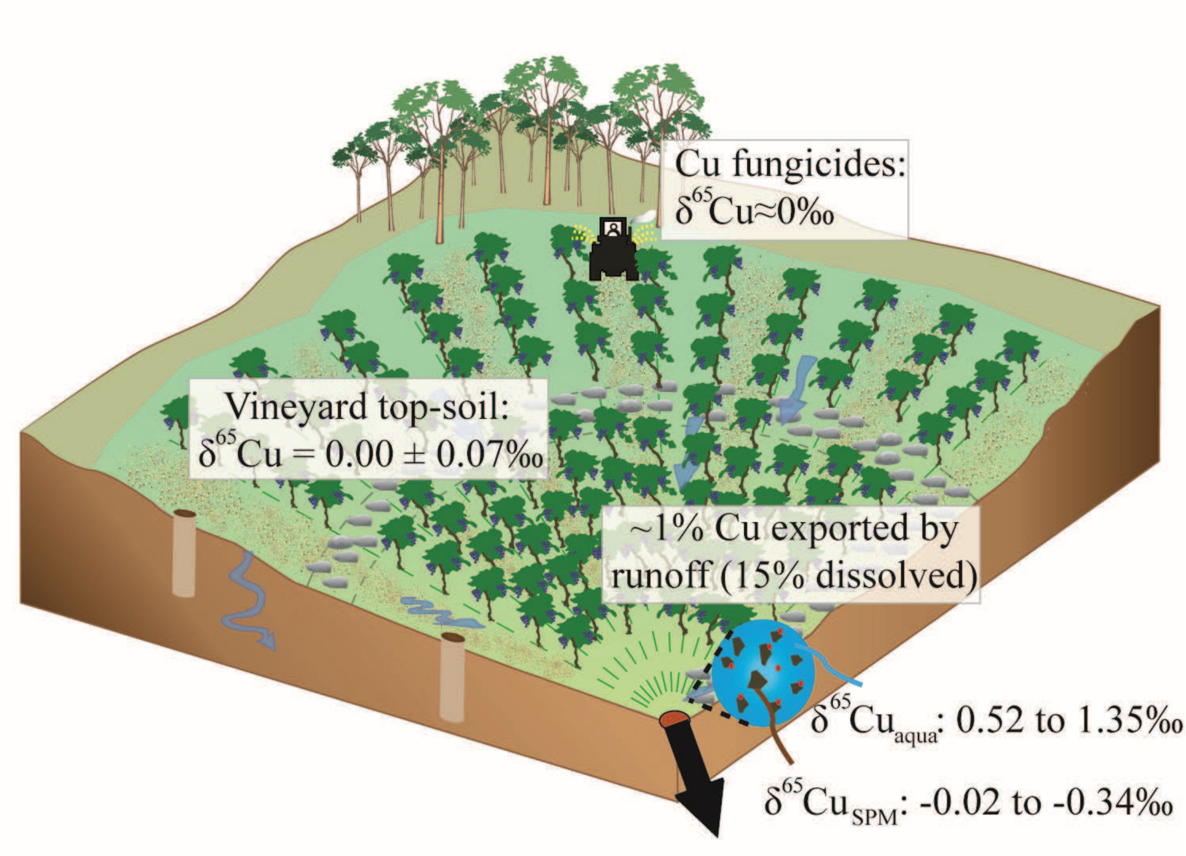
We have applied the Cu isotope analysis method described in Chapter II to trace Cu fungicide accumulation in vineyard soils. In this chapter Cu isotope analysis was combined with particle-size separation of bulk soils to study the distribution of anthropogenic Cu. In addition, we investigated Cu mobilization in soils during rainfall and transport by runoff using Cu isotope ratios of runoff collected at the outlet of a vineyard catchment. Combining isotopic data with hydrological and Cu fungicide application data enabled to evaluate the impact of hydro-climatic conditions and Cu fungicide applications on Cu export from the catchment.

GRAPHICAL ABSTRACT



ABSTRACT

Copper (Cu) stable isotopes were used to investigate Cu retention in soils and runoff transport in a vineyard catchment (Rouffach, France) in relation to the use of Cu fungicides and hydro-climatic patterns. The δ^{65}Cu varied moderately along the soil profiles (-0.12 to 0.24‰ ± 0.08‰), possibly due to similar δ^{65}Cu for the fungicides (~0‰) and background Cu (0.08‰). In contrast, δ^{65}Cu differed between particle-size soil fractions, ranging from -0.37 ± 0.10‰ in fine clays to 0.23 ± 0.07‰ in silt. Together with the soil mineralogy, these results support the conclusion that Cu is retained by clay minerals and organic phases in the soil. Cu export by runoff from the catchment was controlled by soil erosion, cumulatively accounting for 1% of the Cu applied. The dissolved phase of runoff displayed δ^{65}Cu ranging from 0.52 to 1.35‰ and from -0.34 to -0.02‰ in the suspended particulate matter (SPM), suggesting Cu redistribution and isotope fractionation in runoff, and indicating that clays are the main contributors to the export of SPM-bound Cu. Taken together, this study shows that Cu stable isotopes can be used to trace Cu at the catchment scale and that hydrological, isotopic and geochemical approaches can be combined to characterize Cu behavior at contaminated sites.

1. Introduction

Copper (Cu) is an essential micronutrient, which may accumulate in agricultural soils and adversely impact soil biota and fertility.[1] Vineyard soils are frequently contaminated by Cu due to the use of Cu fungicides since the end of the 19th century. In the European Union, up to 6 kg ha^{-1} y^{-1} of Cu is used in both conventional and organic viticulture.[2,3] Cu mobility and transport from agricultural lands is an important issue because Cu may eventually contaminate vulnerable aquatic ecosystems.[4,5] In addition to traditional approaches, stable Cu isotopes ($^{65}Cu/^{63}Cu$) have been recently used to investigate the sources, behavior and transport of Cu in the environment.[4,6-11] This utility encourages the application of Cu isotopes to evaluate Cu retention and distribution among particle-size fractions in contaminated soils, as well as Cu mobilization by runoff at the catchment scale.

Cu retention in soils is controlled by physico-chemical parameters, such as pH, organic matter content and clay content,[2,12] as well as the speciation of the applied Cu.[13] Soil physico-chemical processes can alter the Cu isotope distribution through adsorption,[14,15] complexation,[16] precipitation-dissolution[17] and oxidation-reduction.[18] In vineyard soils, Cu fungicides can be sorbed to clays, iron (Fe) and aluminum (Al) oxy(hydr)oxides, carbonates, and they can (co)precipitate with secondary minerals and complex with soil organic matter (SOM).[1] Although SOM-Cu complexes may dominate in agricultural soils, as observed in Cu-speciation studies using X-ray absorption,[19-24] Cu can also precipitate with carbonates in calcareous soils.[13,25] SOM efficiently sorbs Cu, while dissolved organic matter (DOM) complexes with and maintains Cu in solution, thus favoring its mobilization by rainfall.[1] In addition, the heterogeneous distribution of Cu among particle-size fractions (i.e., sand, silt, clay) is expected to control Cu mobilization from the soil during rainfall-runoff events.

Cu can accumulate in the coarse particulate SOM,[26,27] as well as in finer soil particles that are preferentially mobilized by runoff.[28] Rainfall and runoff patterns influence soil erosion,[29,30] and thus control Cu export by runoff.[6,31] Therefore, we hypothesized that Cu stable isotopes can serve to evaluate how the different soil particle-size fractions contribute to Cu export by runoff from agricultural catchments. Recently, the natural and anthropogenic sources of Cu were evaluated in a flash flood event in a vineyard catchment using Cu isotope analysis.[4] Cu was mainly associated with Fe oxy(hydr)oxides in the SPM, while Cu bound to SOM was isotopically lighter.[4] In another study, the Cu in SPM was isotopically lighter compared to dissolved Cu in rivers, which may be related to isotopically heavier Cu associated with DOM in the dissolved fraction.[4,32] However, Cu mobilization by rainfall and its transport by runoff have not yet been investigated using Cu stable isotopes.

In the present study, we used Cu stable isotopes to study Cu sources, Cu retention processes in soils and Cu transport in a vineyard catchment (Rouffach, Alsace, France) in relation to the use of Cu fungicides and rainfall-runoff patterns. The δ^{65}Cu values of the Cu fungicides, ^{65}Cu/^{63}Cu fractionation associated with Cu retention in the soils and Cu transport in runoff were evaluated during the period of Cu fungicide use (May to July). The objectives were to evaluate i) the retention and distribution of Cu in particle-size fractions of the vineyard soils, and ii) the mobilization and runoff transport of the dissolved and SPM-bound Cu in relation to Cu fungicide use and hydrological conditions.

2. Materials and methods

2.1. Study catchment and soils

The study site is a 42.7 ha vineyard catchment situated in the Alsatian foothills (Alsace, France; 47°57′9 N, 07°17′3 E).[33] 59% (25.3 ha) of the catchment is occupied by vineyards with a mean slope of 15%. Roads and paths represent 4.6% of the surface.[34] The main soil type is a calcareous clay loam with medium infiltration capacity and a soil bulk density of 1.4 g cm^{-3} developed on a loess basement.[34] The vineyard plots are permanently covered by grass in every second inter-row to limit soil erosion, and weeded inter-rows are ploughed to a depth of 15 cm to increase water infiltration. The grass cover was implemented 3 to 4 years after the planting of grape vines.

The rainfall is monitored by a meteorological station[34] located in the catchment. Rainfall between May and July averaged 187 ± 64 mm (1998-2011) and was 157 mm during the study period. The mean runoff coefficient in the catchment was 1.61% during the study period. Rainfall-runoff events do not generate a permanent stream in the catchment. The surface runoff converges at the outlet of the catchment where runoff samples were collected.[35]

2.2. Cu fungicide applications

Cu fungicide applications were estimated based on surveys addressed to the wine producers of the catchment. Cu fungicides were applied on the catchment from May to August in quantities of 2.2 ± 0.3 kg (of the fungicide preparation) ha^{-1} y^{-1} (2008-2011) in the forms of Cu-sulfate (Bordeaux mixture, $CuSO_4 + Ca(OH)_2$), Cu-oxide (Cu_2O), Cu-hydroxide ($Cu(OH)_2$) and Cu-oxychloride ($3Cu(OH)_2 \cdot CuCl_2$). Application rates of the Cu substances ranged from 0.09 to 1.50 kg ha^{-1} in 2011. Cu was sprayed directly on the vine canopy to combat downy mildew.[2] The study was performed from May 11 to July 20, 2011, covering most of the Cu application period and corresponding to 63% of the annual application of Cu. The estimated input of atmospheric

Cu to the catchment was ~83 g,[36] and represented ≤0.5% of the amount of Cu fungicides used during the study period.

2.3. Soil and runoff collection

Four soil cores were collected in two weeded and two grassed inter-rows, as well as one soil sample at a local forested reference site at 70 cm depth (Figures III-1 and III-2). Soil cores were collected up to a depth of 157 cm at site 1 and 105 cm at site 2 using a hand auger. The depth resolution of the soil sampling ranged from 10 to 20 cm. The soil physico-chemical characteristics were described elsewhere (Table III-S1).[37] Four soil samples under grassed inter-rows near the surface were selected for Cu isotope analysis. Historically, vine growing started in 1973 at site 1 (previously grassland) and in 1962 at site 2 (Figures III-1 and III-2). The width between grassed vine inter-rows was 170 cm at site 1 (as well as on 77% of the vineyard catchment), 240 cm at site 2, while the width between weeded inter-rows was 140 cm. The soils are Cambisol Hypereutric Clayic (site 1) and Haplic Cambisol Calcaric Siltic (site 2) (IUSS Working Group WRB, 2006).[37] Soils were mainly composed of calcite, quartz, and minor amounts of feldspars, dolomite and clay minerals (Table III-1).[37] Surface soils at site 1 had less carbonates, and slightly higher OM compared to site 2 (Figure III-2 & Table III-S1).[37] The soil profiles have been characterized previously.[38] Briefly, the A horizon extends until 30/45 cm depth, the B horizon until 80/100 cm, and the C horizon until the bedrock (loess) situated at ~210 cm.[38] The soil samples were stored in polyethylene bags. Composite topsoil samples were also collected along three transects across the catchment (~50 g of topsoil soil was collected each ~100 meters) on April 6, May 11 and June 8, 2011.

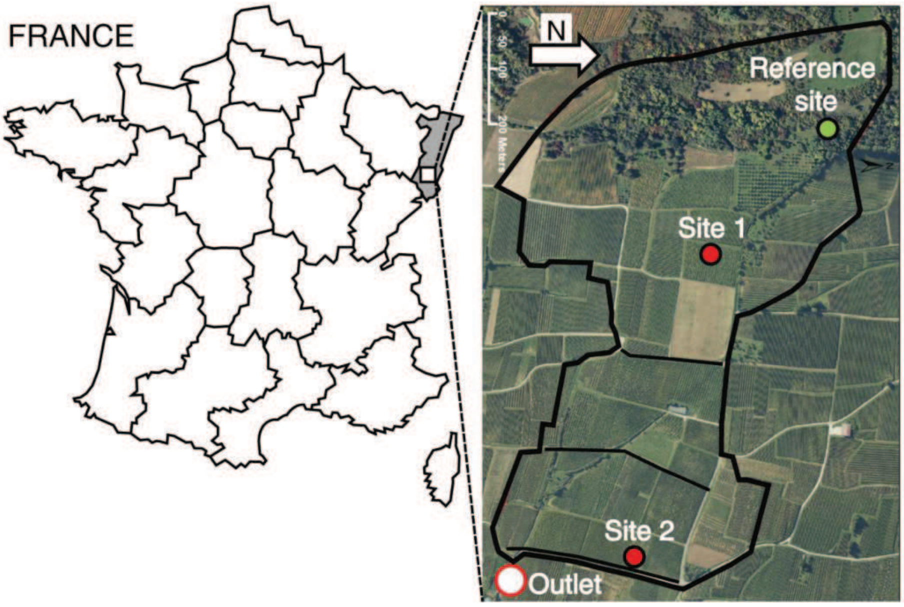


Figure III-1: *The map of the study site (Rouffach, Alsace, France) with the sampling sites and the outlet displayed. The composite topsoils were collected along the three displayed transects.*

Samples	Mineral phases
Bulk soil	calcite, quartz, minor amounts of feldspars, dolomite, clay minerals
Silt	quartz, calcite, dolomite, feldspars (albite, microcline, orthoclase), traces of clay minerals (illite, chlorite and/or kaolinite)
Clay	kaolinite, illite, traces of chlorite and fine fractions of quartz, calcite, goethite and feldspars (albite, microcline, orthoclase)
"Fine clay"	illite, kaolinite and interstratified organic matter?

Table III-1 : *Mineral phases detected by XRD in bulk soil and the different particle-size soil fractions of the vineyard topsoils in weeded inter-rows in the study catchment at Rouffach (Aslace, France).*

The water discharge at the outlet of the catchment was continuously monitored using a bubbler flow module (Hydrologic, Canada) combined with a Venturi channel. Flow-proportional samples of runoff water were collected during each runoff event (300 mL every 3 m^3) using a 4010 Hydrologic automatic sampler (Hydrologic, Canada),[35] and samples were combined weekly into a single composite sample. Samples were refrigerated and transported to the laboratory.

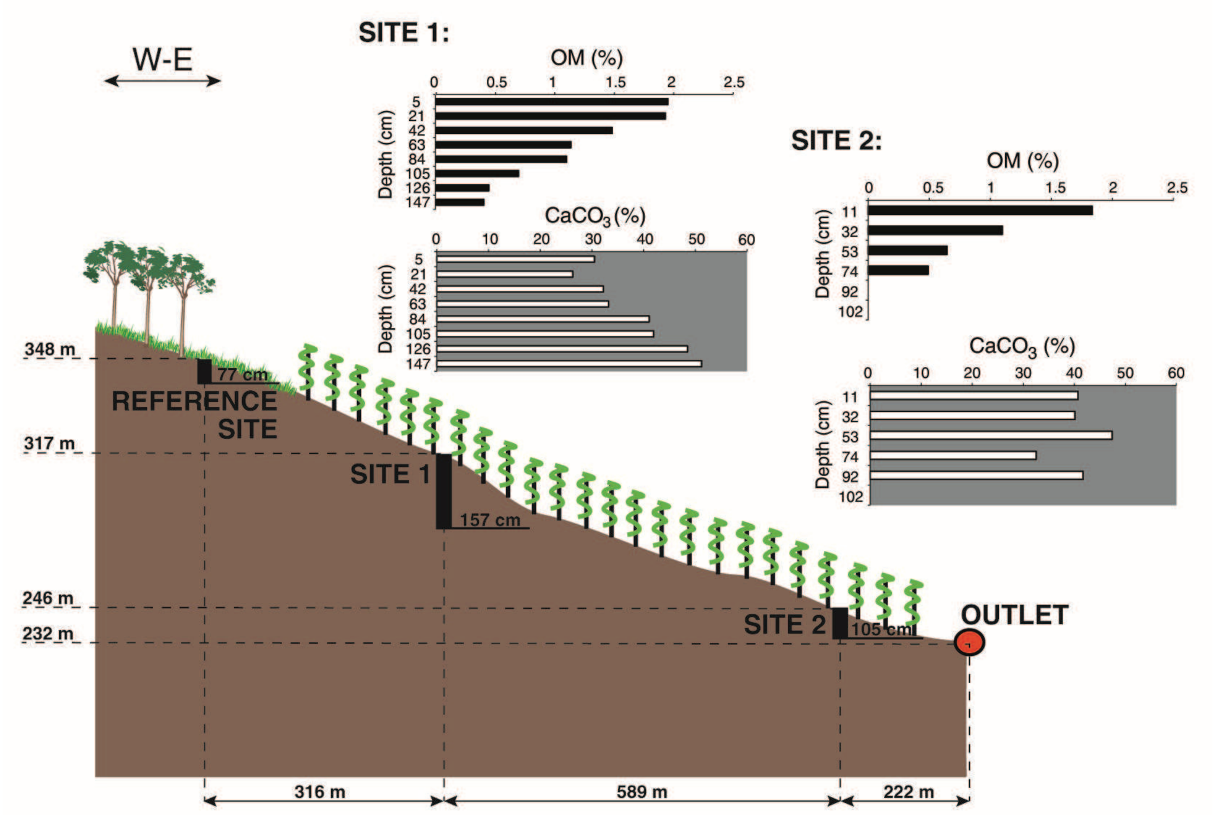


Figure III-2: *Schematic of the vineyard catchment at Rouffach (Alsace, France), showing the soil sampling locations (1 and 2) and the reference site, as well as the runoff sampling at the outlet of the catchment. The altitudes of the sampling sites and their distance from each other are also displayed. The depth variations of the carbonate (CaCO$_3$) and the organic matter (OM) contents in the soil under weeded inter-rows at the two sampling sites are also shown.*

2.4. Sample preparation and analysis

Soil samples were air-dried at 20°C and sieved (2 mm) as described previously.[37] Dried samples were powdered using an agate disk mill (<100 µm) prior to alkaline fusion and acid digestion for chemical composition analysis.[39] The geological standards BCR-2 (US Geological Survey, Reston, VA, USA) and CRM-7003 (Analytika, Prague, Czech Republic) were used to quantify the Cu recovery following acid digestion, which was always >97%. Soil physico-chemical characteristics were determined as previously described.[37]

A composite topsoil sampled on June 8 and weeded topsoils at site 1 and 2 were selected to determine the Cu distribution among particle size fractions (i.e., sand, silt, clay and fine clay) and analyzed in duplicate. Approximately 20 g of sieved soil (<2 mm) were suspended in 200 mL of 18.2 MΩ water (Millipore, Billerica, MA, USA) and disaggregated by high intensity sonication as described previously (see the SI for the detailed protocol).[40] Mineral phases present in bulk soil and particle-size fractions were determined by X-ray diffraction (XRD) (Brüker D5000, Karlsruhe, Germany).[37]

The runoff water samples were filtered using a 0.45 µm cellulose acetate membrane (Millipore) and the filtrate (named below 'dissolved phase') was acidified to pH ~2 using double-distilled nitric acid. Hydrochemical analyses were performed as described elsewhere.[41]

2.5. Cu isotope analysis

For Cu isotope analysis, 100 mg of soil was weighed in PFA vials. Cu in water samples (runoff and the solution following particle size separation) was pre-concentrated by evaporation to obtain 200 ng Cu. The SPM and fine clays were digested on filter membranes. All samples were dissolved in successive acid baths: HF-HNO_3 acid (v:v 4:1) at 70°C, $HClO_4$ acid at 150°C and a mixture of HCl and H_3BO_3 at 110°C.

A protocol for double Cu purification was adapted from Maréchal et al.[42] for each sample matrix.[6] The total procedural blank (sample digestion and purification) for Cu was ≤2 ng (n=3) for water samples (≤1% of Cu in samples) and 6.3 ± 4.4 ng ($\bar{x}$ ± 2s, n=5) for soil and SPM samples, including the Cu on the filter membranes (2.5 ng). The latter value was ≤1% of the Cu in the soil and SPM samples.

Cu isotope ratios were measured in both wet and dry plasma modes with a Finnigan-Neptune MC-ICP-MS (Thermo Scientific, Waltham, MA, USA) using a nickel standard (NIST SRM 986) and a Cu standard (NIST 976) for correction of instrumental mass biases as previously described.[43] The measurement uncertainty for the entire procedure (digestion, purification and isotope analysis) was ≤0.08‰ (2 s, *n*=15), evaluated on a soil reference material CRM-7003 (0.18 ± 0.08‰, including 3 separately digested samples and 15 isotope measurements). This error was adopted for all measured Cu isotope values. The Cu isotope ratios were expressed as δ^{65}Cu [‰] values relative to the NIST 976 reference material.

2.6. Data analysis

Changes in the Cu levels in the studied soils were evaluated using an enrichment factor (EF) that compared the Cu levels at site 1 and 2 to soil at 70 cm depth from a reference forested site upslope of the vineyards:

$$\mathrm{EF_{Cu}} = \frac{\left(\frac{\mathrm{Cu_{sample}}}{\mathrm{Fe_{sample}}}\right)}{\left(\frac{\mathrm{Cu_{reference}}}{\mathrm{Fe_{reference}}}\right)} \quad (1)$$

where Cu and Fe are, respectively, the concentrations of Cu and Fe (mg kg^{-1} d.w.) in the vineyard soil sample and the reference soil (Figures III-1 and III-2). Fe was selected as the reference element due to its low variation coefficient over the soil profiles, thereby limiting EF variations due to local heterogeneities.[44]

A seasonal export coefficient (EC) was calculated as the ratio of the total Cu load exported from the vineyard catchment by runoff over the total Cu mass applied from May 11 to July 20, 2011.

The mean δ^{65}Cu of the applied Cu fungicides was calculated by an isotopic mass balance accounting for each individual Cu fungicide:

$$\begin{aligned}\delta^{65}\mathrm{Cu_{cumulated}} = {} & \mathrm{f_{CuSO_4}} \times \delta^{65}\mathrm{Cu_{CuSO_4}} + \mathrm{f_{3Cu(OH)_2CuCl_2}} \times \delta^{65}\mathrm{Cu_{3Cu(OH)_2CuCl_2}} + \mathrm{f_{Cu(OH)_2}} \\ & \times \delta^{65}\mathrm{Cu_{Cu(OH)_2}} + \mathrm{f_{Cu_2O}} \times \delta^{65}\mathrm{Cu_{Cu_2O}}\end{aligned} \quad (2)$$

where f is the fraction of the Cu fungicides used and δ^{65}Cu is the Cu isotope composition of the fungicides.

Statistical analysis was carried out within R (www.r-project.org), with *p*-values set at 0.05. Hydro-climatic data and chemical compositions of soils and runoff were compared using the paired non-parametric Spearman rank correlation test.

3. Results

3.1. Cu fungicides

1.24 kg ha^{-1} Cu was applied on the catchment from May 5 until August 1, 2011, which corresponds to 26.07 kg for the entire vine-growing season. 16.50 kg Cu was applied (13 Cu fungicides) during the study period (May to July), which represented ~1% of the initial Cu stock in the top 5 cm of the soil (~1670 kg). The seven Cu fungicides obtained from local farmers (three of which were not in use in 2011: Kocide ($Cu(OH)_2$), Champ flo ($Cu(OH)_2$) and Cursor ($3Cu(OH)_2CuCl_2$)) had δ^{65}Cu ranging from -0.21 to 0.91‰. The δ^{65}Cu of the four Cu fungicides used in 2011 (Bordeaux mixture ($CuSO_4 + Ca(OH)_2$), Nordox (Cu_2O), Syphal ($3Cu(OH)_2 \cdot CuCl_2$), Cuprofix ($CuSO_4$)) varied from -0.21 to 0.11‰ (Figure III-3 & Table III-S2).

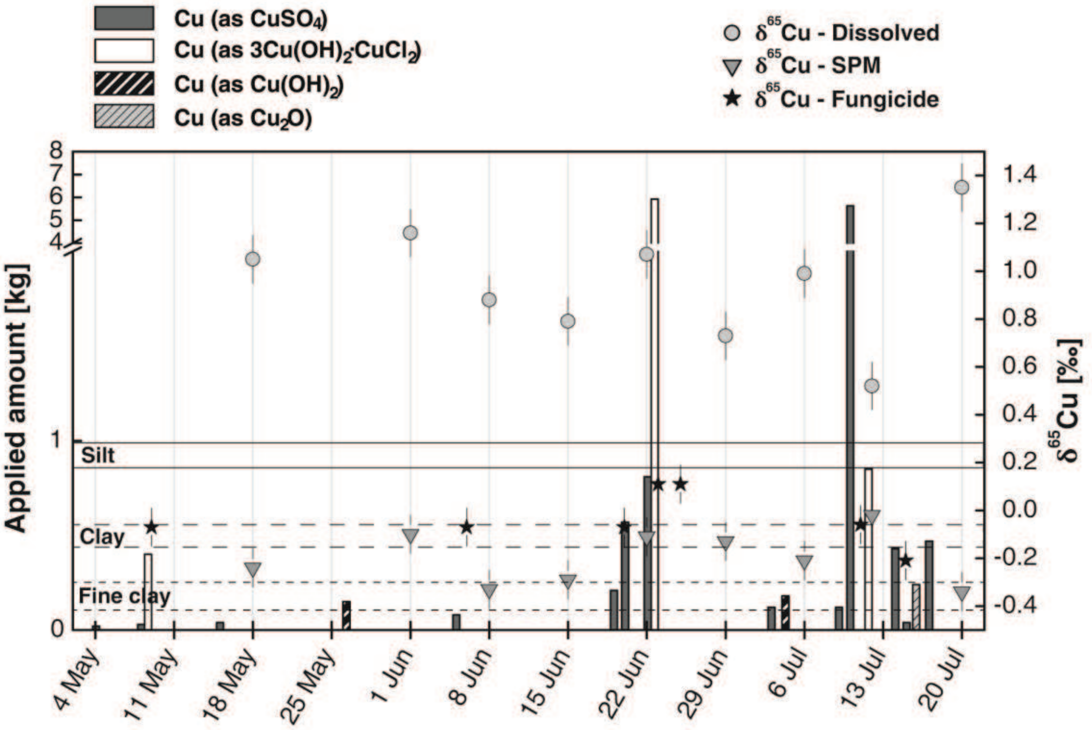


Figure III-3: *Cu amounts and δ65Cu of the applied Cu fungicides and δ65Cu of runoff-associated Cu in the dissolved and the SPM-bound phases from May 11 to July 20 (Rouffach, France). A runoff sample could not be collected on May 25. Horizontal bars represent the δ65Cu range in the soil size fractions in the catchment (i.e. silt, clay, "fine clay"). Error bars correspond to the reproducibility of the Cu isotope measurements evaluated on geological reference materials (±0.08‰).*

3.2. Cu accumulation and δ^{65}Cu in the vineyard soils

Cu accumulated in the surface layers (0 to 50 cm) (Figure III-4). Concentrations in the top 20 cm at site 2 (231 mg kg^{-1}) were higher than in the top 10 cm at site 1 (74 mg kg^{-1}) (Table III-S1), and gradually decreased with depth up to 11.5 mg kg^{-1}. Correspondingly, enrichment factors (EF) were higher at site 2 (4.0 to 27.6) compared to site 1 (1.4 to 9.6) (Figure III-4), which reflects the longer period of Cu fungicide use (i.e., the vine-bearing grapes were planted 11 years earlier at site 2 than at site 1). The Cu concentration of grassed topsoils of both sampling sites was lower than that of the weeded topsoils (Table III-S1).

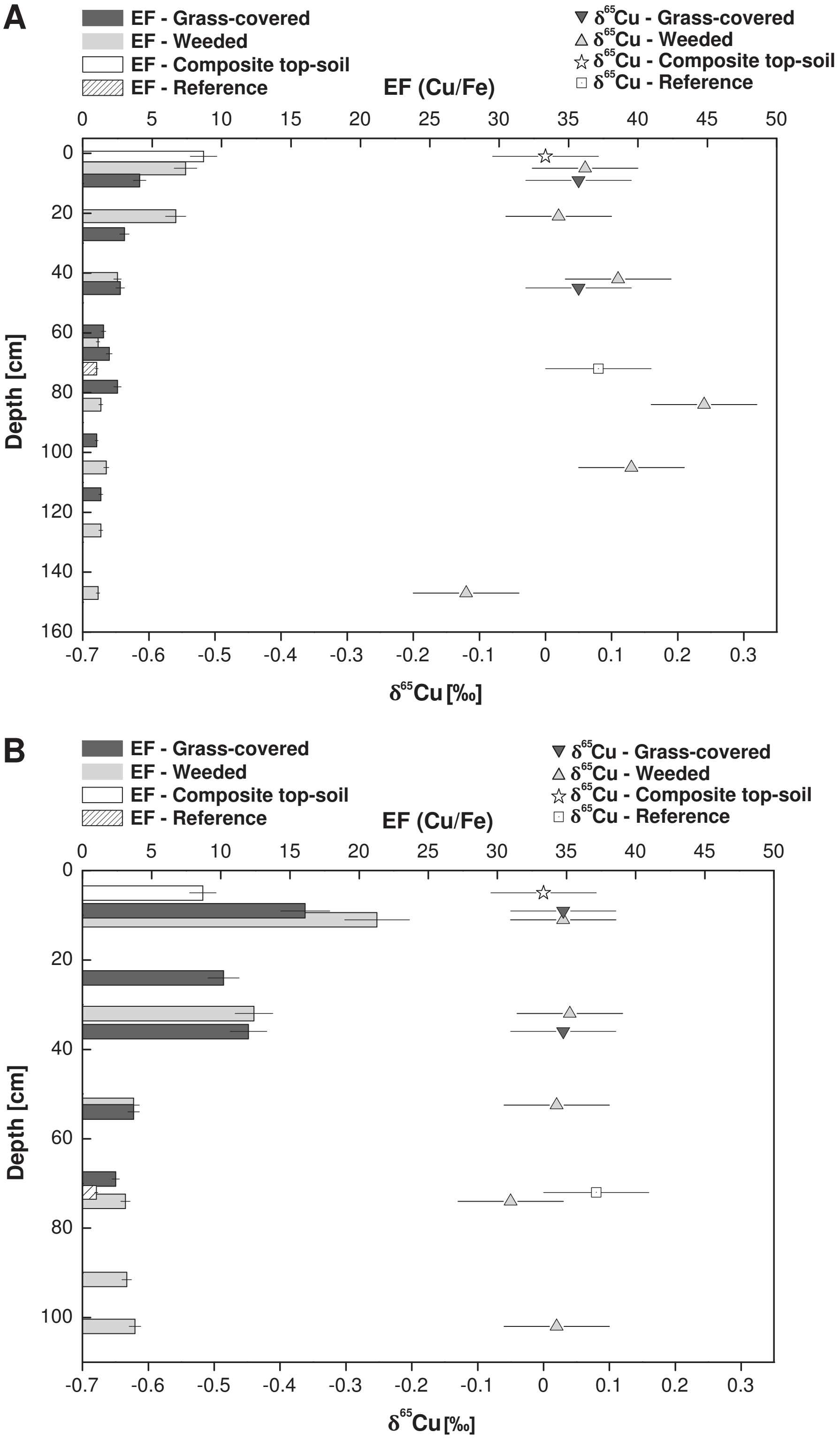


***Figure III-4:** Enrichment factor of Cu (EF) and δ65Cu values in the soil depth profiles at site 1 (**A**) and site 2 (**B**) of the vineyard catchment (Rouffach, France). The deepest soil horizon (67-77 cm) at an uncultivated reference site and the composite top-soil (0-5 cm) are also shown.*

However, the δ^{65}Cu of bulk grassed and weeded topsoils were similar, suggesting that grass cover did not affect Cu isotope fractionation in the bulk soil, possibly due to low plant uptake. Cu in the grass biomass (~4.6 kg) was estimated as ~0.5% of the total Cu stock (~900 kg) in the first 5 cm of the grassed soil of the catchment (based on the mean Cu concentrations in the grass shoot and root following Cu applications: 22.8 and 44.3 mg kg^{-1}),[37] a shoot to root biomass ratio of 5 and a maximal dry grass biomass of 10 t ha^{-1}.[38,45]

The δ^{65}Cu in the soil profile ranged from -0.12 to 0.24‰ at site 1, and did not significantly vary at site 2 (-0.05 to 0.04‰) (Figure III-4). The δ^{65}Cu of the background Cu in the reference soil (0.08‰ at 67-77 cm depth) differed from that in the deepest soil horizon at site 1 (-0.12‰) (Figure III-4). This suggests Cu isotope fractionation during weathering of the bedrock and transport of natural Cu into the soil.[46] Similar δ^{65}Cu along the soil profile at site 2 suggests that anthropogenic Cu can mask the natural variations of δ^{65}Cu (Figure III-4). This contrasts with a previous study showing depletion in ^{65}Cu with depth attributed to the leaching of anthropogenic Cu enriched in ^{63}Cu.[7] This difference of δ^{65}Cu patterns along the soil profile may be due to slightly higher soil pH and lower Cu concentrations in our case, thereby altering Cu behavior.

3.3. Cu distribution and δ^{65}Cu in the soil particle-size fractions

Although the clay fractions represented only 26 to 31 wt% of the bulk soils, Cu concentration was twice as high in the clay fractions (111 to 467 mg kg^{-1}) than in the bulk soils (Tables III-S1 and III-2). Cu concentrations were higher in the fine clay fraction (76 to 408 mg kg^{-1} Cu) than in the silt fraction (62 to 154 mg kg^{-1}), except at site 1. Cu concentrations in the aqueous solution obtained from the clay separations varied from 5 to 32 µg L^{-1} (corresponding to 1.5 to 4.4 mg kg^{-1} in the bulk soil).

At site 1, relative amounts of Cu in the silt fraction (55%) were higher than in the clay fraction (40%), the fine clay fraction (3%) and the solution (2%). Topsoils at site 2 were coarser with less clay and larger sand fractions compared to site 1. At site 2, 51% of Cu accumulated in the clay fractions (47% was in the clay and 4% in the fine clay), and in lower proportions in the silt (27%) and the sand (~20%).

Different Cu concentrations and δ^{65}Cu values among the soil fractions indicated Cu isotope fractionation during distribution of Cu fungicides in the vineyard soils. The fine clay fraction was the most depleted in ^{65}Cu (δ^{65}Cu values from -0.42 to -0.32‰, $\bar{x} \pm 2s$: -0.37 ± 0.10‰), followed by the clay fraction (-0.15 to -0.06‰; $\bar{x} \pm 2s$: -0.08 ± 0.09‰), while the silt fraction was enriched in ^{65}Cu (0.19 to 0.24‰; $\bar{x} \pm 2s$: 0.23 ± 0.07‰) (Figures III-3 & III-5, Table III-2). Cu in the solution was also isotopically heavier (0.04 to 0.44‰).

		Weight % in the soil	**% OM (LOI)**	**Cu [mg kg^{-1}]**	**δ^{65}Cu [‰]**	**2sd**	**n^{a}**
Composite top-soil June 8 (1)	fine clays (> 0.45 µm)	3.1	nd	106.2	-0.42	0.13	3
	clay fractions (< 2 µm)	25.9	6.0	132.8	-0.07	0.11	3
	silt fraction (2 µm < x < 63 µm)	46.3	2.4	61.6	0.28	0.13	3
	Solution (< 0.45 µm)	-	nd	1.9	0.32	0.06	2
Composite top-soil June 8 (2)	fine clays (> 0.45 µm)	4.8	nd	101.1	-0.32	0.07	2
	clay fractions (< 2 µm)	26.1	5.6	112.8	-0.06	0.07	2
	silt fraction (2 µm < x < 63 µm)	47.2	2.6	71.3	0.19	0.11	2
	Solution (< 0.45 µm)	-	nd	1.5	0.44	0.10	2
Surface soil at site 1	fine clays (> 0.45 µm)	2.6	nd	76.4	-0.33	0.08	3
	clay fractions (< 2 µm)	26.6	7.5	110.7	-0.06	0.13	3
	silt fraction (2 µm < x < 63 µm)	49.7	3.5	82.0	0.24	0.04	2
	Solution (< 0.45 µm)	-	nd	4.1	0.12	0.12	2
Surface soil at site 2	fine clays (> 0.45 µm)	3.3	nd	408.3	-0.40	0.07	2
	clay fractions (< 2 µm)	22.7	6.9	467.2	-0.15	0.07	2
	silt fraction (2 µm < x < 63 µm)	41.1	2.7	154.4	0.23		1
	Solution (< 0.45 µm)	-	nd	1.6	0.04	0.02	2

***Table III-2 :** Proportions, OM contents, Cu concentrations and δ^{65}Cu variations of the different particle-size soil fractions of the topsoils in the vineyard catchment (Rouffach, France).*
^{a}n: number of Cu isotope measurements

3.4. Cu export and δ^{65}Cu in SPM-bound and dissolved runoff phases

A total of 43 rainfall events occurred during the study period that generated 27 runoff events. At least 1 rainfall-runoff event occurred weekly (weekly hydrological data are summarized in Table III-S3). Rainfall depth ranged from 0.2 to 23.9 mm with mean intensities from 0.3 to 14.0 mm h^{-1}. The runoff volumes per event ranged from 2.5 to 195.4 m^{3}, with a total volume of 1247.8 m^{3} for the investigation period. SPM-bound Cu concentrations in runoff ranged from 90 to 366 mg kg^{-1} ($\bar{x}$ ± 2s: 164 ± 177 mg kg^{-1}), while dissolved Cu concentrations ranged from 7.7 to 32.0 µg L^{-1} ($\bar{x}$ ± 2s: 21.6 ± 16.0 µg L^{-1}) (Table III-S4), which was 7 to 27 times higher than average global dissolved Cu concentrations in rivers (1.19 µg L^{-1}).[32] Altogether, 144 g of Cu was exported in runoff from the catchment representing 0.9% (EC) of the Cu mass applied during the investigation period.

δ^{65}Cu of the dissolved Cu ranged from 0.52 to 1.35‰ (Figure III-3) and from -0.02 to -0.34‰ in the SPM-bound Cu in runoff (Figure III-3, Table III-S4). δ^{65}Cu values of SPM-bound Cu were similar to those of the clay and the fine clay fractions of the topsoils, suggesting a preferential mobilization of fine soil particles during rainfall-runoff (Figure III-3).

4. Discussion

4.1. Cu retention and distribution in the vineyard soils

Following the foliar application of Cu fungicides, Cu may undergo isotope fractionation during sorption on the vine leaves (~300 mg kg^{-1}) and on the grass (~20 mg kg^{-1}).[37] Cu can also reach the vineyard soil by direct deposition to the soil during Cu applications and by wash-off from the vine leaves and the grass during rainfall.[1] In the present study, Cu isotope fractionation associated with foliar application and subsequent wash-off could not be observed since δ^{65}Cu in the topsoil where Cu accumulated (EF>3) was in the range of the mean δ^{65}Cu of Cu fungicides (~0‰) (Figure III-4).

Similar δ^{65}Cu for the grassed and weeded topsoils, and the low translocation of Cu from the soil to the grass (~0.5%) suggests that the vegetation did not affect the Cu isotope pattern in the vineyard soils. Indeed, plant uptake of Cu is expected to enrich in ^{63}Cu, yielding a $\Delta^{65}\text{Cu}_{\text{plant-nutrient}}$ of -0.1 to -1‰.[47,48] In addition, δ^{65}Cu did not vary significantly along the soil profile at site 2, which suggests that Cu uptake by grape vines is limited in calcareous vineyards.[49] However, enrichment in ^{65}Cu of the soil collected at 80 cm depth at site 1 may indicate root uptake, although the largest vine root density has been previously observed between 20 and 60 cm.[50] The depletion in ^{65}Cu with depth associated with lower Cu levels (EF≤2) below 70 cm at site 1 concurs with Cu isotope distribution patterns of natural Cu observed in oxic weathered soils.[46]

Due to the small variations in the δ^{65}Cu of the bulk vineyard soils, Cu retention and distribution processes are further interpreted based on the Cu isotope ratios in the particle-size soil fractions. It has been shown that surface soils in the Rouffach catchment have from 45 to 50% of the Cu in the residual fraction (i.e., Cu included within the silicate matrix) and the organically bound fraction contains up to 45% Cu at site 1 and 15% at site 2.[37] The quantity of acid-soluble Cu was also significant with 3 to 32% at site 1 and 2, respectively, while negligible amounts of Cu were bound to oxides.[37]

In calcareous soils, precipitation of Cu as carbonates or hydroxides was noted as the prevailing process of Cu retention.[13,25,51] As the precipitation of malachite is expected to enrich the precipitates in ^{63}Cu ($\Delta^{65}\text{Cu}_{\text{Cu(II)aq-malachite}}$ = 0.17 to 0.38‰),[17] the lower δ^{65}Cu values in the clay fractions may indicate Cu carbonate precipitation, provided that the residual non-precipitated Cu was entrapped in the coarser fractions (silt and sand) enriched in ^{65}Cu. However, XRD data showed that carbonate phases (calcite, dolomite) prevailed in the isotopically heavier silt fraction, with only traces of calcite in the clay fractions and no detected carbonates in the isotopically lightest fine clays (Table III-1). This indicates that Cu precipitation as carbonate cannot explain the δ^{65}Cu patterns in the soil particle-size fractions. Therefore, Cu retention processes involving SOM and clay minerals should be considered.

Sorption of Cu to mineral and organic phases is expected to fractionate Cu isotopes between the sorbed and the dissolved Cu phases.[14,52,53] While Cu sorption to Fe and Al oxy(hydr)oxides as well as Cu complexation with OM enriches the sorbed Cu in ^{65}Cu, Cu sorption to clay minerals may produce isotopically lighter sorbed Cu.[14,15,52,53] The isotopically lighter Cu in the clay fractions of the studied soils can be attributed to Cu sorption to clay minerals as indicated by XRD analysis (Table III-1). In addition, the Δ^{65}Cu values between the silt, clay and fine clay fractions are in the range of those obtained experimentally for Cu sorption to kaolinite ($\Delta^{65}Cu_{adsorbed\text{-}solution}$ = -1.5 to -0.2).[15]

The varying δ^{65}Cu values associated with the soil fractions also suggest that Cu sorption into the soil follows a multiple-step process. A higher fractionation was observed at the initial stage of Cu sorption before reaching apparent equilibrium after 60 min.[15] Therefore, the lower δ^{65}Cu values found in the fine clay fraction compared to the clay fraction suggest that the applied Cu may first sorb to the fine clay and then to the clay. The residual non-adsorbed Cu can thus be retained in the silt fraction.

Alternatively, Cu aging in the vineyard soils may also cause isotope fractionation during Cu diffusion into the microstructure of clay minerals or organo-clays, generating a sequence of ^{63}Cu depletion in the clay and fine clay fractions. Aging is the slower stage of Cu retention in soils following a rapid sorption of Cu into the soil.[51,54] Aging may include Cu diffusion into the mineral matrix and act in reducing Cu mobility and availability in the soil.[12] Such Cu redistribution from cation-exchange sites to more strongly binding sites at the surface of clay minerals by aging has been observed in calcareous soils,[54] although Cu isotope fractionation during aging in soils is currently unknown.

In addition, Cu sorption onto qualitatively different SOM may also explain the differences in Cu isotope distribution patterns between the soil size fractions. Cu speciation in agricultural soils (including calcareous soils) may be controlled by the SOM, as highlighted by synchrotron-based X-ray absorption studies.[19-24] In our case, the correlation between the EF of Cu and the SOM content ($r=0.54$, $p \leq 0.05$, $n=15$) along the soil profiles suggests Cu sorption onto SOM. Cu-SOM complexes may be stable in vineyard soils because Cu toxicity towards microorganisms prevents decomposition and mineralization of organic matter.[26,55]

Cu complexation with SOM is expected to enrich the OM-bound Cu in ^{65}Cu relative to the dissolved Cu fraction in the soil solution.[16,53] The extent of isotope fractionation depends on the stability constants of the Cu-OM complexes with a $\Delta^{65}Cu_{complex\text{-}free}$ typically ranging from 0.14 to 0.84‰,[16,53] which is in the range of Δ^{65}Cu values observed between the silt, clay and fine clay fractions (0.5 to 0.7‰).

Cu isotope fractionation between the soil fractions may be explained by Cu binding to qualitatively different SOM. The quality of the SOM may vary among the soil fractions. Less degraded SOM, such as plant fragments and polysaccharides, is more common in the coarser fractions (silt), while more altered (oxidized) SOM is more common in the clay fractions.[26,27,55] Labile N-containing and aromatic compounds of microbial metabolites and highly altered organic matter (e.g., fulvic acid) may accumulate and can be stabilized in the clay fractions in the form of organo-mineral complexes.[56,57] In organic matter, Cu(II) is typically bonded to carboxyl and carbonyl O and amino N ligands.[58] Cu binding to N atoms is suggested to form less stable Cu-OM complexes compared to Cu-O bonds and the lower stability of the Cu-OM complexes may decrease the ^{65}Cu enrichment of the OM-complexed Cu.[16] Therefore, the higher relative abundance of N-groups and Cu-N interactions may explain the isotopically lighter Cu accumulation in the clay fractions compared to the silt fractions associated with larger amounts of polysaccharides and potential Cu-O binding.[57]

Overall, the clay minerals and SOM can both explain Cu isotope patterns in the studied vineyard soils. The two scenarios are probably the extreme models for explaining Cu behavior in these soils. However, as soils are mixtures of organic and mineral constituents, the most likely model of Cu fixation into the soil is situated in between the two scenarios. Isotopically lighter Cu suggests a more mineral-controlled Cu sorption in the clay fractions, while isotopically heavier Cu supports more Cu-OM formation in the silt fraction. In addition, mechanisms of Cu retention and distribution among the particle-size fractions may also determine the mobility of Cu from the vineyard soils.

4.2. Cu export from the vineyard catchment by surface runoff

The relatively small export of Cu by surface runoff (~1%) underscores the low mobility of anthropogenic Cu in the vineyard soils. The Cu export in runoff was mainly associated with SPM (56 to 93%), indicating that erosion was the major process mobilizing anthropogenic Cu during rainfall-runoff events from vineyard soils. The negative δ^{65}Cu of the SPM-bound Cu in runoff suggests that Cu was bound to the clay and fine clay fractions (Figure III-3). More precisely, the relationship between δ^{65}Cu and the molar ratios of Fe/Cu in the SPM (Figure III-5) suggests a mixing between the clay fractions at site 2 and the clay to silt fractions of the topsoil of the catchment or the topsoil at site 1 as endmembers. Hence, the mixing diagram (Figure III-5) indicates the contribution of the different sites within the catchment and the soil fractions to the SPM-bound Cu exported by runoff.

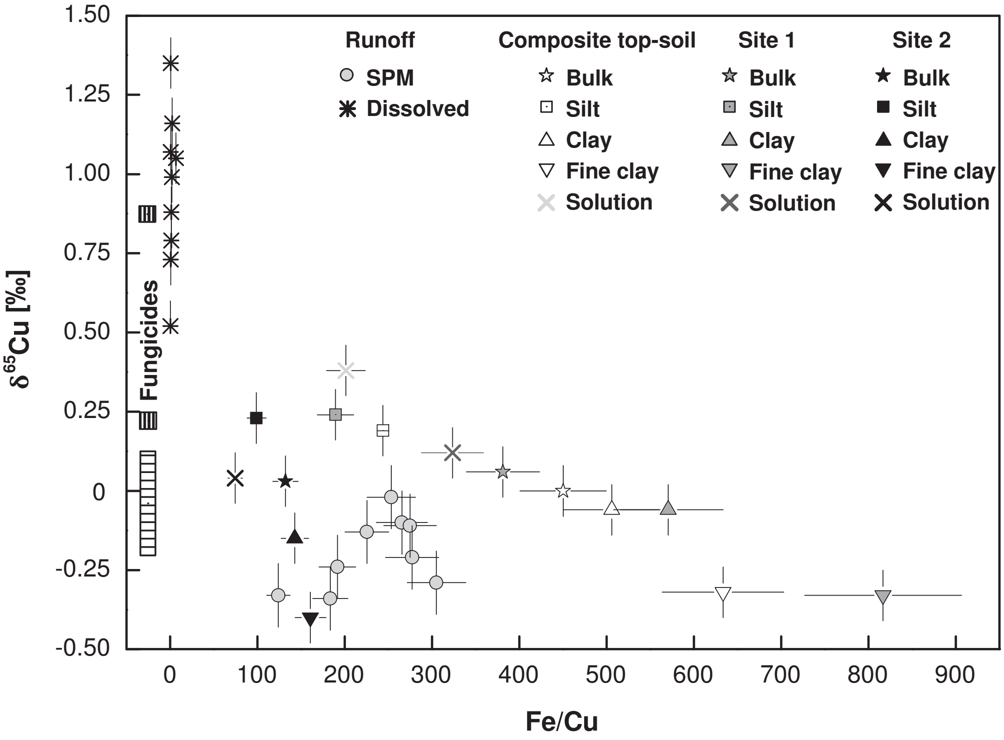


Figure III-5: *δ^{65}Cu values of the SPM-bound and dissolved Cu in runoff collected at the outlet of the catchment (Rouffach, France). The soil fractions of a composite top-soil and the surface soils at the two sites in weeded inter-rows are also displayed as a function of the Fe/Cu molar ratios. The vertical bars represent the range of δ^{65}Cu values of the Cu-fungicides applied in 2011 (horizontal hatching) and not applied in 2011 (vertical hatching). Note that the difference between site 1 and 2 is in the Cu concentrations of the soils (Fe concentrations are similar).*

The Al/Ca ratios can be used as an index for particle-size distribution in the SPM, as the silt contained high Ca (due to carbonates) and the clay particles were enriched in Al (due to clay minerals).[59] Al/Ca ratios indicate that SPM (0.5 to 2.0) in runoff consisted of a mixture of silt (0.2 to 0.4) and clay (2.2 to 2.8), while the contribution of the fine clay fraction remained low (4.1 to 4.9) (Figure III-6). These results contradict the isotopic data, showing that the δ^{65}Cu of the SPM was located between the δ^{65}Cu of fine clays and clays (Figure III-6). However, based on a mass balance calculation including the dissolved and SPM-bound Cu, the δ^{65}Cu of the total Cu in runoff varied from -0.16 to 0.21‰, which is in the range of the δ^{65}Cu of the clay and silt fractions (Table III-S4). This suggests that Cu isotope fractionation occurred in runoff during partitioning between the dissolved and SPM-bound Cu, resulting in the depletion of ^{65}Cu in the SPM with concomitant enrichment in ^{65}Cu in the dissolved phase.

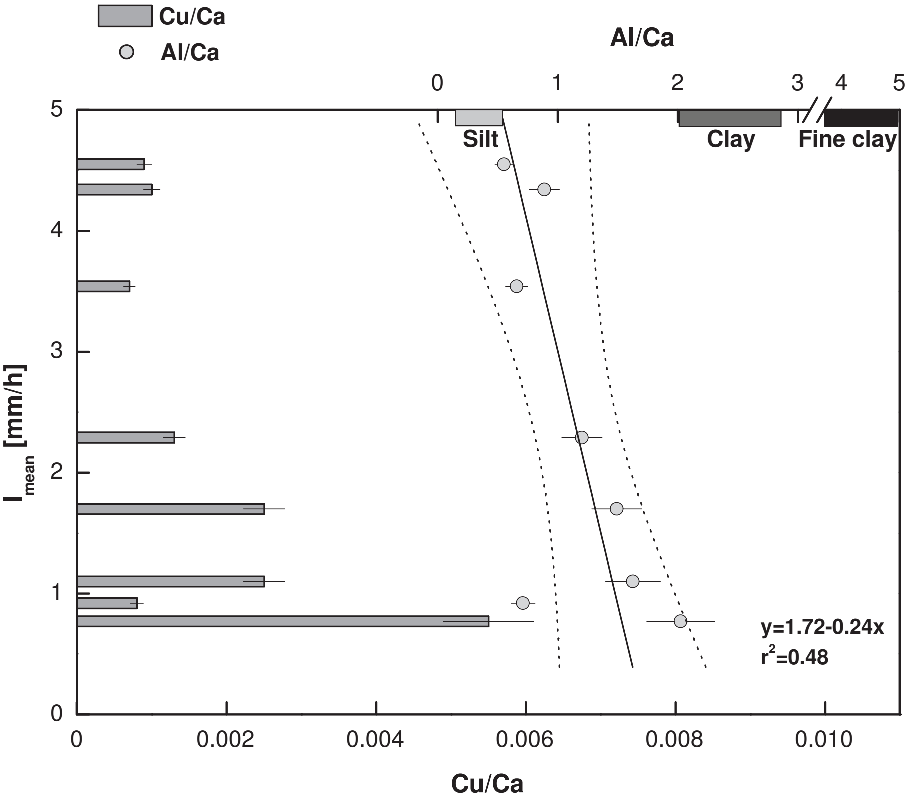


Figure III-6: *Weekly mean rainfall intensity as a function of the Ca normalized Cu concentrations and Al to Ca molar ratios of the SPM in runoff at the outlet of the vineyard catchment (Rouffach, France). Error bars correspond to the propagated analytical uncertainties.*

Several studies have shown that the dissolved phase of runoff was enriched in ^{65}Cu compared to the suspended solids in rivers due to the equilibrium partitioning of Cu isotopes between the organically complexed Cu and the Cu sorbed to the SPM phase.[4,32] In this study, the observed δ^{65}Cu values in the dissolved phase of runoff may result from one of two processes. The first process is the complexation of Cu(II) with organic ligands that strongly complex Cu, such as siderophores (excreted by plants or microorganisms).[32,60] The second possible process is the oxidation of Cu(I)oxides fungicides (Nordox, δ^{65}Cu: -0.21‰) which is expected to release isotopically heavier Cu(II) in solution as found in the case of Cu(I) sulfide re-oxidation.[61]

Cu isotope fractionation during Cu mobilization and transport in runoff is also supported by the absence of a correlation between the δ^{65}Cu of the applied Cu fungicides and that of the runoff-associated Cu (Figure III-3). This correlation would be expected because freshly applied Cu is known to be more mobile than older ("aged") Cu in the soil, as evidenced by larger weekly dissolved loads of Cu in runoff following Cu fungicide applications (Figure III-7). Isotope fractionation during fast partitioning of the applied Cu among the soil fractions following wash-off from vine leaves and mobilization of reacted Cu (i.e., Cu sorbed to the soil) during rainfall may explain the lack of correlation between the δ^{65}Cu values of the applied Cu and the dissolved Cu in runoff.

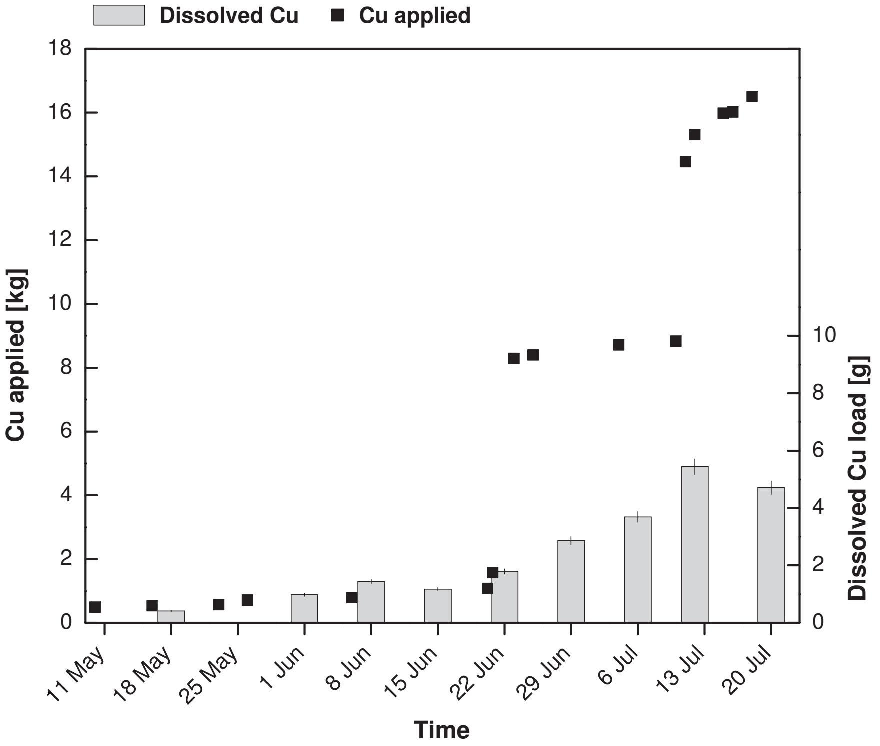


Figure III-7: *The relationship between Cu fungicide applications (cumulated) and weekly dissolved Cu loads in runoff collected at the outlet of the vineyard catchment (Rouffach, France).*

Moreover, rainfall characteristics may control Cu export from the vineyards and impact erosion processes responsible for Cu mobilization. The Cu/Ca ratios show that during intense rainfall events, along with Cu-enriched soil particles (such as clays), coarser materials containing less Cu (such as silt and sand) were also mobilized (Figure III-6). This mobilization resulted in a dilution effect of the coarser soil particles (i.e., the amount of Cu per unit weight of SPM decreases with larger particles) as their greater contribution to the Cu export decreased the total concentration of SPM-bound Cu in runoff. However, the coarser soil particles increased the load of exported SPM and thus SPM-bound Cu, but to a proportionally lower extent than if an equivalent mass of Cu-enriched clays was exported (Figure III-8). Intense rainfall events also correlated with concentrations of dissolved Cu in the runoff (r=0.96, p=0.003, *n*=7, without the June 8 sample) due to the contribution of the coarser fractions of the vineyard soils.

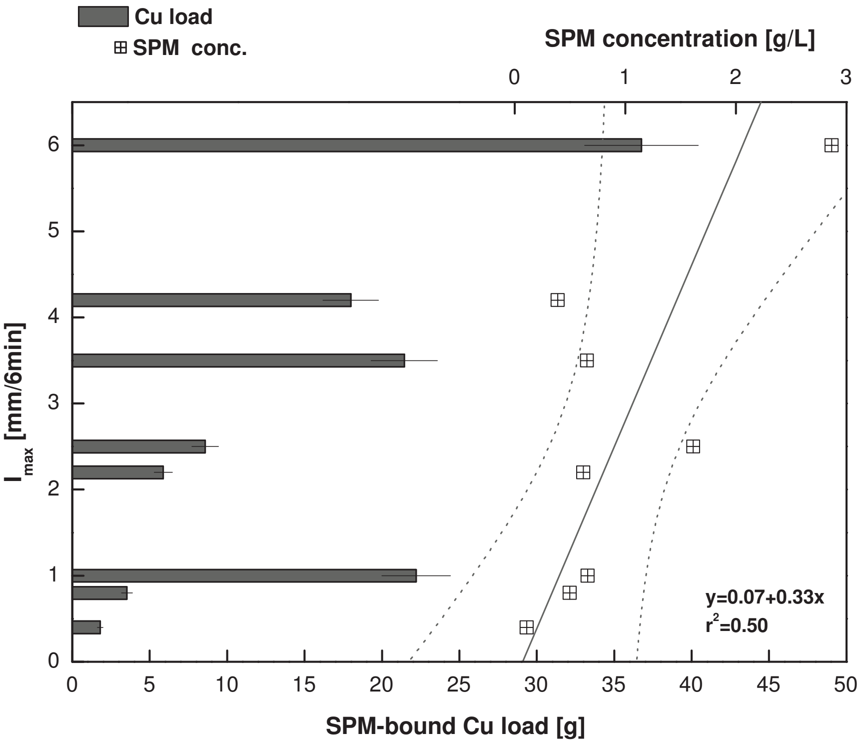


Figure III-8: *The export of SPM and SPM-bound Cu loads in runoff collected at the outlet of the vineyard catchment (Rouffach, France) as influenced by the rainfall intensity.*

5. Implications for evaluating Cu behavior at the catchment scale

The agricultural use of Cu is extensive (fertilizer, food additive, pesticide) because it is used for the protection of both plants (fruit and grape orchards)[62] and animals (livestock and poultry).[63] Successive Cu applications to agricultural soils, in some cases for many decades, may result in Cu accumulation in soils. Cu-contaminated runoff from agricultural catchments may then enter into aquatic ecosystems during rainfall-runoff events. In this context, stable isotopes can be used to investigate the sources, fate and sinks of metal contaminants in the environment, although metal stable isotopes are rarely used at the catchment scale to evaluate metal transport in relation to hydrological patterns. Here, the major Cu sources (Cu fungicides) and sinks (soils) in a vineyard catchment were investigated using Cu stable isotopes and related to rainfall-runoff controlling Cu export.

Our results highlight that the δ^{65}Cu in the bulk soil may not be useful in tracing Cu retention and transport if Cu fungicides signatures are in the same range than those of background Cu. In contrast, the δ^{65}Cu in particle-size fractions of vineyard surface soils revealed that the retention processes of Cu fungicide implies a heterogeneous distribution of Cu among soil fractions. Cu seems to be mainly transported by clay in runoff, although coarser soil particles may be also contributive during intense rainfall events. The δ^{65}Cu in the soil fractions suggest that clay minerals and OM govern the Cu isotope distribution in the vineyard soils.

Following Cu-fungicide application, Cu reaching the soil may rapidly react with soil constituents, as the δ^{65}Cu in runoff could not be directly related to the fungicide treatments. Our field study also suggests that the isotopically distinct pools found in the surface waters are formed in the soils during Cu distribution between the soil constituents and Cu mobilization by rainfall. Indeed, dissolved Cu in rivers has been found to be isotopically heavier, while SPM contains isotopically lighter Cu.[32] This also underscores the relevance of processes occurring at the agricultural catchment scale that contribute to the Cu load transported in rivers for interpreting the δ^{65}Cu in hydrological systems. In the future, the isotope fractionation caused by individual processes influencing Cu partitioning in soils, especially those driving the long-term Cu transformation,[51,54] will be needed to constrain field observations of Cu behavior at the catchment scale.

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SUPPORTING INFORMATION

							%										mg kg^{-1}				
	Depth [cm]	%OM (LOI)[a]	%Corg	%OM (WBC)	%$CaCO_3$	pH_{H2O}	SiO_2	Al_2O_3	MgO	CaO	Fe_2O_3	MnO	TiO_2	Na_2O	K_2O	P_2O_5	S	Cu	Ni	Zn	EF (Cu/Fe)
Site 1, soil under grass	0-18	6.1	na	na	31.6	7.80	47.7	8.16	1.18	15.5	3.47	0.09	0.51	0.44	1.80	0.29	723	39.8	21.9	74.9	4.6
	36-54	4.5	0.626	1.251	34.7	7.80	48.2	8.66	1.34	17.5	3.77	0.10	0.54	<dl	2.26	0.26	1126	28.9	27.9	64.3	3.1
Site 1, soil under weed inter-row	0-10	6.6	0.975	1.949	30.5	7.60	46.9	8.30	1.37	15.7	3.56	0.10	0.52	0.37	2.39	0.31	753	74.2	33.6	99.7	8.4
	10-31	4.2	0.965	1.929	26.3	8.15	48.6	8.95	1.32	16.7	3.81	0.10	0.54	0.71	1.96	0.21	743	72.5	45.0	111.2	7.6
	31-52	4.7	0.740	1.480	32.2	7.70	47.6	9.08	1.51	16.2	3.85	0.09	0.56	0.43	2.38	0.26	750	27.2	36.6	66.2	2.8
	52-73	3.5	0.567	1.134	33.2	8.24	40.7	9.65	1.49	19.7	3.95	0.08	0.53	0.44	2.10	0.16	na	12.0	29.0	49.0	1.2
	73-94	4.0	0.549	1.097	41.1	7.80	36.3	10.13	1.63	23.0	3.75	0.06	0.53	0.15	2.30	0.15	1336	14.2	17.5	42.4	1.5
	94-115	3.1	0.346	0.692	41.9	8.23	32.5	9.85	1.94	24.3	3.37	0.05	0.52	<dl	3.00	0.17	997	16.6	30.5	45.9	2.0
	115-136	3.0	0.221	0.442	48.5	na	28.3	9.26	1.70	27.1	3.78	0.06	0.46	<dl	2.10	0.13	na	14.0	35.0	43.0	1.5
	136-157	3.1	0.199	0.399	51.2	7.80	28.6	9.59	1.73	27.4	3.67	0.06	0.47	<dl	2.34	0.13	793	11.5	35.2	45.9	1.3
Site 2, soil under grass	0-18	5.0	0.822	1.645	38.7	7.80	37.5	6.83	1.48	22.1	3.87	0.07	0.38	0.37	1.81	0.46	1427	175.3	18.5	77.4	18.2
	30-42	4.0	0.578	1.157	39.0	8.00	37.7	6.86	1.70	22.7	3.80	0.07	0.37	0.43	2.45	0.36	664	129.1	46.2	70.3	13.6
Site 2, soil under weeded inter-row	0-21	4.8	0.917	1.834	40.7	8.00	40.3	7.34	1.74	20.4	3.83	0.08	0.42	0.59	2.58	0.47	690	230.7	35.6	93.8	24.2
	21-42	3.7	0.549	1.098	40.1	8.10	41.4	8.29	1.63	20.4	4.30	0.08	0.45	0.40	2.09	0.36	656	150.6	29.9	78.1	14.1
	42-63	3.1	0.322	0.645	47.4	8.10	36.4	7.26	1.89	23.5	4.20	0.06	0.41	0.05	2.38	0.23	917	43.5	23.6	56.3	4.2
	63-85	2.5	0.245	0.490	32.5	na	26.2	5.21	1.29	33.6	3.32	0.05	0.28	0.13	1.24	0.15	1420	29.1	40.2	56.1	3.5
	85-98	2.2	na	na	41.7	na	30.7	6.74	1.45	28.4	3.97	0.06	0.36	<dl	1.57	0.18	na	36.0	28.0	49.0	3.6
	98-105	na	na	na	na	na	23.4	4.60	1.56	33.9	2.99	0.05	0.24	<dl	1.08	0.16	1110	32.1	20.4	43.1	4.3
Composite top-soil April 6	0-5	na	na	na	na	na	43.0	7.07	1.59	20.0	3.67	0.09	0.44	0.63	1.63	0.33	na	104.5	36.0	95.3	11.4
Composite top-soil May 11	0-5	na	na	na	na	na	42.4	7.40	1.64	20.9	3.82	0.11	0.46	0.66	1.71	0.38	na	105.8	38.0	98.9	11.1
Composite top-soil June 8	0-5	4.8	0.837	1.674	32.2	8.03	46.9	7.99	1.53	17.7	4.08	0.10	0.49	0.60	1.80	0.35	na	72.1	41.0	103.6	7.1
Reference soil	67-77	na	na	na	na	na	38.4	6.90	0.73	25.1	4.62	0.06	0.41	0.05	1.56	0.13	886	11.5	39.0	50.7	1.0

Table III-S1: *The physico-chemical characteristics of the soil depth profiles in the studied vineyard catchment, of the composite top-soils and of the reference soil (Rouffach, Alsace, France) and Cu enrichment factors (EF).*

[a] *Note that the higher organic matter (OM) values quantified by loss on ignition (LOI)[1] compared to the Walkley and Black C_{org} (organic carbon) can be due to the partial oxidation of carbonates and dehydroxylation of clay minerals during the LOI process.[2] na: non-analyzed;<dl: non-detected*

Particle-size separation protocol (modified from the protocol of Genrich and Bremner, 1974)[3]

The soil suspension was vibrated for 30 min using a probe type sonicator (Vibra-Cell™ VC505, Sonics & Materials Inc., Newtown, CT, USA) adjusted to 55 W and delivering ~100 000 J to the samples (~500 J mL^{-1}) to disperse soil aggregates without destroying organo-mineral complexes.[4] The soil suspension was maintained <20°C in an ice bath during the sonication. The sand fraction was wet sieved (63 µm) and carefully rinsed with 18.2 MΩ water. The silt-clay fractions were successively separated 9 times by centrifugation (Hearus Megafuge 40R, Thermo Scientific, Waltham, MA, USA), and the supernatant was re-centrifuged (4000 rpm, 35 min) to collect clay particles. The supernatant of the second centrifugation step was filtered using a 0.45 µm (cellulose acetate membranes) filter to separate the "solution" from the "fine clay fraction" that could not be separated by centrifugation. The four fractions were then dried at 60°C, weighed and checked for recovery (≥91%). The dried clay particles were re-powdered with an agate mortar prior to further treatments and analyses.

Product name	**Cu form**	**δ^{65}Cu [‰]**	**Applied in 2011**
Champ flo	$Cu(OH)_2$	0.22	no
Kocide	$Cu(OH)_2$	0.91	no
Cursor	$3Cu(OH)_2 \cdot CuCl_2$	0.22	no
Bordeaux mixture	$CuSO_4 + Ca(OH)_2$	-0.06	yes
Nordox	Cu_2O	-0.21	yes
Syphal	$3Cu(OH)_2 \cdot CuCl_2$	0.11	yes
Cuprofix	$CuSO_4$	-0.07	yes

Table III-S2: *The δ^{65}Cu of the measured Cu-based fungicides applied in Rouffach during the vine-growing season in 2011 or earlier.*

Date	Rainfall depth [mm]	Rainfall duration [mn]	Mean intensity [mm h^{-1}]	Max. intensity [mm $6mn^{-1}$]	Quiescent period [d]	Mean runoff discharge [L s^{-1}]	Max. runoff discharge [L s^{-1}]	Min. time between application and runoff [d]
May 18	12.7	420	1.70	0.8	3.89	2.60	4.64	3
June 1	16.3	636	-	-	8.83	1.51	2.50	5
June 8	8.0	744	0.77	0.4	6.71	1.41	2.56	1
June 15	10.8	318	3.54	2.5	6.18	3.55	9.61	8
June 22	15.3	672	4.34	2.2	7.10	2.78	15.67	15
June 29	29.7	384	4.55	6.0	6.53	8.90	34.58	1
July 6	9.7	582	0.92	1.0	7.46	6.70	44.69	4
July 12	28.5	798	2.29	3.5	4.34	2.54	18.58	0
July 20	26.3	1668	1.10	4.2	5.63	1.80	18.36	0

Table III-S3: *Rainfall data monitored by the meteorological station situated at the study catchment (Rouffach, Alsace, France) and hydrological measurements at the outlet of the catchment.*

		SPM [%]						**SPM [mg kg^{-1}]**												
Date	**Dissolved Cu [µg L^{-1}]**	**Al**	**Mg**	**Ca**	**Fe**	**Mn**	**Ti**	**Na**	**K**	**P**	**Cu**	**Zn**	**S**	**EF in SPM (Cu/Fe)**	**% dissolved Cu in runoff**	**log Kd**	**δ^{65}Cu dissolved**	**δ^{65}Cu in SPM**	**δ^{65}Cu of total Cu in runoff**	**$\Delta^{65}Cu_{SPM-dissolved}$**
May 18	7.7	3.41	0.69	3.4	2.27	0.039	0.185	0.22	0.54	0.157	135	279	1844	21	10	4.2	1.05	-0.24	-0.11	-1.29
June 1	14.8	3.09	0.60	6.4	2.09	0.044	0.211	0.26	0.27	0.123	90	165	1142	15	23	3.8	1.16	-0.10	0.19	-1.26
June 8	32.0	5.71	1.02	4.2	3.99	0.092	0.273	0.25	1.70	0.319	366	455	2969	33	44	4.1	0.88	-0.33	0.21	-1.21
June 15	22.6	3.97	0.85	9.0	2.76	0.073	0.244	0.33	1.14	0.138	103	182	1357	13	12	3.7	0.79	-0.29	-0.16	-1.08
June 22	22.9	4.36	0.84	7.3	2.93	0.070	0.243	0.28	0.39	0.149	121	166	1180	15	23	3.7	1.07	-0.11	0.17	-1.18
June 29	30.8	3.72	0.92	10.0	2.73	0.077	0.279	0.43	0.15	0.140	138	144	1087	18	7	3.7	0.73	-0.13	-0.07	-0.86
July 6	13.8	4.74	0.96	9.9	3.07	0.087	0.297	0.47	0.59	0.162	126	166	1251	15	14	4.0	0.99	-0.21	-0.04	-1.20
July 12	24.2	5.62	0.84	7.0	3.25	0.082	0.270	0.33	1.51	0.178	146	152	1294	16	20	3.8	0.52	-0.02	0.09	-0.54
July 20	25.3	6.83	0.98	6.2	4.01	0.089	0.288	0.29	1.99	0.228	249	232	1755	22	21	4.0	1.35	-0.34	0.01	-1.69

Table III-S4: *Chemical composition of SPM, Cu concentrations and δ^{65}Cu of runoff-associated Cu phases collected at the outlet of the vineyard catchment (Rouffach, France).*

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IV. Chapter: Copper Stable Isotopes to Trace Copper Behavior in Wetland Systems

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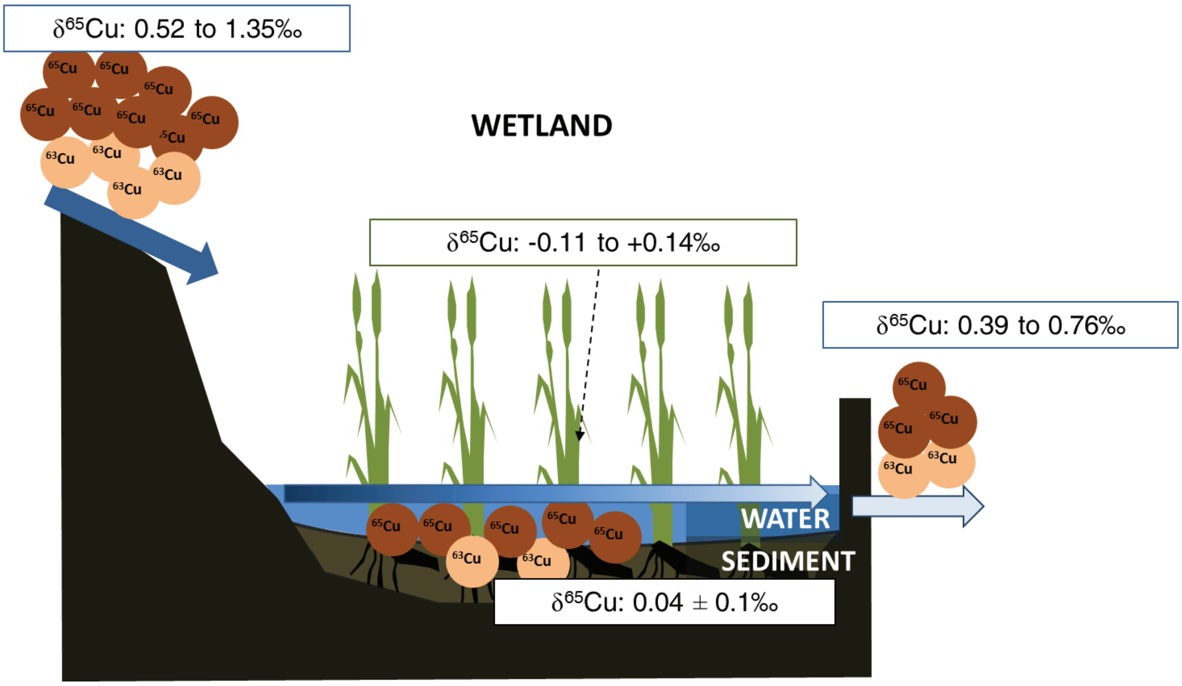
In Chapter III we have shown that approximately 1% of Cu applied as fungicide preparations in the vineyards was transported downstream by runoff. In this chapter we evaluated the functioning of a stormwater wetland collecting in collecting and retaining the Cu-contaminated runoff from the vineyards during the fungicide application period (May – July). Cu stable isotope ratios, hydrochemical and geochemical analyses were combined with a mass balance approach to evaluate the transport and transformation of dissolved and particulate Cu in the wetland, under the variable hydrological and redox conditions occurring.

GRAPHICAL ABSTRACT



ABSTRACT

Wetlands are reactive zones of the landscape that can sequester metals released by industrial and agricultural activities. Copper (Cu) stable isotope ratios (δ^{65}Cu) have recently been used as tracers of transport and transformation processes in polluted environments. Here, we used Cu stable isotopes to trace the behavior of Cu in a stormwater wetland receiving runoff from a vineyard catchment (Alsace, France). The Cu loads and stable isotope ratios were determined in the dissolved phase, suspended particulate matter (SPM), wetland sediments and vegetation. The wetland retained >68% of the dissolved Cu and >92% of the SPM-bound Cu, which represented 84.4% of the total Cu in the runoff. The dissolved Cu became depleted in ^{65}Cu when passing through the wetland ($\Delta^{65}\text{Cu}_{\text{inlet-outlet}}$ from 0.03‰ to 0.77‰), which reflects Cu adsorption to aluminum minerals and organic matter. The δ^{65}Cu values varied little in the wetland sediments (0.04 ± 0.10‰), which stored >96% of the total Cu mass within the wetland. During high-flow conditions, the Cu flowing out of the wetland became isotopically lighter, indicating the mobilization of reduced Cu(I) species from the sediments and Cu reduction within the sediments. Our results demonstrate that the Cu stable isotope ratios may help trace Cu behavior in redox-dynamic environments such as wetlands.

1. Introduction

Copper (Cu) is produced in large quantities for diverse applications, including the use of Cu-based fungicides in agriculture, and can be released into the environment. Vineyards worldwide are Cu-contaminated agroecosystems because large quantities of Cu are applied yearly (up to 6 kg ha^{-1} in the European Union[1]) resulting in topsoil Cu concentrations in vineyards of 15 to 3200 mg kg^{-1} [2]. Large amounts of Cu are removed from agricultural land by runoff, and this Cu may eventually contaminate aquatic ecosystems[2]. Wetland systems are dynamic interfaces between the geosphere and the biosphere that provide important ecosystem service functions (i.e., natural benefits to households, communities, and economies[3]) through their ability to sequester metals released during industrial or agricultural activities[4]. Wetland systems can intercept Cu-contaminated runoff from agricultural land or urban areas before Cu fluxes enter into aquatic ecosystems[5]. However, quantitative knowledge of the hydrological and biogeochemical processes governing the transport of Cu in wetlands that receive metal-contaminated runoff remains scarce.

The fate of Cu in wetlands is governed by the interplay between physical, physico-chemical and biological processes[6]. Cu retention is mainly controlled by sedimentation, adsorption to organic matter or mineral phases, (co-)precipitation as minerals and plant uptake[4,7]. Depending on the pH and interacting compounds, the Cu is found as hydroxo-, (hydrogen)carbonato-, sulfato-, chloro- and organo-complexes in freshwaters[8]. The dissolved Cu may strongly bind to oxyhydroxides or adsorb onto clay minerals and organic matter, and Cu is therefore predominantly transported in association with suspended particulate matter (SPM) in runoff[9]. The reduced Cu(I)-species (Cu_xS_y, Cu(I)-S_{org}) and metallic Cu(0) are formed under reducing conditions in wetland sediments[10-12]. Under oxidizing conditions, reduced sulfur species can be partially re-oxidized with the concomitant release of dissolved Cu[7]. In addition to traditional approaches, stable isotopes of transition metals (e.g., Fe, Cu, Zn, Mo and Cr) have recently been used[13-16] to evaluate the sources, transport and cycling processes of metals in redox-dynamic environments.

The stable isotopes of Cu ($^{65}Cu/^{63}Cu$) are potentially powerful geochemical tracers for transport and transformation processes of anthropogenic Cu[17]. Cu isotope fractionation has been observed during Cu adsorption onto minerals[18], complexation with organic matter[19], oxidation-reduction[20] and Cu-uptake by plants[21] or microorganisms[22]. Stable Cu isotope analysis has been utilized to trace Cu behavior in living organisms[21,22], soils[23,24], rivers[25] and sediments[26,27]. Recently, the natural and anthropogenic sources of Cu were evaluated in a flash flood event from a vineyard catchment using a combination of sequential extraction and Cu isotope analysis[24]. However, Cu isotope fractionation during metal retention processes by wetlands has not yet been studied. Research into the partitioning of stable Cu isotopes in wetland systems with respect to hydrological and biogeochemical conditions might aid understanding of Cu behavior and transport in natural redox-dynamic environments.

In this study, we used Cu stable isotopes to investigate Cu behavior in a stormwater wetland that collected Cu-contaminated runoff from a vineyard catchment (Rouffach, Alsace, France). The wetland provides a natural laboratory for studying the $^{65}Cu/^{63}Cu$ fractionation associated with Cu behavior in a redox-dynamic environment under field conditions. Our objectives were i) to evaluate the retention and distribution of Cu among wetland compartments (i.e., in the water, the SPM, the bed sediments and the vegetation) during the application period of Cu-based fungicides (May to July) and ii) to characterize $\delta^{65}Cu$ isotopic values in wetland compartments and to trace the behavior of Cu with respect to the prevailing hydrological and biogeochemical processes.

2. Materials and methods

2.1. Study wetland

The artificial stormwater wetland (constructed in 2002) is situated at the outlet of the 42.7 ha Hohrain vineyard catchment (Rouffach, Alsace, France; 47°57’9 N, 07°17’3 E)[28]. Our study was performed between 4 May and 20 July, 2011, during the period of use of Cu-based fungicides. The amount of Cu-based fungicides applied to the catchment was estimated based on yearly surveys addressed to the winegrowers. From May to August, 2.2 ± 0.3 kg ha^{-1} y^{-1} (2008-2011) of Cu-fungicides were applied in the forms of Cu-sulfate (Bordeaux mixture, $CuSO_4$ + $Ca(OH)_2$), Cu-oxide (Cu_2O), Cu-hydroxide ($Cu(OH)_2$) and Cu-oxychloride ($3Cu(OH)_2\cdot CuCl_2$) (Figure IV-1A). The rainfall between May and July averaged 187 ± 64 mm (1998-2011), and the total rainfall during the study period in 2011 was 214 mm. The rainfall-runoff events do not generate a permanent stream in the catchment.

During rainfall events, the surface runoff converges at the outlet of the catchment and is collected by a vegetated stormwater wetland (Figure IV-2)[29,30]. The stormwater wetland has a surface area of 319 m^2 and a total volume of 1500 m^3. It is composed of a sediment deposition forebay (215 m^2) and a gravel filter (104 m^2) that enhances the hydraulic residence time. The sediments and the vegetation of the stormwater wetland were removed in February 2009 as part of the local maintenance scheme. The wetland vegetation covered 80% of the forebay area, and *Phragmites australis* (Cav.) Steud. represented 90% of the total vegetation cover during the investigation period. The water depth in the forebay varied from 0.2 to 0.7 m during the investigation period, depending on the runoff volume.

2.2. Sample collection

The water discharges entering and leaving the wetland were continuously monitored using bubbler flow modules (Hydrologic, Canada) combined with a Venturi channel at the wetland inlet and a V-notch weir at the outlet. Flow-proportional water samples were collected at the inlet using a 4010 Hydrologic automatic sampler (Hydrologic, Canada) and at the outlet using a 6712FR Teledyne Isco automatic sampler (Isco Inc., Lincoln, NE, USA)[29]. The flow-proportional water samples were collected during each runoff event (300 mL every 3 m^3) and were combined weekly into a single composite sample to establish weekly mass balances. The water samples could not be collected on May 25, on May 11 at the inlet and on June 1 at the outlet.

A grid-cell sampling was applied by dividing the forebay area into four equal rectangular cells (9 × 6 m) to collect Cu in the various wetland compartments. Samples of the water, bed sediments and vegetation were collected on 6 April, 11 May and 8 June at the center of each cell and separately pooled to obtain a composite sample per sample type. The vegetal biomass was determined based on the estimates of the vegetation cover and the plant density in the wetland forebay. The physico-chemical properties (T in °C, pH and dissolved oxygen) of the wetland water were measured in situ using WTW multi-parameter 350i portable sensors (WTW, Weilheim, Germany). The top 10 cm of the bed sediments were collected in each wetland cell using a coring tube. The chemical and textural characteristics of the bed sediments are described in the supporting information (Table IV-S1). X-ray diffraction analysis revealed that the wetland sediments were mainly composed of quartz, calcite, feldspars, dolomite and clay minerals (data not shown).

2.3. Sample preparation and analysis

The water samples were filtered using 0.45 μm cellulose acetate membranes (Millipore, Billerica, MA, USA). The obtained SPM was dried at 60°C. The organic/inorganic carbon, alkalinity, conductivity, anions and major and trace elements were quantified in the collected samples as described elsewhere[31], using the standards SLRS-4 (National Research Council, Ottawa, Ontario, Canada) and TM-24.3 (Environment Canada, Department of the Government of Canada, Gatineau, Quebec, Canada) for quality control. The SPM collected on 20 July was analyzed by scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) (Tescan Vega 2 with Edax Pegasus, SEAL Laboratories, El Segundo, CA, USA). The dried bed sediment samples were powdered using an agate disk mill (<60 μm) prior to alkaline fusion and chemical composition analysis[32]. The geological standards BCR-2 (US Geological Survey (USGS), Reston, VA, USA) and CRM-7003 (Analytika, Praha, Czech Republic) were used to quantify the Cu recovery, which was always >97%. The plant samples were separated into below-ground (rhizome and roots) and above-ground (stem and leaves) subsamples. The plant samples were washed for 5 min with 18.2 MΩ water (Millipore), dried at 60°C and powdered with an agate disk mill prior to acid digestion. The Cu recovery (>99%) from the digested plants was quantified using the spinach leaves standard (National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1570a).

2.4. Cu isotope analysis

The water samples were concentrated by evaporation to yield 200 ng of Cu. For the Cu isotope analysis, 50 mg of sediment and 200 mg of plant samples were prepared. The SPM samples were digested on filter membranes. All of the samples were dissolved on a hotplate using HF-HNO_3 acid (v:v 4:1) at 70°C, $HClO_4$ acid at 150°C and a mixture of HCl and H_3BO_3 acids at 110°C successively.

A protocol for double Cu purification was adapted from Maréchal et al.[33] for each sample type (see Table IV-S2 for the detailed protocol). Samples with a recovery rate lower than 90% were discarded to preclude isotopic fractionation during ion exchange chromatography[34]. High ratios (>10,000) of Ca and Mg to Cu in the outlet water samples resulted in Cu recoveries <90%. Therefore, the Cu purification was successful for only four out of the nine water samples collected at the wetland outlet. The total procedural blank (sample digestion and purification) for Cu was $\leq$2 ng (n=3) for liquid samples ($\leq$1% of Cu in samples) and 5.3 ± 4.1 ng ($\bar{x}$ ± 2s, n=7) for solid samples, including the Cu in the filter membranes (2.5 ng). The latter value was generally less than 1% of the Cu in the SPM (ranging from 400 ng to 126 µg) or in the vegetation (800 ng to 15 µg).

Cu isotope ratios were measured with a Finnigan-Neptune MC-ICP-MS (Thermo Scientific, Waltham, MA, USA) using the geological standards CRM-7003 and BCR-2 for quality control and a Ni standard (NIST SRM 986) for correction of instrumental mass biases during measurements of the $^{65}Cu/^{63}Cu$ ratios[35]. The measurement uncertainty for the entire procedure (digestion, purification and isotope analysis) was $\leq$0.10‰ (n=3, 2s), which was adopted for all of the measured Cu isotope values. The Cu isotope ratios were expressed as $\delta^{65}Cu$ [‰] values relative to the NIST 976 isotope reference material (see the SI).

2.5. Data analysis and modeling

The retention by the wetland of an element (X) in the dissolved phase ($\mathrm{Ret}_{X,t}$) was calculated based on the inlet-outlet mass balance according to Eq. 1:

$$\mathrm{Ret}_{\mathrm{X,t}}[\%] = \frac{\mathrm{V}_{\mathrm{In,t}} \times \mathrm{Conc}^{\mathrm{X}}_{\mathrm{In,t}} - \mathrm{V}_{\mathrm{Out,t}} \times \mathrm{Conc}^{\mathrm{X}}_{\mathrm{Out,t}}}{\mathrm{V}_{\mathrm{In,t}} \times \mathrm{Conc}^{\mathrm{X}}_{\mathrm{In,t}}} \times 100 \qquad (1)$$

where Conc^{X} is the concentration of the dissolved species X, and V is the water volume at the inlet (In) and the outlet (Out) of the wetland during the week t.

The apparent Cu isotope fractionation ($\Delta^{65}\mathrm{Cu}_{(\mathrm{Out,t\text{-}Retained,t})}$) between the outflowing residual dissolved Cu ($\delta^{65}\mathrm{Cu}_{\mathrm{Out,t}}$) and the dissolved Cu load retained by the wetland ($\delta^{65}\mathrm{Cu}_{\mathrm{Retained,t}}$) was calculated using Eq. 2 [36]:

$$\delta^{65}\mathrm{Cu}_{\mathrm{Out,t}} = \delta^{65}\mathrm{Cu}_{\mathrm{In,t}} + \Delta^{65}\mathrm{Cu}_{(\mathrm{Out,t-Retained,t})} \times \mathrm{f}_{\mathrm{Retained,t}} \qquad (2)$$

where $\delta^{65}\mathrm{Cu}_{\mathrm{In,t}}$ is the Cu isotope composition in the dissolved load at the inlet, and $\mathrm{f}_{\mathrm{Retained,t}}$ denotes the fraction of the dissolved Cu load retained in the wetland during the week t as calculated in Eq. 1. Because $\delta^{65}\mathrm{Cu}_{\mathrm{Out,t}} \approx \delta^{65}\mathrm{Cu}_{\mathrm{Wetland,t}}$ in our case, the Cu isotope ratios of the water samples collected in the wetland forebay were used for all of the calculations.

The weekly retention of Cu in the SPM was calculated according to Eq. 3:

$$\mathrm{Ret}_{\mathrm{SPM,Cu,t}}[\%] = \frac{\mathrm{M}^{\mathrm{SPM}}_{\mathrm{In,t}} \times \mathrm{Conc}^{\mathrm{Cu}}_{\mathrm{SPM,In,t}} - \mathrm{M}^{\mathrm{SPM}}_{\mathrm{Out,t}} \times \mathrm{Conc}^{\mathrm{Cu}}_{\mathrm{SPM,Out,t}}}{\mathrm{M}^{\mathrm{SPM}}_{\mathrm{In,t}} \times \mathrm{Conc}^{\mathrm{Cu}}_{\mathrm{SPM,In,t}}} \times 100 \qquad (3)$$

where $\mathrm{M}^{\mathrm{SPM}}_{\mathrm{t}}$ stands for the SPM mass [kg dry weight (d.w.)], and $\mathrm{Conc}^{\mathrm{Cu}}_{\mathrm{SPM,t}}$ represents the SPM-bound Cu concentration [mg Cu kg^{-1} d.w.] at the inlet (In) and the outlet (Out) of the wetland for week t.

The hydrological and hydrochemical variables were compared using the paired non-parametric Wilcoxon signed rank and the Spearman rank correlation tests (with a significance level of $p \leq 0.05$) using R (version 2.14.2). The speciation modeling of Cu was performed using Visual Minteq (version 3.0, compiled by J.P. Gustafsson, KTH, Stockholm). The implemented Nica-Donnan and Stockholm Humic (SH) models were used to consider the Cu-dissolved organic carbon (DOC) interactions (see Table IV-S3 for the detailed Visual Minteq modeling).

3. Results and discussion

3.1. Hydro-biogeochemical conditions and Cu retention by the wetland

During the investigation period, 49 rainfall events generated a total of 33 runoff events in the upstream vineyard catchment. The runoff entering the wetland ranged from 2.5 to 195.4 m^3 representing a total volume of 1448 m^3 (Figure IV-1B and Table IV-S4 in SI).

Cu fungicides were used on the vineyard catchment from 4 May to 1 August (Figure IV-1A). During the investigation period, 150 g of Cu (dissolved Cu and Cu in the SPM) entered into the wetland via runoff. The Cu in the SPM represented 83% of the entering Cu load, which is in agreement with previous studies[37]. Both the dissolved and the SPM-bound Cu fractions were efficiently retained by the wetland throughout the investigation period (Figure IV-1C and Table IV-1). The weekly dissolved Cu concentrations decreased from the inlet ($\bar{x} \pm 2s$: 21.6 ± 16.0; range: 7.7 - 32.0 µg L^{-1}) to the outlet (4.2 ± 7.1; range: 1.1 - 10.6 µg L^{-1}) (Table IV-2). The dissolved Cu concentrations in the wetland ranged from 1.3 to 12.3 µg L^{-1} and did not differ from the concentrations observed at the outlet ($p>0.05$), which indicates retention of dissolved Cu in the wetland forebay. The weekly retention rates of dissolved Cu ($Ret_{Cu,t}$) ranged from 68 to 95% (88 ± 18%), and the retention rates tended to be lower (≤85%) during high-flow conditions (runoff discharges >18 L s^{-1} versus the average discharge of 4 L s^{-1}). The retention by the wetland of the SPM ($Ret_{SPM,Cu,t}$) ranged from 94.4% to 99.7% (97.5 ± 3.8%) and was similar to the retention of particulate Cu (97.9 ± 3.2%). This result emphasizes the high sediment-trapping efficiency of stormwater wetlands. The concentrations of Cu in the SPM entering the wetland tended to be higher (164 ± 177 mg kg^{-1}) than the concentrations in the SPM leaving the wetland (126 ± 100 mg kg^{-1}), except for the weeks of 29 June and 6 July (Figure IV-3A, Table IV-S5). The Cu concentrations increased from April to July in the wetland sediments (Table IV-S1). The Cu stock in the bed sediments, estimated based on Cu concentrations in the top 10 cm and its apparent density, was 971 ± 494 g (Table IV-S1 and Figure IV-S1), which reflected the Cu accumulation over 2.5 years. The estimated contribution of Cu in the direct rainfall to the total Cu mass balance in the wetland was 225 mg[38], which represents less than 1% of the dissolved Cu load entering the wetland by runoff.

	Dissolved Cu								**Cu in SPM**			
Date of sampling	**δ^{65}Cu inlet [‰]**	**Inlet Cu load [g]**	**δ^{65}Cu wetland [‰]**	**δ^{65}Cu outlet [‰]**	**Outlet Cu load [g]**	**Retained Cu [g]**	**Retention rate [%]**	**Δ^{65}Cu (Retained – Outflowing dissolved)**	**δ^{65}Cu inlet [‰]**	**δ^{65}Cu wetland [‰]**	**δ^{65}Cu outlet [‰]**	**Retention of Cu in SPM [%]**
May 11[1]	n.a.	n.a.	0.76	0.50	0.12	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
May 18	1.05	0.41	0.54	n.a.	0.09	0.32	78.88	0.65	-0.24	-0.20	-0.11	98.42
June 1[2]	1.16	0.98	0.61	n.a.	0.10	0.88	89.65	0.61	-0.10	n.a.	n.a.	n.a.
June 8	0.88	1.44	0.53	n.a.	0.11	1.33	92.31	0.38	-0.33	n.a.	n.a.	99.99
June 15	0.79	1.17	0.76	n.a.	0.11	1.06	90.70	0.03	-0.29	n.a.	-0.23	96.71
June 22	1.07	1.79	0.58	n.a.	0.18	1.61	89.82	0.55	-0.11	-0.53	n.a.	95.29
June 29	0.73	2.86	0.51	0.43	0.61	2.25	78.54	0.28	-0.13	n.a.	-0.51	97.61
July 6	0.99	3.69	0.39	0.23	1.19	2.50	67.76	0.89	-0.21	n.a.	-0.88	96.90
July 12	0.52	5.44	0.55	0.36	0.79	4.64	85.40	-0.04	-0.02	-0.46	-0.77	98.56
July 20	1.35	4.71	0.49	n.a.	0.21	4.50	95.44	0.90	-0.34	-0.55	n.a.	99.92

Table IV-1 : *δ^{65}Cu values and Cu loads in the dissolved phase (<0.45 µm) and the SPM. Fractionation values between the outflowing residual dissolved Cu and the retained Cu were calculated using eq 3. Analytical uncertainty for δ^{65}Cu values is ± 0.10‰ (n=3).*

[1] *No sample at the inlet was collected.*

[2] *Cu retention was calculated using Cu concentration in the water from the forebay. No water sample at the wetland outlet was collected for this date.*

n.a.: not assessed

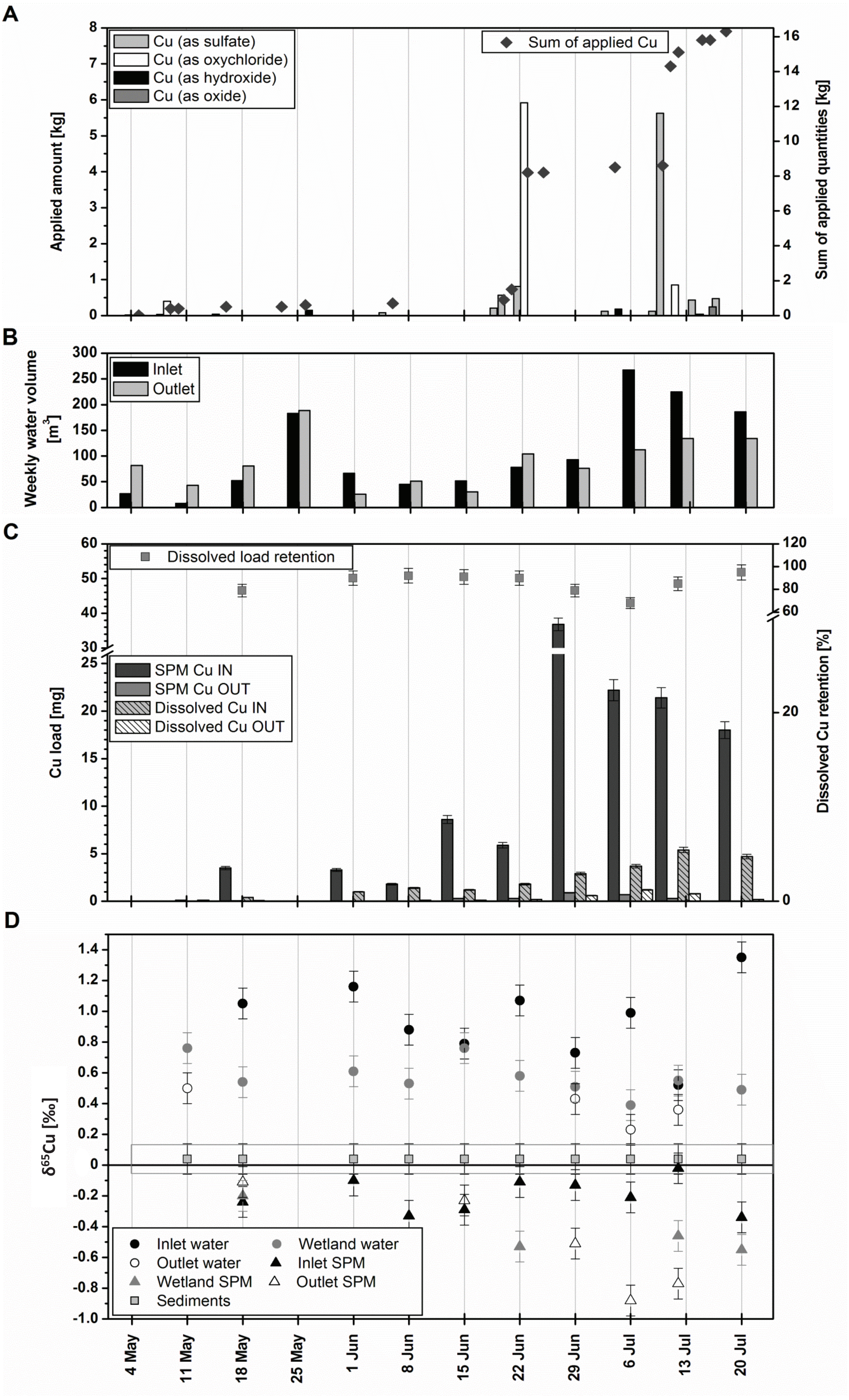


Figure IV-1: *Use of Cu-based fungicides (in terms of metallic Cu) (A) and temporal change of hydrological conditions (B), Cu loads (C) and δ^{65}Cu values (D) at the stormwater wetland (Rouffach, France) from May 4 to July 20 2011. The horizontal bar in figure D represents the δ^{65}Cu of sediments-bound Cu. Error bars correspond to the total analytical uncertainty (± 0.10 ‰, n=3 for δ^{65}Cu measurements).*

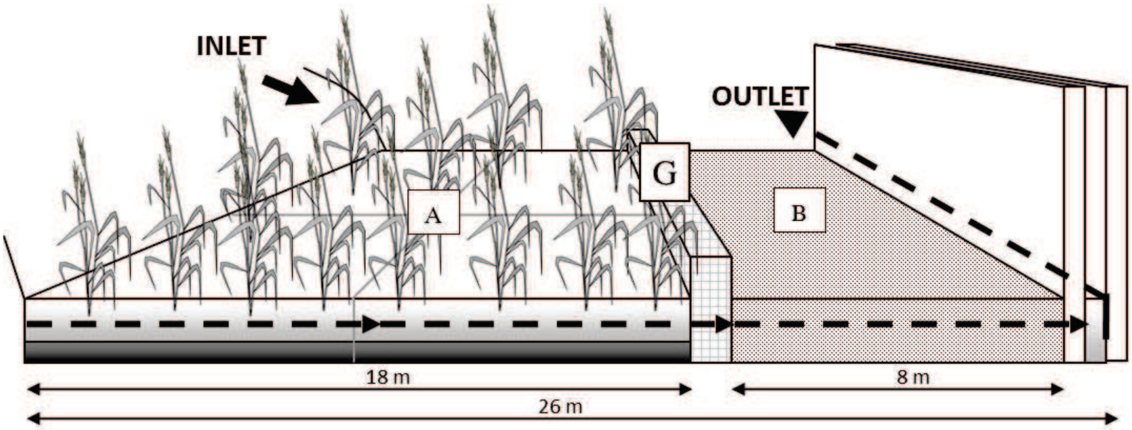


Figure IV-2: *Scheme of the constructed stormwater wetland (Rouffach, France, year of construction 2002). The wetland is composed of a vegetated forebay (A) and the gravel filter (B), separated by a gabion barrier (G), a box filled with rocks whose role is to enhance the dispersion of water ahead of the gravel filter.*

The hydrochemical characteristics significantly changed between the wetland inlet and the outlet (Table IV-2). The average pH value in the wetland forebay during the investigation period was 7.14 ± 0.38, and the pH differed significantly between the wetland inlet (5.97 ± 0.42; range: 5.60 to 6.32) and the outlet (7.73 ± 0.44; range: 7.39 to 8.11) (Table IV-2). The dissolved oxygen concentrations decreased in the wetland forebay between May and July from 1.7 mg L^{-1} to 0.23 mg L^{-1}, respectively, and higher temperatures prevailed during the summer (15°C in May and 19°C in July) (Table IV-2). A gradual change in the prevailing terminal electron-accepting processes (TEAPs), from oxidizing to more reducing conditions in summer, was also supported by the budgets of nitrate, iron and sulfate in the wetland (Figure IV-3B).

Nitrate dissipation ranged from -10 to 97% in the wetland, and denitrification occurred during most of the investigation period (Figure IV-3B). Dissolved iron was retained in the wetland until 15 June and released thereafter when reducing conditions prevailed (Figure IV-3B), most likely through reductive dissolution of Fe oxyhydroxides[7]. Sulfate was released from the wetland until 29 June. Chemical and biological re-oxidation of reduced sulfur species into sulfate likely occurred when oxidizing conditions prevailed in the wetland due to the rapid re-oxidation rates of metal sulfides[39]. The complete dissolution of sulfate-bearing minerals such as the barite ($BaSO_4$) detected by SEM/EDS in the inflowing SPM collected on 20 July (Figure IV-S2) would be quantitatively negligible for the sulfate budget (<1%) compared with sulfide oxidation.

		Inlet	Wetland	Outlet	p (Inlet – Outlet)
Ca	[mg L^{-1}]	36.9 (30.7 - 49.1)	100.3 (59.6 - 156.2)	79.5 (47.2 - 134.2)	≤ 0.01
Mg	[mg L^{-1}]	1.2 (0.9 - 1.4)	24.7 (5.8 - 51.7)	20.5 (8.4 - 40.6)	≤ 0.001
Al	[µg L^{-1}]	18.9 (10.2 - 37.4)	5.3 (0.2 - 11.8)	10.8 (n.d. - 47.3)	≤ 0.05
Fe	[µg L^{-1}]	24.1 (7.7 - 44.4)	32.2 (19.5 – 86.0)	31.7 (2.2 - 86.6)	n.s.
Mn	[mg L^{-1}]	0.07 (n.d. - 0.16)	0.12 (0.01 - 0.38)	0.27 (n.d. - 0.88)	n.s.
Zn	[µg L^{-1}]	6.6 (1.2 - 14.5)	5.2 (1.1 - 15.3)	2.2 (0.9 - 3.4)	≤ 0.05
Cu	[µg L^{-1}]	21.6 (7.7 – 32.0)	5.8 (1.3 - 12.3)	4.2 (1.1 - 10.6)	≤ 0.001
P	[µg L^{-1}]	209.1 (75.9 - 319.2)	131.4 (7.8 - 294.8)	43.8 (12.5 - 88.4)	≤ 0.001
S	[mg L^{-1}]	3.7 (2.1 - 5.5)	29.4 (2.2 - 79.3)	16.8 (1.5 - 64.9)	n.s.
Cl^-	[mg L^{-1}]	3.5 (1.2 - 14.8)	11.9 (1.9 - 26.6)	9.3 (3.8 - 23.3)	≤ 0.01
NO_3^-	[mg L^{-1}]	0.8 (n.d. - 1.9)	0.4 (n.d. - 2.5)	0.2 (n.d. - 0.5)	≤ 0.05
SO_4^{2-}	[mg L^{-1}]	7.2 (3.8 – 12.0)	91.0 (3.5 - 223.8)	66.7 (1.9 - 214.4)	n.s.
PO_4^{3-}	[mg L^{-1}]	0.57 (n.d. - 1.05)	0.16 (n.d. - 0.38)	n.d.	-
TOC	[mg L^{-1}]	28 (12 – 48)	15 (7 – 64)	11 (6 – 29)	≤ 0.001
DOC	[mg L^{-1}]	12.7 (5.1 - 20.6)	7.9 (3.5 - 18.2)	7.3 (4.8 - 13.5)	≤ 0.05
Alkalinity	[meq L^{-1}]	1.8 (1.1 - 2.3)	4.6 (3.1 - 6.7)	4.2 (2.9 - 7.0)	≤ 0.001
Conductivity	[µS cm^{-1}]	163 (113 - 214)	501 (293 - 739)	436 (266 - 739)	≤ 0.001

		May		June		July	
		Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
T	[°C]	16.2 (15.5 – 18.0)	14.9 (14.2 – 15.7)	19.7 (18.3 – 21.6)	17.1 (15.9 – 18.1)	20.0 (19.2 – 20.7)	19.3 (18.0 – 20.7)
pH	[-]	5.83 (5.60 – 6.09)	7.78 (7.62 – 8.06)	6.00 (5.75 – 6.22)	7.81 (7.58 – 8.11)	6.10 (5.96 – 6.32)	7.45 (7.39 – 7.50)
DO*	[mg L^{-1}]	2.14 (1.34 – 3.06)	1.96 (1.15 – 2.48)	1.04 (0.12 – 1.77)	1.51 (0.45 – 2.08)	2.08 (1.88 – 2.44)	2.53 (1.81 – 3.14)

Table IV-2 : *Hydrochemical characteristics of water samples collected at the stormwater wetland and in situ measured physico-chemical parameters between May 4 and July 20 2011. Values are provided as the mean and ranges (min-max). n.d. and n.s. stand respectively for non-detected and non-significant. TOC: total organic carbon, DOC: dissolved organic carbon, DO: dissolved oxygen, S and P refer to total dissolved sulfur and phosphorous respectively.*
**DO was monitored in the water discharge at the inlet and the outlet of the wetland.*

The microbial sulfate-reduction at the end of June resulted in sulfate removal by the wetland. The weekly estimates of microbial sulfate-reduction in the wetland forebay (0.2 to 31.6 mol SO_4^{2-}), based on typical sulfate reduction rates in freshwater lake sediments (0.12 to 21 mmol SO_4^{2-} m^{-2} d^{-1})[40], corresponded to the observed sulfate removal by the wetland (0.5 to 11.9 mol SO_4^{2-}). Other losses of sulfate in the wetland (i.e., gypsum precipitation, sulfate adsorption to wetland sediments and uptake by wetland plants) are expected to be quantitatively lower[41,42].

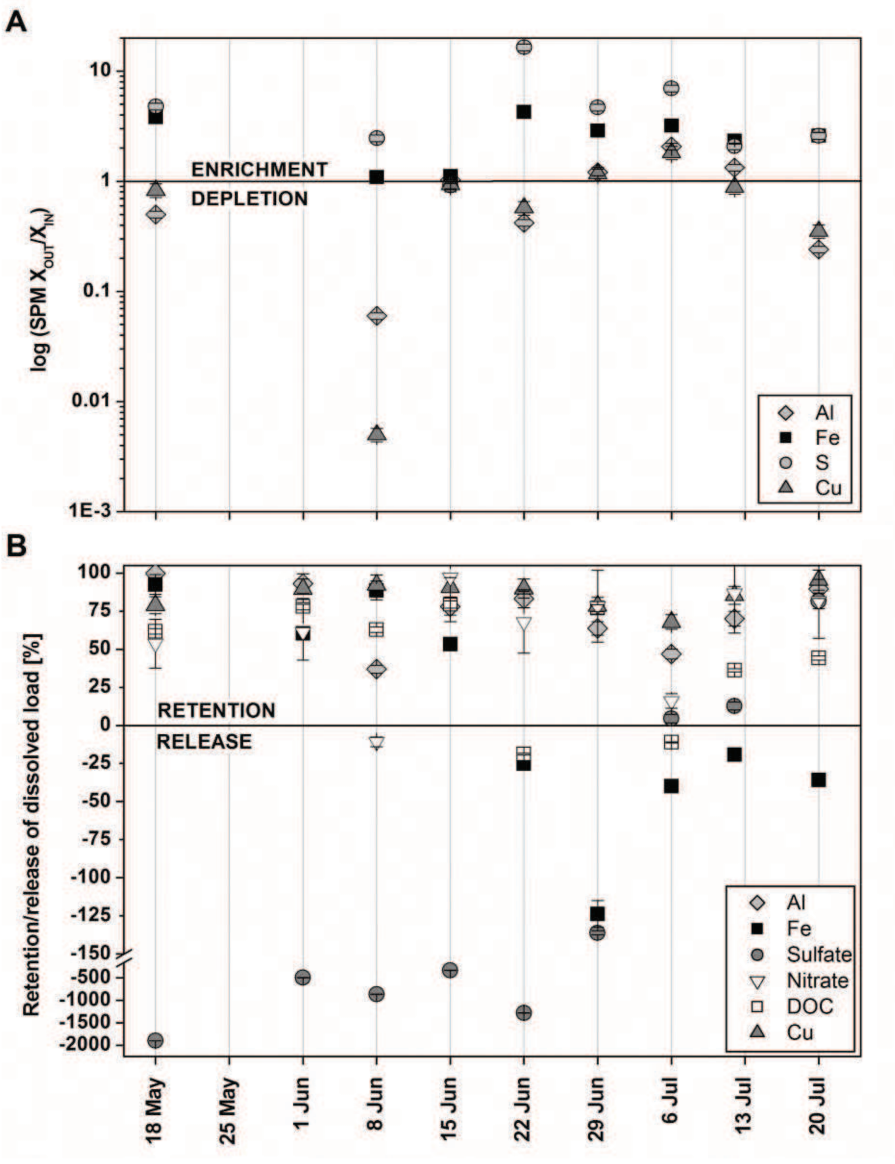


Figure IV-3: *Transport of Al, Fe, Cu, S, sulfate, nitrate and dissolved organic carbon (DOC) in the stormwater wetland. Outlet/inlet ratios of elemental concentrations in suspended particulate matter (SPM) (A) and retention or release of dissolved load in the wetland (B). The errors bars given for the ratios were calculated via error propagation and incorporates analytical uncertainties of concentration measurements of elements and chemical species.*

3.2. Cu behavior and isotope fractionation in the wetland

The observed δ^{65}Cu value in the dissolved phase of runoff entering the wetland was 0.95 ± 0.50‰, and the value in the SPM was -0.20 ± 0.23‰ (Figure IV-1D and Table IV-1). These values are in accordance with the literature results, insofar as the dissolved Cu (<0.2 µm) in rivers was found to be isotopically heavier (δ^{65}Cu = 0.68‰) than Cu bound to suspended solids (δ^{65}Cu = -1.02 to -0.24 ‰)[25]. In a recent study, the Cu in the SPM of a stream connected to a vineyard catchment was isotopically lighter (δ^{65}Cu = 0.09‰) than the dissolved Cu (δ^{65}Cu = 0.31‰)[24].

The $\delta^{65}Cu$ values of the dissolved Cu entering the wetland ($\delta^{65}Cu_{intlet}$) ranged from 0.52 to 1.35‰ and were generally higher than the values found in the wetland forebay (with $\delta^{65}Cu_{wetland}$ values ranging from 0.39 to 0.76‰, $\delta^{65}Cu_{wetland} = 0.57 \pm 0.24$‰) (see Figure IV-1D and Table IV-1). The change in the isotopic composition of the dissolved Cu between the inlet and the wetland forebay ($\Delta^{65}Cu=\delta^{65}Cu_{inlet} - \delta^{65}Cu_{wetland}$) ranged from -0.03 to 0.86‰ ($\Delta^{65}Cu = 0.40 \pm 0.57$‰). The $\delta^{65}Cu$ values of the dissolved Cu in the wetland did not significantly differ from the values at the outlet ($p>0.05$).

The $\delta^{65}Cu$ values of the SPM-bound Cu ranged from -0.55 to -0.20‰ in the wetland forebay and from -0.88 to -0.11‰ at the outlet (Figure IV-1D and Table IV-1). The $\delta^{65}Cu$ values of the SPM at the inlet significantly differed from the values at the outlet at the end of June and in July. This period corresponded to reducing conditions in the wetland. The largest change in the Cu isotope composition between the wetland inlet and outlet for both the dissolved and particulate Cu fractions occurred during the high-flow and reducing conditions in July. The change in the Cu isotope composition between the inlet and the outlet for both the dissolved and particulate Cu indicates that Cu retention by the wetland resulted in Cu isotope fractionation. The $\delta^{65}Cu$ values in the wetland sediments (0.04 ± 0.10‰) corresponded to the values calculated based on an isotopic mass balance that accounted for the total Cu trapped by the wetland (particulate and dissolved) ($\delta^{65}Cu = -0.02 \pm 0.12$‰).

In conjunction with the hydrological conditions, the processes governing Cu behavior in wetland systems are as follows[7]: i) Cu adsorption onto mineral phases (e.g., Fe and Al oxyhydroxides and clays) and complexation with organic matter; ii) Cu reduction and the formation of reduced Cu(I) species under reducing conditions; iii) Cu (co-)precipitation with minerals (e.g., carbonates); iv) the biological uptake of Cu; and v) the sedimentation of particulate Cu. These processes may result in Cu isotope fractionation between the reacting phases, which may help elucidate the Cu behavior and the retention processes in the wetland.

The apparent Cu isotope fractionation between the residual dissolved Cu flowing out of the wetland and the Cu retained in the wetland ($\Delta^{65}Cu_{(Retained-Out)} = -0.04$ to 0.90‰) indicates Cu adsorption in the wetland (see Tables IV-S5 and IV-S7). The significant correlation between the dissolved loads of Cu and Al, Fe and DOC at the wetland outlet ($r \geq 0.83$, $p<0.01$, n=9) suggests that the transport of dissolved Cu was driven by Al- and Fe-rich organic colloids[43]. Al and Fe oxyhydroxides are important sinks of trace elements in sediments and can trap Cu through adsorption or co-precipitation[7]. Cu adsorption onto Fe/Al oxyhydroxides ($\Delta^{65}Cu_{(solid-solution)}=0.24$ to 1.25‰[18,44,45]) results in Cu isotope fractionation similar to the fractionation observed between retained and residual Cu flowing out of the wetland. However, the release of dissolved

Fe from the wetland sediments under reducing conditions did not result in significantly lower Cu retention by the wetland, underscoring the minor role of Fe species in the sequestration of the dissolved Cu. Al species, in the form of oxyhydroxides or other Al-bearing minerals, may be involved in Cu retention in the wetland. Speciation modeling using Minteq indicates that Al and Fe oxyhydroxides (diaspore and hematite) were over-saturated in the wetland water column, which suggests the occurrence of Al and Fe colloidal forms (Table IV-S3). The retention of dissolved Cu by Al species is consistent with the results of the SPM. The enrichment in Cu and Al in the outflowing SPM were significantly correlated (r=0.93, p=0.002, n=8) (Figure IV-3A), which suggests that Al-bearing minerals such as Al oxyhydroxides and clay minerals were involved in Cu sequestration.

The speciation modeling also indicates that Cu was mainly found as organocomplexes in the water phase (Table IV-S3). While Cu mobilization through complexation with dissolved organic matter may occur at elevated pH values (>7)[46], Cu may also be trapped by adsorption and complexation onto wetland organic materials, such as living or decaying plant material or associated biofilms[7,47]. Complexation of Cu with insoluble humic acid may produce small Cu isotope fractionations ($\Delta^{65}Cu_{(solid-solution)}$ = 0.26 ± 0.11‰[19]) in the range of the fractionations observed in this study. In addition, the surface adsorption of Cu onto dead bacteria may also produce solid-phase enrichment in ^{65}Cu [22].

Cu retention may also occur in calcareous wetland sediments through precipitation with crystallizing mineral phases such as carbonates (>20% of $CaCO_3$ in the wetland bed sediments; see Table IV-S1). The precipitation of dissolved Cu(II) in the form of malachite and hydroxide may slightly enrich the dissolved Cu(II) fraction in ^{65}Cu ($\Delta^{65}Cu_{(solution-solid)}$ = ~0.2 to ~0.4‰[20,48]). Since Cu in the dissolved forms becomes lighter while passing through the wetland, the precipitation of dissolved Cu as carbonates or hydroxides is not a likely process for Cu retention in the wetland as it would result in an enrichment in ^{65}Cu. The larger Ca concentrations, higher alkalinity and higher quantities of inorganic carbon at the wetland outlet compared with the inlet indicate carbonate dissolution in the wetland sediments (Table IV-2). Moreover, increasing CO_2 pressure originating from microbial decomposition of organic matter in sediments may displace the equilibrium between carbonate and bicarbonate species toward carbonate dissolution[7]. Therefore, both the isotopic and concentration data support the finding that (co-)precipitation with carbonates was not a significant Cu retention process in the wetland.

		April	**May**	**June**	**July**
Vegetation cover	[%]	80	80	80	80
Above-ground biomass	[kg]	277	252	245	305
Below-ground biomass	[kg]	43	194	464	593
Cu in below-ground biomass	[mg kg^{-1}]	56.6	75.3	32.3	-
Cu in above-ground biomass	[mg kg^{-1}]	5.9	4.3	4.3	-
δ^{65}Cu in below-ground biomass	[‰]	-0.12	-0.21	0.05	-
δ^{65}Cu in above-ground biomass	[‰]	0.28	0.62	0.48	-
δ^{65}Cu in whole plants	[‰]	-0.11	-0.17	0.14	-

***Table IV-3 :** Monthly change of the wetland vegetation, Cu concentrations and δ^{65}Cu values in the plants.*

Wetland vegetation did not take up significant amounts of Cu during the investigation period (Figure IV-S1). Although 400 kg of total vegetal biomass was produced in the wetland between April and June, larger Cu loads in biomass could not be observed due to the dilution effect of biomass growth (Figure IV-S1, Table IV-3). The Cu concentrations decreased between April and June in the above-ground (i.e., leaves and stems) and the below-ground (i.e., roots and rhizomes) biomass (Table IV-3). Altogether, Cu stored in the biomass represented less than 4% of the total Cu budget in the wetland, which is in agreement with previous studies that included *P. australis*[49]. In total, 80 to 98% of the Cu stored in the biomass was found in the below-ground tissues. The isotope data show that the wetland vegetation preferentially took up isotopically lighter Cu when both below- and above-ground biomasses were accounted for ($\delta^{65}Cu_{vegetation}$, which includes $\delta^{65}Cu_{below\text{-}ground}$ and $\delta^{65}Cu_{above\text{-}ground,}$ = -0.17 to 0.14‰) (see Table IV-3). This indicates that the uptake of dissolved Cu by the vegetation was negligible for the isotopic mass balance because a significant uptake of dissolved Cu by the vegetation would have enriched the residual dissolved Cu at the outlet in ^{65}Cu. The Cu source for the vegetation was likely the bed sediments, which had a slightly higher δ^{65}Cu value ($\delta^{65}Cu_{sediments}$ = 0.04 ± 0.10‰) compared with the below-ground tissues ($\delta^{65}Cu_{below\text{-}ground}$ = -0.21 to 0.05‰). This is in agreement with recent findings showing that plants are enriched in ^{63}Cu compared with the source due to active absorption mechanisms[21,50]. The above-ground parts (stems and leaves) of the vegetation were enriched in ^{65}Cu compared with the below-ground biomass (rhizomes and roots) (Table IV-3). This suggests a preferential translocation of ^{65}Cu to the above-ground vegetal tissues in agreement with Ryan et al.[50]. The adsorption of Cu dissolved in the water column onto submerged stems and leaves was not a significant process because most of the vegetal biomass remained above the water level and was not in direct contact with the dissolved Cu, even during high-flow conditions. Surface adsorption and incorporation of Cu into living bacterial cells may enrich biological solids in ^{63}Cu, leaving the solution enriched in ^{65}Cu ($\Delta^{65}Cu_{(solution-solid)}$ = ~0.2 to ~4‰). Based on the observed isotopic signatures, this suggests that active vegetal or microbial uptake mechanisms, which are expected to be larger in summer, were not predominantly involved in the Cu-sequestration process in the wetland. However, the sampling approach used in this study did not enable specific assessment of the isotope fractionation between microorganisms and the dissolved phase.

The sedimentation of SPM-bound Cu in the wetland was likely a major Cu-retention process[4]. This process may be associated with Cu isotope fractionation due to the size-selective retention of particles bearing different Cu isotope signatures by the wetland. Coarser particles are deposited with a higher efficiency in wetlands compared with finer ones[51], which may change the Cu isotope composition of the SPM-bound Cu during transport. However, this study does neither provide a detailed size distribution of the SPM in the wetland nor the δ^{65}Cu values associated with the particle size fractions.

3.3. Mobilization of reduced Cu(I)-species during high-flow conditions

During high-flow and reducing conditions (in July), the lower apparent dissolved Cu retention rates corresponded to larger changes in the Cu isotope composition between the wetland inlet and the outlet for both the dissolved Cu and the Cu in the SPM (Figure IV-1).

We propose that the release of an isotopically lighter pool of Cu from the wetland contributed to ^{63}Cu enrichment at the wetland outlet in both the dissolved and particulate phases (Figure IV-4). Indeed, reducing conditions in sediments and soils can cause the mobilization of reduced forms of Cu[11,23,27,52]. The precipitation of Cu as covellite can result in an isotopic fractionation of $\Delta^{65}Cu(I)_{solid}$-$Cu(II)_{aqu}$ = -3.06 ± 0.14‰, and the large isotopic change was attributed to the reduction step from Cu(II) to Cu(I)[20]. The reduction of Cu(II) by reduced sulfur species to form Cu(0), Cu_xS_y and Cu-S_{org} is a well-known process in oxygen-depleted environments[11,12]. The Cu(II) in the wetland sediments likely underwent reduction to Cu(I) during the reducing periods when significant sulfate-removal was observed. The low solubility of Cu_xS_y species (K_{sp} = 10^{-22} to 10^{-35}) supports the occurrence of Cu_xS_y precipitation in the wetland sediments. Less than 1‰ of the sulfate load retained by the wetland would enable Cu-sulfide precipitation to occur following sulfate reduction. However, the massive release of dissolved sulfate during the oxidizing conditions was not associated with a lower retention of dissolved Cu (Figure IV-3B). This indicates that reduced sulfur species may not exclusively control Cu sequestration in wetland sediments. Other reducing agents may also play an important role in Cu(II) reduction, such as Fe(II) and organic matter[10,53]. During high-flow and reducing conditions, the isotopically lighter Cu(I)- bearing colloids or Cu(0) nanoparticles could be mobilized from the wetland sediments, thus lowering the isotopic signature of the outflowing Cu and leaving the remaining sediment heavier.

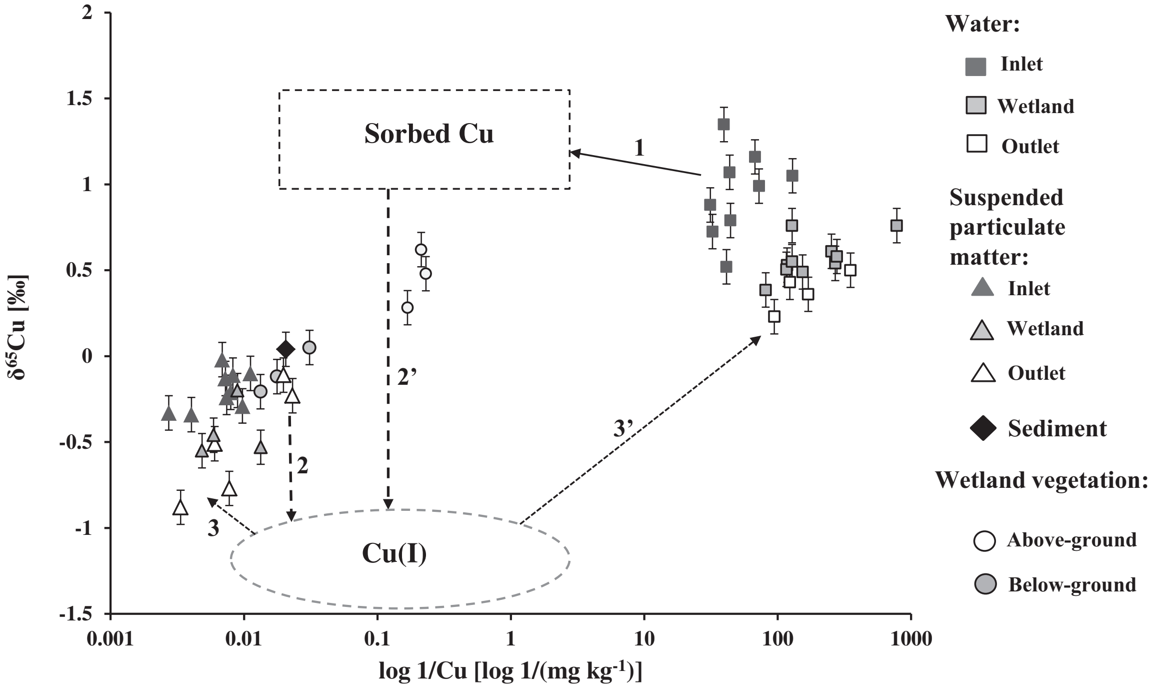


Figure IV-4: *Conceptual model of Cu transport and transformation in the wetland.* ***1)*** *Sorption of dissolved Cu to the sediment and enrichment in ^{65}Cu of the sorbate,* ***2+2')*** *Reduction of Cu(II) within the sediment of the sorbed (2') and retained particulate (2) Cu forming an isotopically lighter Cu(I) pool,* ***3+3')*** *Contribution of the isotopically lighter Cu(I) to Cu associated with the dissolved phase (3') and SPM (3) outflowing the wetland under high-flow conditions.*

4. Implications for wetlands receiving Cu-contaminated runoff

This study adds to a scarce body of findings that explore the use of Cu stable isotope ratios in field conditions and provides a rational basis for using Cu stable isotopes as a biogeochemical tracer of Cu behavior in natural redox-dynamic environments.

The data presented in this study highlight that wetlands are important Cu sinks and that wetlands can efficiently retain both dissolved and particulate Cu from contaminated water. The dissolved Cu concentrations in runoff from the vineyard catchment exceeded the US Environmental Protection Agency (EPA) eco-toxicological recommendations for aquatic environments in 90% of samples (acute level: 13 µg L^{-1}; chronic level: 9 µg L^{-1}), which indicates that the runoff may threaten aquatic ecosystems when Cu-based fungicides are transported from agricultural land. Cu concentrations can be reduced in wetlands to an eco-toxicologically less harmful level, which illustrates the capacity of natural or engineered wetlands to retain runoff-associated Cu. However, wetlands may also act as potential Cu sources because reduced Cu species can be mobilized depending on hydrological and prevailing redox conditions. Therefore, transport of runoff-associated Cu through wetland systems, and in particular wetland systems used as a management practice targeting pesticide attenuation, should be carefully considered.

Our results also support that dissolved Cu in a wetland system can be retained by rapid adsorption processes onto organic matter and minerals present in the wetlands (mainly Al species) depending on the hydrological regime, whereas particulate Cu is retained by sedimentation. Cu adsorption is a rapid process that can reach near-equilibrium within the time frame of the hydraulic water residence in the wetland, leading to enrichment of ^{65}Cu in the adsorbed phase. The Cu isotope fractionation in the dissolved phase between the wetland inlet and outlet is suggested to result from the adsorption and/or co-precipitation processes that drive Cu retention. During low-flow and/or oxidizing conditions, the Cu bound to the SPM is almost quantitatively deposited in the wetland, without significant Cu isotope fractionation between the inlet and the outlet. In contrast, during high-flow and reducing conditions, the reduced Cu(I)-species may be mobilized from the sediment, and isotopically lighter dissolved (colloidal) and particulate Cu may be released from the wetland. In the future, Cu stable isotope values can complement mass-balance approaches to elucidate Cu retention and transformation processes in redox-dynamic systems.

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SUPPORTING INFORMATION

Additional description of the study wetland

The hydraulic residence time of the wetland was estimated based on the water volume contained in the wetland and the outlet flow rate and averaged 14.0 ± 18.3 hours during the investigation period. The minimum water volume stored in the wetland forebay was approximately 50 m^3. The hydrological budget of the wetland was balanced when direct rainfall and evapotranspiration were included. Due to the clayey wetland bed (permeability (k_s)<10^{-10} m s^{-1}) and based on the water balance, water loss by vertical infiltration was negligible.

Sampling date	**LOI**	**SiO_2**	**Al_2O_3**	**MgO**	**CaO**	**Fe_2O_3**	**MnO**	**TiO_2**	**Na_2O**	**K_2O**	**P_2O_5**	**Cu**	**Zn**
	[%]							**[mg kg^{-1}]**					
March 23	15.10	52.3	10.7	2.24	10.6	4.31	0.074	0.498	0.52	2.32	0.197	48.0	86.6
April 6 (1)	14.06	50.6	12.0	2.37	11.2	4.95	0.075	0.575	0.56	2.63	0.221	47.9	92.7
April 6 (2)	14.87	50.6	12.0	2.52	10.6	4.74	0.074	0.556	0.63	2.62	0.203	53.1	92.7
May 11	14.77	49.0	12.9	2.77	11.4	5.43	0.082	0.612	0.59	2.72	0.199	30.2	103.0
June 8	16.33	49.8	11.1	2.34	11.4	4.61	0.078	0.541	0.61	2.44	0.248	64.0	101.1
July 12	15.25	49.8	11.5	2.05	12.8	4.75	0.080	0.570	0.51	2.38	0.280	66.5	107.9

Table IV-S1: *Chemical and textural characteristics of the wetland bed sediments. Elements are expressed in oxides. Analytical uncertainty for the proportions of major elements is 5% and for trace elements [mg kg^{-1}] is <10%. LOI stands for loss on ignition at 1000 °C.*

The sediment texture was (%): clay 44, fine silt 33, coarse silt 10, fine sand 5 and coarse sand 8. Carbonates content was 29±11%.

The pH_{water} value of the sediments was 7.6 and the apparent sediment density was 875.1 kg $m^{3\ -1}$.

Cu isotope analysis

Sample preparation and digestion were performed in a clean room using double sub-boiled acids, 18.2 MΩ water (Milli-Q, Merck Millipore, MA, US) and suprapur reagents. Sample digests were re-dissolved in the adequate acid (7 mole L^{-1} HCl + 0.01% H_2O_2) for Cu purification.

Sample type	Resin volume	Resin wash	Conditioning	Sample load	Matrix elution	Cu elution
			7 mole L^{-1} HCl + 0.01% H_2O_2			
Water (×2)	1.6 mL	3×7 mL 0.5 mole L^{-1} HNO_3 alternating with 7 mL H_2O, 1×7 mL 7 mole L^{-1} HCl, 1×7 mL H_2O	6 mL	1 mL	4×1 mL	26.5 mL
Solids 1st purification	3 mL	3×10 mL 0.5 mole L^{-1} HNO_3 alternating with 10 mL H_2O, 1×9 mL 7 mole L^{-1} HCl, 1×9 mL H_2O	10 mL	1 mL	5×1 mL	45 mL
Solids 2nd purification	2 mL	3×7 mL 0.5 mole L^{-1} HNO_3 alternating with 7 mL H_2O, 1×7 mL 7 mole L^{-1} HCl, 1×7 mL H_2O	8 mL	1 mL	4×1 mL + 2×2 mL	26 mL
Plant samples (×2)	2 mL	3×7 mL 0.5 mole L^{-1} HNO_3 alternating with 7 mL H_2O, 1×7 mL 7 mole L^{-1} HCl, 1×7 mL H_2O	8 mL	1 mL	4×1 mL + 2×2 mL	26 mL

Table IV-S2: *Sequences of Cu purification for different environmental samples using 10 mL Bio-Rad columns (Bio-Rad, CA, US) and AGMP1M resin (Bio-Rad).*

Each purification step was followed by a fast digestion of samples in 1 mL HNO_3 to mineralize the organic matter leached from the AGMP1M resin. Purified samples and standards were re-dissolved in 0.5 mole L^{-1} HNO_3 to reach concentrations of 100 µg L^{-1} Cu. Ni standard (NIST SRM 986) was added (200 µg L^{-1} Ni) to purified samples for correction of instrumental mass biases during measurements of $^{65}Cu/^{63}Cu$. The introduction system was a desolvating nebulizer (Apex HF, ESI, NE, USA). One measurement consisted of 40 cycles of 8 s integration.

Cu isotope ratios were expressed as $\delta^{65}Cu$ [‰] values relative to NIST 976 isotope reference material according to eq 1

$$\delta^{65}\mathrm{Cu} = \left(\frac{\mathrm{R_{sample}}}{\mathrm{R_{NIST\ 976}}} - 1\right) \times\ 1000 \tag{1}$$

where R denotes the Cu isotope ratios ($^{65}Cu/^{63}Cu$) of the sample and the NIST 976 standard.

Cu isotope analysis of geological standards yielded 0.18 ± 0.10‰ (n=3, 2SD) for CRM-7003 (Bigalke et al. obtained 0.21 ± 0.11‰, (n=5, 2SD))[1] and 0.20 ± 0.10‰ (n=2, 2SD) for BCR-2 (Bigalke et al. obtained 0.22 ± 0.05‰ (n=5, 2SD))[1].

The maximal bias during Cu isotope measurements caused by the blank would be ~0.07‰ considering the observed Cu isotopic variations in nature (~-4.5 to ~+8‰)[2].

A	**Inlet 18/05**					
Component	**Dissolved inorganic**	**Bound to DOM**	**Total dissolved**	**% dissolved**	**Total precipitated**	**% precipitated**
/HFA(6)	0	1.8E-04	1.8E-04	100	0	0
Al+3	4.7E-10	6.1E-07	6.1E-07	100	0	0
Ba+2	2.8E-07	3.1E-09	2.9E-07	100	0	0
Ca+2	1.1E-03	4.0E-05	1.1E-03	100	0	0
Cl-1	5.8E-05	0	5.8E-05	100	0	0
CO3-2	2.0E-05	0	2.0E-05	100	0	0
Cu+1	5.2E-17	2.5E-20	5.2E-17	100	0	0
Cu+2	3.3E-10	1.2E-07	1.2E-07	100	0	0
Fe+2	7.0E-14	5.8E-15	7.6E-14	100	0	0
Fe+3	1.3E-12	3.3E-07	3.3E-07	40.961	4.7E-07	59.039
H+1	4.1E-05	-1.1E-04	-6.4E-05	100	0	0
H4SiO4	6.3E-05	0	6.3E-05	100	0	0
HS-1	8.3E-95	0	8.3E-95	100	0	0
K+1	1.5E-04	7.7E-08	1.5E-04	100	0	0
Mg+2	5.2E-05	1.6E-06	5.4E-05	100	0	0
N2 (aq)	5.0E-06	0	5.0E-06	100	0	0
Na+1	4.2E-05	2.1E-08	4.2E-05	100	0	0
NH4+1	1.2E-37	6.0E-41	1.2E-37	100	0	0
NO2-1	2.0E-30	0	2.0E-30	100	0	0
NO3-1	4.7E-30	0	4.7E-30	100	0	0
O2 (aq)	4.7E-30	0	4.7E-30	100	0	0
PO4-3	9.5E-07	0	9.5E-07	100	0	0
SO4-2	1.2E-04	0	1.2E-04	100	0	0
Sr+2	1.2E-06	1.8E-08	1.3E-06	100	0	0
Ti(OH)4	2.4E-08	0	2.4E-08	95.726	1.1E-09	4.274
Zn+2	1.7E-07	5.4E-08	2.2E-07	100	0	0

B	**Wetland 18/05**					
Component	**Dissolved inorganic**	**Bound to DOM**	**Total dissolved**	**% dissolved**	**Total precipitated**	**% precipitated**
/HFA(6)	0	2.4E-04	2.4E-04	100	0	0
Al+3	2.5E-10	7.2E-09	7.4E-09	100	0	0
Ba+2	1.4E-07	8.4E-10	1.4E-07	32.294	3.0E-07	67.706
Ca+2	3.0E-03	5.2E-05	3.1E-03	99.950	1.5E-06	0.050
Cl-1	5.4E-04	0	5.4E-04	100	0	0
CO3-2	4.1E-04	0	4.1E-04	100	0	0
Cu+1	1.9E-24	7.6E-28	1.9E-24	100	0	0
Cu+2	6.0E-13	4.2E-08	4.2E-08	100	0	0
Fe+2	3.2E-24	1.1E-25	3.3E-24	100	0	0
Fe+3	1.1E-14	1.5E-08	1.5E-08	3.144	4.6E-07	96.856
H+1	4.2E-04	-1.9E-04	2.3E-04	100	0	0
H4SiO4	7.3E-05	0	7.3E-05	94.519	4.2E-06	5.481
HS-1	1.1E-147	0	1.1E-147	100	0	0
K+1	2.6E-05	1.7E-08	2.6E-05	100	0	0
Mg+2	1.5E-03	2.2E-05	1.5E-03	100	0	0
N2 (aq)	3.9E-26	0	3.9E-26	100	0	0
Na+1	4.1E-04	2.7E-07	4.1E-04	100	0	0
NH4+1	1.3E-68	8.0E-72	1.3E-68	100	0	0
NO2-1	1.2E-19	0	1.2E-19	100	0	0
NO3-1	9.7E-07	0	9.7E-07	100	0	0
O2 (aq)	4.4E-05	0	4.4E-05	100	0	0
PO4-3	3.2E-08	0	3.2E-08	3.385	9.2E-07	96.615
SO4-2	1.8E-03	0	1.8E-03	99.984	3.0E-07	0.016
Sr+2	3.9E-05	2.9E-07	3.9E-05	100	0	0
Ti(OH)4	2.4E-08	0	2.4E-08	49.800	2.4E-08	50.200
Zn+2	5.3E-09	3.8E-08	4.3E-08	100	0	0

C	**Inlet 29/06**					
Component	**Dissolved inorganic**	**Bound to DOM**	**Total dissolved**	**% dissolved**	**Total precipitated**	**% precipitated**
/HFA(6)	0	1.8E-04	1.8E-04	100	0	0
Al+3	5.4E-10	3.6E-07	3.6E-07	49.891	3.7E-07	50.109
Ba+2	2.9E-07	3.0E-09	2.9E-07	100	0	0
Ca+2	1.2E-03	4.7E-05	1.2E-03	100	0	0
Cl-1	4.2E-04	0	4.2E-04	100	0	0
CO3-2	2.4E-05	0	2.4E-05	100	0	0
Cu+1	5.5E-22	1.7E-25	5.5E-22	100	0	0
Cu+2	3.2E-10	4.9E-07	4.9E-07	100	0	0
Fe+2	3.4E-21	3.1E-22	3.7E-21	100	0	0
Fe+3	2.7E-13	5.5E-08	5.5E-08	18.099	2.5E-07	81.901
H+1	5.1E-05	-1.2E-04	-6.6E-05	100	0	0
H4SiO4	8.1E-05	0	8.1E-05	100	0	0
HS-1	3.1E-142	0	3.1E-142	100	0	0
K+1	1.7E-04	7.8E-08	1.7E-04	100	0	0
Mg+2	5.8E-05	1.9E-06	6.0E-05	100	0	0
N2 (aq)	1.2E-19	0	1.2E-19	100	0	0
Na+1	2.5E-05	1.1E-08	2.5E-05	100	0	0
NH4+1	1.0E-62	4.7E-66	1.0E-62	100	0	0
NO2-1	9.7E-18	0	9.7E-18	100	0	0
NO3-1	3.0E-05	0	3.0E-05	100	0	0
O2 (aq)	4.4E-05	0	4.4E-05	100	0	0
PO4-3	6.0E-06	0	6.0E-06	100	0	0
SO4-2	7.5E-05	0	7.5E-05	100	0	0
Sr+2	1.2E-06	1.8E-08	1.3E-06	100	0	0
Ti(OH)4	2.4E-08	0	2.4E-08	60.421	1.6E-08	39.579
Zn+2	2.8E-08	1.4E-08	4.1E-08	100	0	0

D	**Outlet 29/06**					
Component	**Dissolved inorganic**	**Bound to DOM**	**Total dissolved**	**% dissolved**	**Total precipitated**	**% precipitated**
/HFA(6)	0	7.7E-05	7.7E-05	100	0	0
Al+3	7.4E-10	2.0E-07	2.0E-07	48.736	2.0E-07	51.264
Ba+2	3.3E-07	1.0E-09	3.3E-07	100	0	0
Ca+2	2.1E-03	1.7E-05	2.1E-03	100	0	0
Cl-1	1.4E-04	0	1.4E-04	100	0	0
CO3-2	2.3E-05	0	2.3E-05	100	0	0
Cu+1	3.5E-22	7.9E-26	3.5E-22	100	0	0
Cu+2	9.2E-10	1.3E-07	1.4E-07	100	0	0
Fe+2	9.4E-21	1.6E-22	9.6E-21	100	0	0
Fe+3	5.1E-13	2.0E-08	2.0E-08	4.763	3.9E-07	95.237
H+1	4.2E-05	-5.8E-05	-1.5E-05	100	0	0
H4SiO4	7.8E-05	0	7.8E-05	48.320	8.0E-05	51.680
HS-1	1.6E-143	0	1.6E-143	100	0	0
K+1	8.4E-05	2.1E-08	8.4E-05	100	0	0
Mg+2	5.1E-04	3.6E-06	5.2E-04	100	0	0
N2 (aq)	2.2E-20	0	2.2E-20	100	0	0
Na+1	1.2E-04	3.0E-08	1.2E-04	100	0	0
NH4+1	2.2E-63	5.6E-67	2.2E-63	100	0	0
NO2-1	1.1E-18	0	1.1E-18	100	0	0
NO3-1	4.0E-06	0	4.0E-06	100	0	0
O2 (aq)	1.9E-05	0	1.9E-05	100	0	0
PO4-3	9.5E-07	0	9.5E-07	100	0	0
SO4-2	2.7E-04	0	2.7E-04	100	0	0
Sr+2	1.1E-05	4.1E-08	1.1E-05	100	0	0
Ti(OH)4	2.4E-08	0	2.4E-08	35.851	4.3E-08	64.149
Zn+2	1.2E-07	9.4E-09	1.3E-07	100	0	0

Tables IV-S3 A, B, C, D: *Visual Minteq (version 3.0) modelling of the dissolved species at the inlet, in the wetland forebay and at the outlet of the stormwater wetland. Measured concentrations of chemical species and in situ measured physico-chemical parameters (T, pH) were used as input data. Speciation calculations were performed for five selected water samples: Inlet 18/05 (A), Wetland 18/05 (B), Inlet 29/06 (C) and Outlet 29/06 (D). Both SHM and NICA-Donnan models were tested for considering interactions with dissolved organic matter (as fulvic acid) and gave similar results. Alkalinity, pH and T values were specified, oversaturated solids were allowed to precipitate when the final answer was reached, default databases were used and redox pairs of Cu+/Cu+2, Fe+2/Fe+3, HS-/SO4-2, N2/NO3-, NH4+/NO3- and NO2-/NO3- were taken into account. Concentrations are provided in mol* L^{-1}.

Weekly periods	Quiescent period [d]	Hydraulic residence time [h]	Q_{max} of runoff [L s^{-1}]	Inlet volume [m^3]	Outlet volume [m^3]
May 4 - 11	8.07	6.1	2.14	7.9	43.1
May 11- 18	3.89	4.7	4.64	52.4	80.8
May 18 - 25	6.59	4.3	47.06	183.2	188.6
May 25 – June 1	8.83	31.1	2.50	66.3	25.7
June 1 - 8	6.71	14.0	2.56	44.9	51.4
June 8 - 15	6.18	27.1	9.61	51.7	30.2
June 15 - 22	7.10	7.8	15.67	78.2	104.2
June 22 - 29	6.53	17.9	34.58	93.0	75.9
June 29 – July 6	7.46	21.0	44.69	267.3	112.2
July 6 - 12	4.34	10.2	18.58	224.6	134.5
July 12 -20	5.63	10.1	18.36	186.2	134.2

Table IV-S4: *Hydrological data in the wetland for the weekly sampling periods.*

		Inlet	**Wetland**	**Outlet**
Si[1]	[%]			
Al	[%]	4.61 (3.09 - 6.83)	3.35 (1.53 - 4.26)	3.55 (0.32 - 9.82)
Mg	[%]	0.85 (0.6 - 1.02)	1.10 (0.68 - 1.83)	0.80 (0.29 - 1.61)
Ca	[%]	7.03 (3.39 - 9.98)	6.33 (4.2 - 10.83)	6.37 (1.93 - 10.56)
Fe	[%]	3.01 (2.09 - 4.01)	6.01 (5.66 - 6.93)	7.56 (3.07 - 12.51)
Mn	[%]	0.07 (0.04 - 0.09)	1.31 (0.62 - 1.78)	2.91 (0.20 - 6.32)
Ti	[%]	0.25 (0.19 - 0.30)	0.11 (0.11 - 0.12)	0.16 (0.05 - 0.26)
Na	[%]	0.32 (0.22 - 0.47)	3.85 (0.14 - 12.96)	0.53 (0.16 - 1.87)
K	[%]	0.92 (0.15 - 1.99)	1.79 (1.26 - 2.58)	1.01 (0.17 - 2.88)
P	[%]	0.18 (0.12 - 0.32)	1.01 (0.68 - 1.19)	0.66 (0.20 - 1.14)
Sr	[mg kg^{-1}]	169 (107 - 217)	1029 (481- 2358)	1012 (332 - 1939)
Ba	[mg kg^{-1}]	314 (226 - 369)	457 (325- 571)	847 (294 - 1434)
Co	[mg kg^{-1}]	14 (10 - 17)	-	59 (18 - 134)
Cr	[mg kg^{-1}]	101 (75 - 136)	142 (116 - 167)	159 (86 - 314)
Cu	[mg kg^{-1}]	164 (90 - 366)	245 (167 - 375)	126 (97 - 229)
Ni	[mg kg^{-1}]	42 (26 - 67)	25 (- - 25)	46 (0.5 - 115)
Zn	[mg kg^{-1}]	216 (144 - 455)	320 (158 - 721)	441 (198 - 716)
S	[g kg^{-1}]	1.54 (1.09 - 2.97)	30.89 (4.35 - 91.82)	7.38 (1.24 - 19.40)

Table IV-S5: *Elemental composition of suspended particulate matter (SPM) at the inlet (n=9), in the wetland (n=4) and at the outlet (n=9). Values are provided as the mean and the ranges. Analytical uncertainty for proportion of major elements is 5% and for trace elements [mg kg^{-1}] is <10%.*

[1] *Silicon was volatilized during acid digestion with HF.*

Processes		**$\Delta^{65}Cu = \delta^{65}Cu_A - \delta^{65}Cu_B$**		**References**
	A-B	**min**	**max**	
Apparent Cu isotope fractionation during dissolved Cu retention in the wetland ($\delta^{65}Cu_{retained} - \delta^{65}Cu_{residual\ dissolved}$)		***-0.04***	***0.90***	***This study***
Adsorption on Fe oxyhydroxides	Fe oxyhyrdoxide-solution	0.24	0.98	Clayton, 2005[3]
				Pokrovsky, 2008[4]
				Balistrieri, 2008[5]
Adsorption onto gibbsite	Gibbsite-solution	0.75	1.25	Pokrovsky, 2008[4]
Copper reduction and precipitation	Cu(I)solid- Cu(II)aq	-2.92	-4.07	Zhu, 2002[6]
				Ehrlich, 2004[7]
Copper oxidative dissolution	Cu(I)solid- Cu(II)aq	-2.7	0.21	Mathur, 2005[8]
				Wall, 2011[2]
				Asael, 2005[9]
Copper precipitation	Precipitate-solution	-0.4	-0.2	Maréchal & Sheppard, 2002
(malachite: $Cu_2(CO_3)(OH)$, $Cu(OH)_2$)				reported in Ehrlich, 2004[7]
Uptake by plants	Roots-solution	-0.11	-0.84	Jouvin, 2012[10]
	Shoot-solution	-0.33	-1.06	Jouvin, 2012[10]
	Shoot-soil	-0.33	-0.94	Weinstein, 2011[11]
	Plant - solution	-0.11	-1.05	Ryan, 2013[12]
	Shoot -solution	-0.08	-0.43	Ryan, 2013[12]
Translocation of Cu	Shoots-germinated seeds	-0.33	-0.34	Weinstein, 2011[11]
	Shoot-root	-0.08	-0.72	Jouvin, 2012[10]
	Shoot -root	-0.04	1.03	Ryan, 2013[12]
Complexation with humic acid	Humic acid-solution	0.15	0.37	Bigalke, 2010[13]
Interaction with bacteria	Bacteria-solution	1.97	2.43	Mathur, 2005[8]
		0.44	0.76	Borrok, 2008[14]
		-1.7	0.4	Pokrovsky, 2008[4]
		-4.4	-0.2	Navarrete, 2011[15]
Interactions with dead bacteria cells	Bacteria-solution	-0.3	0.69	Navarrete, 2011[15]

Table IV-S6: *Cu isotope fractionation observed in the wetland and reported ranges (min – max) of experimental Cu isotope fractionation for wetland processes.*

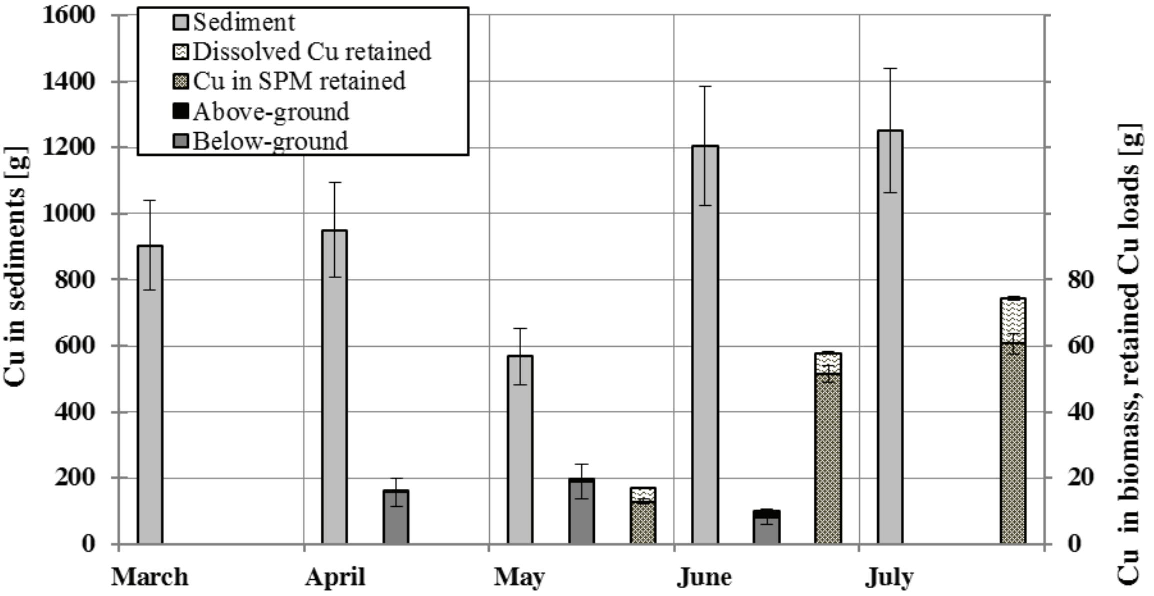

Figure IV-S1: *Estimated Cu stocks in wetland sediments, wetland vegetation (above- and below-ground stocks) and dissolved Cu and Cu in suspended particulate matter (SPM) retained by the wetland. Error bars account for the reproducibility of Cu measurements in replicates (n=2).*

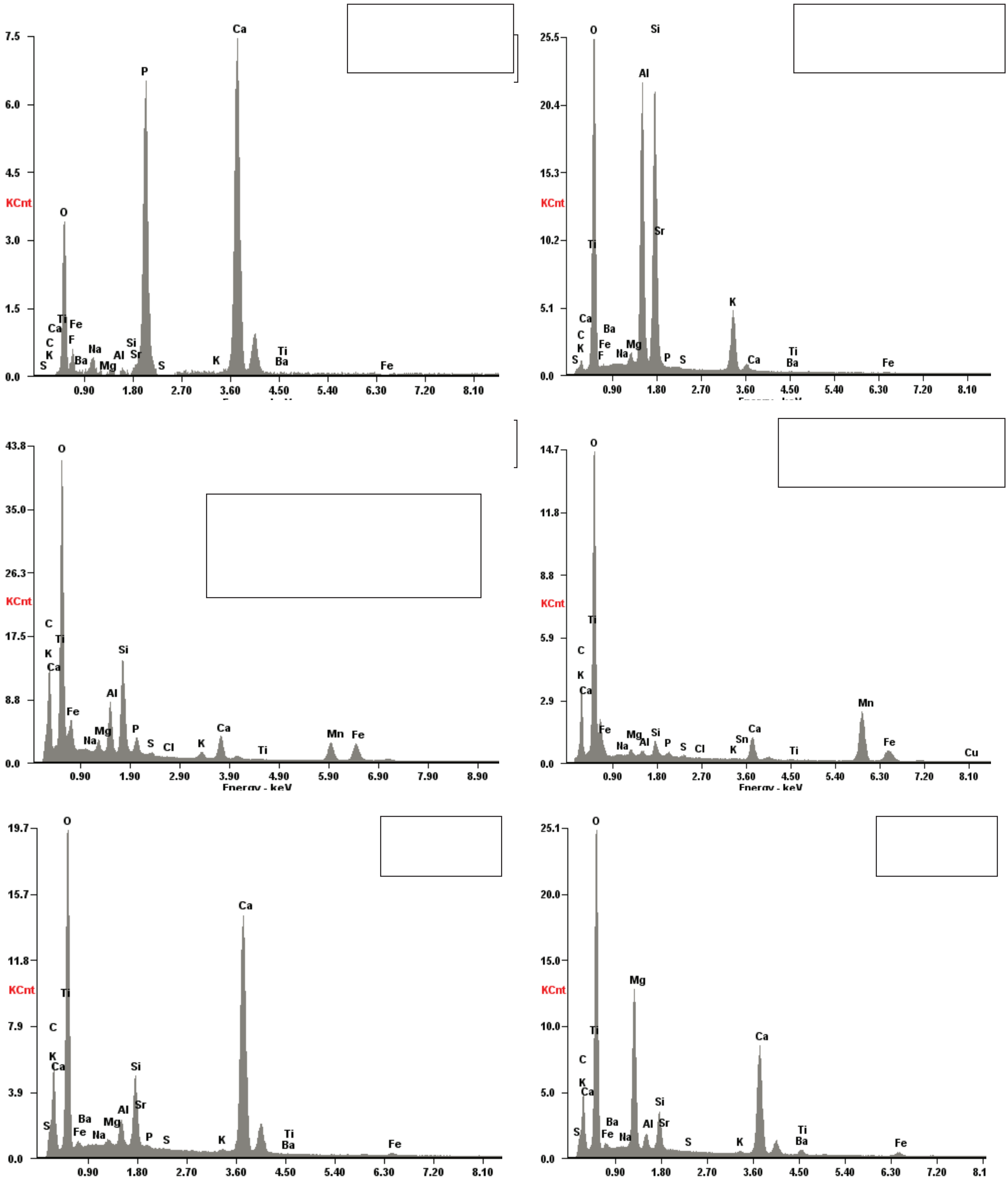

Figure IV-S2: *EDS spectra of mineral and organic particles in SPM at the stormwater wetland (Rouffach, France) on July 20 2011.*

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V. Chapter: Copper transformation at biogeochemical gradients in Winogradsky columns

In Chapter IV, the Cu isotope results emphasized the potential role of Al (and less Fe) oxy(hydr)oxides in the sorption of Cu in the wetland sediments, as well as the reduction of Cu(II) within the sediments. We set up a laboratory experiment (Wingoradsky columns) to investigate the Cu transformation under reducing conditions in the sediments. In the Winogradsky columns the microbially mediated terminal electron accepting processes (TEAPs) create biogeochemical gradients in the sediment depth profile, enabling to explore the long-term transformations of the sediment-bound Cu under contrasting biogeochemical conditions. We combined sequential chemical extractions of Cu and Cu isotope analysis to evaluate how Cu transformation evolved in the wetland sediments.

CHAPTER V: COPPER TRANSFORMATION AT BIOGEOCHEMICAL GRADIENTS IN WINOGRADSKY COLUMNS

ABSTRACT

Knowledge of copper (Cu) biotransformation along redox gradients in polluted wetland sediments is scare. We investigated over 520 day the impact of biogeochemical processes on the Cu transformation and ageing in Winogradsky columns, a fundamental experiment in microbial ecology on the sulfur cycle in redox-dominated environments. Photosynthetic algae released dissolved oxygen in the water overlying the sediments while sulfide and ferrous iron concentrations gradually increased over the columns' depth. Sequential extraction revealed that sediment evolution at different depths of the columns altered the chemical composition, including the Cu distribution, and could be related to terminal electron accepting processes (TEAPs). Concomitant Fe and Cu enrichments were observed at the 6-10 cm below the water-sediment interface after 520 days, suggesting that Cu cycle was linked to the Fe redox processes. Cu partitioning into the residual phases of the wetland sediments increased over time, which may reduce Cu mobility. The Cu stable isotope ratios changed in the Cu-enriched layer of the sediment compared to the initial sediment ($\delta^{65}Cu_{initial}$ - $\delta^{65}Cu_{C4,6\text{-}8cm}$= -0.19‰), suggesting the release and the subsequent accumulation of isotopically heavier Cu during sediment ageing. Together, the results highlight reductive dissolution of Fe oxy(hydr)oxides with concomitant release of Cu bound to the Fe-bearing phases. However, re-oxidation of reduced Cu^+ species cannot be excluded at the oxic water-anoxic sediment interface. In addition, silicon (Si) and calcium (Ca) release near the water-sediment interface indicated sediment ageing as a result of biogeochemical processes. Overall, this study shows that microbially mediated processes significantly changed the geochemical composition of the aged sediments that highlight the powerful role of microbes in shaping anoxic environments. Knowledge on the composition of microbial communities using high-throughput sequencing techniques In the future will enable to elucidate the relationship between biogeochemical processes, Cu transformation and bacterial community change along the redox gradients.

1. Introduction

Wetlands are interfacial environments between terrestrial and aquatic ecosystems, and are important sinks for heavy metals.[1] Wetlands provide ecological functions including water quality improvement by metal pollution reduction, and are thus finding increasing application for the treatment of polluted water.[2] Copper (Cu) transformation and mobility are strongly impacted by biogeochemical conditions in redox-dominated compartments, such as wetland sediments.[3] Redox conditions may in turn influence availability of terminal electron acceptors (TEAs) for microbial metabolism which may eventually affect Cu cycle in the wetland sediment. Although wetland systems may intercept Cu-contaminated runoff from agricultural or urban areas and limit Cu transport into surface waters, knowledge on the long-term transformation of Cu in wetland sediments with respect to biogeochemical processes is still scarce.

The Winogradski columns is a fundamental miniature ecosystem experiment in microbial ecology to expand the volume of natural processes in sediments,[4,5] including the microbially mediated sulfur cycle. The Winogradski columns may be used to elucidate the relationship between the Cu transformations, the sulfur cycle and the biogeochemical gradients in wetland sediments.[6] A redox gradient established along the Winogradsky columns during algal oxygen production in the overlying water and gradual depletion of TEAs during microbial consumption downwards in the sediments.[6] In the anoxic sediments, sulfate-reducing bacteria produce H_2S diffusing upwards in the sediments, thereby creating a sulfide gradient.[6] Cu is a chalcophile element with high affinity to sulfur, leading to the formation of various Cu sulfide species, such as chalcocite (Cu_2S), covellite (CuS), and Cu-iron-sulfides, such as chalcopyrite ($CuFeS_2$).[7] Under sulfate reducing conditions, the formation of Cu sulfides in wetland sediments may thus reduce Cu mobility, although Cu may be released when colloidal Cu^0 and Cu sulfides are formed.[8] The re-oxidation of reduced Cu species (such as Cu sulfides) or by the reductive dissolution of the Cu-sorbing Fe oxy(hydr)oxides may also mobilize Cu from the sediment.[9,10]

In this context, we embarked on a study to characterize the long-term transformation of Cu along biogeochemical gradients established in Winogradsky columns consisting of wetland sediments. Aging is the process by which Cu mobility and bioavailability is reduced in soils and sediments over time following Cu sorption.[11] Analysis of the geochemical composition of the sediments and TEAs, sequential chemical extractions of Cu[12] and Cu stable isotopes were coupled to investigate how biogeochemical processes control Cu transformation over time. Cu isotopes were applied previously to investigate Cu-bacteria interactions,[13] sorption to mineral phases,[14,15] complexation with organic matter[16,17] and redox processes.[,18,19] In particular, copper stable isotopes have been to trace bacterially-driven Cu sulfide dissolution in acid mine drainage[20] and Cu sulfide formation in geological settings.[21] However, the influence on the Cu cycle of TEAPs and related isotope fractionation in redox gradient established in wetland sediments remains largely unexplored.

The aim of the present study is to evaluate the behavior and the aging of Cu at biogeochemical gradients formed in Cu-polluted wetland sediments based on a Winogradsky column experiment. Four Winogradski columns were set-up with Cu-polluted sediments from a stormwater wetland that collected runoff from a vineyard catchment in Rouffach (Alsace, France).[22] Cu originates from the vineyard soils, which are frequently contaminated due to the use of Cu fungicides (see Chapter III). One column was sacrificed at day 40 and 80 and two at day 520 to collect water and sediment samples at high resolution over depth. The Cu transformation and partitioning in the sediment and the porewater phases was evaluated over depth and related to the geochemical composition of the sediment and microbial processes.

2. Materials and methods

2.1. Experimental set-up and sampling

Cu-contaminated sediments from a stormwater wetland situated at the outlet of a vineyard catchment in Rouffach (Alsace, France) were collected in December 2012. The physical properties and chemical characteristics of the sediments have been described elsewhere (see also Table V-S1).[2] The sediments were mixed with the nutrient mixture for preparing the Winogradski columns. Sulfur (4.5% sediment Na_2SO_4), inorganic carbon (0.2% sediment Na_2CO_3) and pH-buffers (of 0.5% K_2PO_4 and 0.5% NH_4SO_4) were added to the sediments.[4] Sediments were taken as grab samples (0-10 cm) from four locations of the 319 m^2 stormwater wetland of Rouffach, were transported in sealed plastic boxes and kept refrigerated until filling the columns. Sediments were homogenized using a mixer before addition of nutrients. Four borosilicate glass columns (inner diameter: 15 cm, height: 65 cm) were filled with ~6.2 kg of sediments on a height of 40 cm (~7.1 L) (Figure V-1). 0.7% of pure cellulose (100 g sediment) was also added in the 19 cm bottom sediments as an additional organic carbon source to favor fermentative organisms.[4] A 19 cm 18 MΩ water (Millipore, Billerica, MA, USA) layer was added at the top of the sediment column, leaving 4 cm headspace at the top of the columns. The columns were sealed with a transparent Plexiglas cover disk to limit atmospheric contamination and water evaporation, and were kept in an air conditioned room at 20°C ± 0.5 °C. The columns were exposed daily to light from four projectors each of 150 W tungsten-halogen lamps (R7S, 150 W, Phillips, Eindhoven, Netherlands) for 12 hours per day to favor photosynthetic activity. Dissolved oxygen concentrations were monitored using non-invasive oxygen sensors (Presens®, Regensburg, Germany) mounted inside the columns at 15, 25-30, 38, 47 and 56 cm depth from the water-sediment interface (Figure V-1), with the first spot situated in the water phase and the four others in the sediment layers. The columns were regularly (1 per week in the beginning and 1 per month later on) photographed during the experiment for visual comparison.

All the materials used for the construction and the sampling of the Winogradsky columns were soaked in a hydrochloric acid bath (HCl, 20 %) for 24h and rinsed for 24 h with 18 MΩ water, and with ethanol (96%) for sterilization.

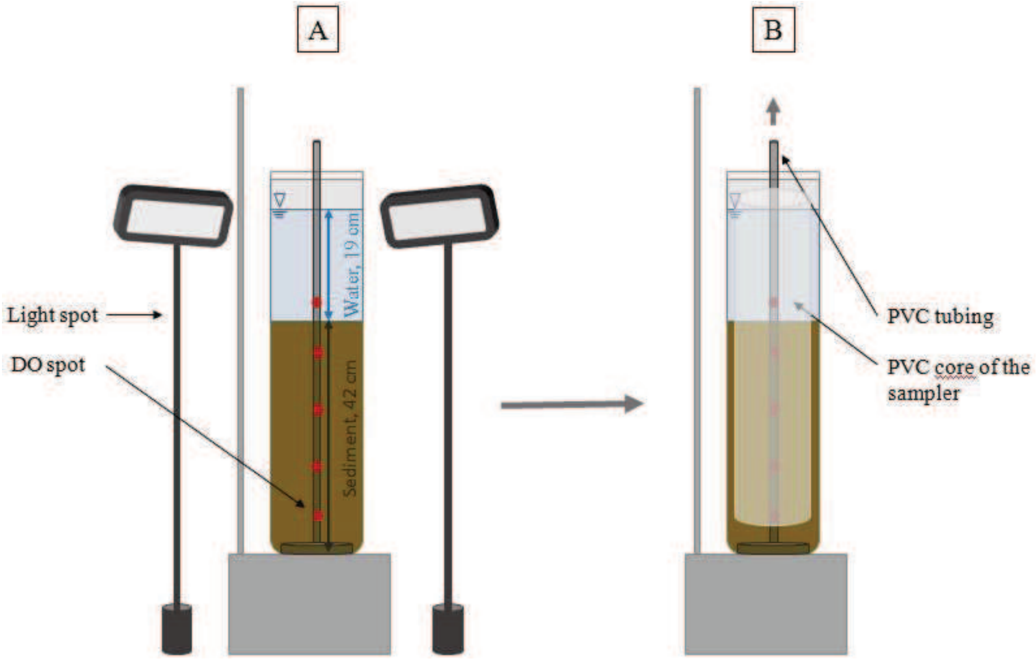

***Figure V-1:** Schematics of the constructed Winogradsky columns with the position of the dissolved oxygen spots (A), and the sampling set-up consisting of a PVC tube and a PVC support (B).*

The first column ('C1') was sampled after 40 days, the second column after 80 days ('C2') and the two last columns were sampled after 520 days ('C3' and 'C4'). The first two columns (C1 and C2) were characterized with sequential chemical extraction for Cu, and the columns C3 and C4 were additionally characterized using Cu and sulfur stable isotopes.

The water overlying the sediments was collected using Teflon tubing and plastic syringes. Water samples from the final columns (C3 and C4) were collected for algal (100 mL), microbiological and hydrochemical analyses. For algal characterization, water samples were filtered through 12 µm cellulose nitrate filters (CN AE 100, Schleiler & Schuell, Germany) and preserved in a 0.25% glutaraldehyde solution. For microbial DNA extraction, water samples were filtered through sterile 0.2 µm cellulose nitrate filters (Whatman, Maidstone, England), and stored in sterile 50 mL plastic Falcon tubes at -20°C until further processing. Top and bottom water samples (1680 mL, corresponding each to 9.5 cm from the top of the overlying water layer towards the bottom) were collected for hydrochemical analyses.

A sediment core at the center of each column was collected by inserting a PVC tube (diameter of 12.5 cm) and fixing it on a PVC support placed at the bottom of the columns at the beginning of the experiment (Figure V-1). A Teflon bar was affixed on the support to retrieve an undisturbed sediment core (Figure V-1). The sediment cores were immediately frozen at -20 °C until further analysis. The frozen sediment cores were cryo-sliced into layers with a tungsten saw. The columns C1 and C2 were cut into 5 layers of 3, 10, 10, 9 and 8 cm depth from the top of the sediments towards the bottom, whereas columns C3 and C4 were cut into 5 layers of 2 cm for the top 10 cm and into 5 layers of 6 cm for bottom part of the sediment core. The depth resolution of the core slicing was chosen to characterize with a higher depth resolution the top layers as gradients in the columns were expected to be steeper below the oxygenated overlying water. The frozen sediment layers were thaw in a glovebox (Jacomex BS531, Dagneux, France) under nitrogen atmosphere (<1 ppm O_2). 1g of bulk sediment was collected before centrifugation for microbial DNA extraction and stored in sterile Eppendorf tube at -20°C until further processing. Each sediment layers were centrifuged at 1700 g for 30 min (Jouan B4, Thermo Electron Corp., Waltham, MA, USA) to extract porewater. Approximately 2.5 mL of the porewater through 0.22 µm hydrophilic PVDF membrane filter (Millipore) under nitrogen atmosphere using syringe filters for Fe^{2+} analysis. The remaining porewater fraction was filtered through 0.2 µm hydrophilic PVDA membrane filters (Millipore) in the laboratory for hydrochemical analyses.

2.2. Algal analyses

The algal analysis in the overlying water samples consisted of enumeration (± 10%), taxonomic identification and algal biomass determination (± ~10%) performed at the GreenWater Laboratory (Palatka, FL, USA) according to standard sample preparation protocols using an inverted microscope equipped with phase contrast optics (Nikon Eclipse TE200, Tokyo, Japan).[23]

2.3. Hydrochemical analyses

pH was measured with a combined electrode (Aquatrode plus, Metrohm AG, Herisau, Switzerland). Alkalinity, carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) concentrations were determined by acid–base titration (905 Titrando, Metrohm AG). Sulfide ions (S^{2-}) (ArrowH2S™, Lazar Research Laboratories, Inc., Los Angeles, CA, USA, ± 0.5%), and redox potential (Eh) (ArrowDOX™, Lazar Research Laboratories, Inc., ± 0.2 mV) were measured in the unfiltered porewater samples under N_2 atmosphere. Nitrate ions (NO_3^-) were tentatively measured

(ArrowSTRAIGHT™, Lazar Research Laboratories, Inc., ± 4%) but interferences, possibly due to the presence of chloride ions, preclude any quantification. Ferrous iron (Fe^{2+}) analysis was done by UV spectrophotometer (Shimadzu UV-1700 Pharmspec, Kyoto, Japan) at 522 nm (± 3%) immediately after exposing samples to the atmosphere to avoid artifact due to Fe^{2+} re-oxidation. The dissolved organic carbon (DOC) concentration in the waters samples was analyzed using a carbon analyzer (Shimadzu TOC – VCPH). Acetate, major anions and cations (listed in the Table V-S2) were quantified using ion chromatography (ICS 3000 Dionex, San Diego, CA, USA, ± 2%). Major and trace element analyses, including Cu, were carried out by an ICP-AES (iCAP 6000, Thermo Scientific, Waltham, MA USA, ± 10%) and an ICP-MS (Thermo Finnigan X-Series II, Thermo Scientific, Waltham, MA USA, ± 5%) as previously described.[2] The validity and the reproducibility of hydrochemical measurements was verified using certified standards SLRS-4 (National Research Council, Ottawa, Canada) and TM-24.3 (Environment Canada, Gatineau, Canada).

2.4. Geochemical analyses

Sequential chemical extractions was performed on the centrifuged sediment samples to quantify the Cu and Fe partitioning according to a modified and previously tested protocol from Tessier et al.[24,25] Five extraction phases were defined as follows: 1) exchangeable, 2) acid-soluble (carbonate-bound), 3) reducible (Fe- and Mn-oxide bound), 4) oxidable (organic matter- and sulfide-bound), and 5) residual (included within the silicate matrix and more refractory sulfides). Samples were treated in a glovebox until the oxidable step to avoid change in the oxidation state of the redox-sensitive species (e.g. reduced sulfides). The Cu recovered in the washing solutions was systematically added to the previously extracted fractions. Standard deviations were < 10% for the acid-soluble, reducible, oxidable and residual fractions (n=3).

Sediments were oven-dried (at 60°C) and powdered (<63 µm) for the chemical composition analysis following alkaline fusion and acid digestion by ICP-AES and ICP-MS. For quality control, geological standards were processed in parallel for each analytical series (BCR-2, SCL-7003). Organic matter (OM) (SOL-0401), organic carbon (C_{org}) (NF ISO 10694), inorganic carbon (C_{inorg}) (NF ISO 10694), pH (in water) (NF ISO 10390), total carbon (C_{tot}) (NF ISO 10694), total nitrogen (N_{tot}) (NF ISO 13878) and the mineral nitrogen forms ($N\text{-}NO_3^-$, $N\text{-}NH_4^+$) (SOL-1402) in the fresh sediment samples (transported frozen on "dry ice") were quantified at the INRA Soil Analysis Laboratory (Arras, France) accredited by the French National Accreditation Authority (COFRAC), recognized by the European calibration services (EA — European Cooperation for Accreditation). X-ray diffraction (XRD) analysis (Brüker D5000, Brüker Corp, Billerica, MA, USA) (3–65° 2θ scanning angle, 1 s–0.02° upwards step, Cu anticathode, wavelength λ Kα1= 1.54056 Å, current 40 kV voltage and 30 mA) was performed the initial sediment and for sediment layers listed in Table V-1 that have been selected based on their contrasted chemical compositions.

2.5. Cu and sulfur stable isotope analysis

The sample preparation protocol for Cu isotope analysis and the operating conditions of the MC-ICP-MS for the Cu isotope measurements are described elsewhere.[2] The porewater samples had high matrix to Cu ratios and thus could not be purified for Cu isotope analysis (see Chapter II for further details on the problematic about "critical samples").

Sulfur (S) isotope ($\delta^{34}S$) analysis was carried out by Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS) at the Iso-Analytical Ltd. (Crewe, UK) as described elsewhere.[26] The dried and powdered sediment samples were acidified with 1 N HCl and left overnight to remove inorganic carbon. The samples were then neutralized by repetitively washing with distilled water and oven dried at 60 °C prior to isotope analysis. The analysis proceeds in a batch process by which a reference is analyzed followed by a number of samples and then another reference. For quality control purposes test samples of IA-R061 (in-house standard) and IAEA-SO-5 a certified international standard (barium sulfate, $\delta^{34}S_{V\text{-}CDT}$ = +0.50 ‰) were measured.

3. Results

3.1. The carbon (C) and nitrogen (N) cycles in the columns

Carbon (C) dynamics are controlled by the mineralization of sediment organic matter (SOM) and the CO_2 incorporation into the biomass of autotrophic microorganisms (Figure V-2). The lower C_{inorg} concentrations in the top sediment layers suggests the dissolution of the carbonate phases from the sediment (i.e., calcite and dolomite), whereas enrichment in C_{inorg} underneath, in particular in the C4 between 4 and 8 cm depth under the water-sediment interface (WSI), may indicate re-precipitation of carbonate phases (Figure V-2). Dissolved carbonates can serve for sustaining the growth of algae that proliferated both in the overlying water and in the top of the sediment layers exposed to light. Some carbonates may also have leached downward and re-precipitate as supported by the higher C_{inorg} concentrations below the top sediment layers (Figure V-2).

In contrast, higher concentrations of organic carbon (C_{org}) were observed in the top sediment layers and C_{org} decrease underneath (Figure V-2). This corresponds to both algal development with concomitant C_{org} accumulation in the surface layers of the sediment, and the decaying organic matter following algal death and flocculation. The dynamics of C_{org} is also controlled by heterotrophic bacteria that utilize C_{org} as electron donor in their metabolic activities and degrade organic compounds up to mineralization into CO_2.[1] The highest SOM mineralization was observed between 4 to 8 cm below the WSI in C4 and from 2 to 10 cm in C3 with a parallel increase in C_{inorg} (Figure V-2), highlighting microbial activity at those depths. The acetate profiles in the porewater indicate fermentation at the bottom sediment layers containing the cellulose (Figure V-3).[27] Higher acetate concentrations were also observed near the surface sediment layers (Figure V-3), suggesting lower consumption rates by bacteria using acetate as an electron donor, such as Fe^{3+}-reducing and sulfate-reducing microorganisms.[28]

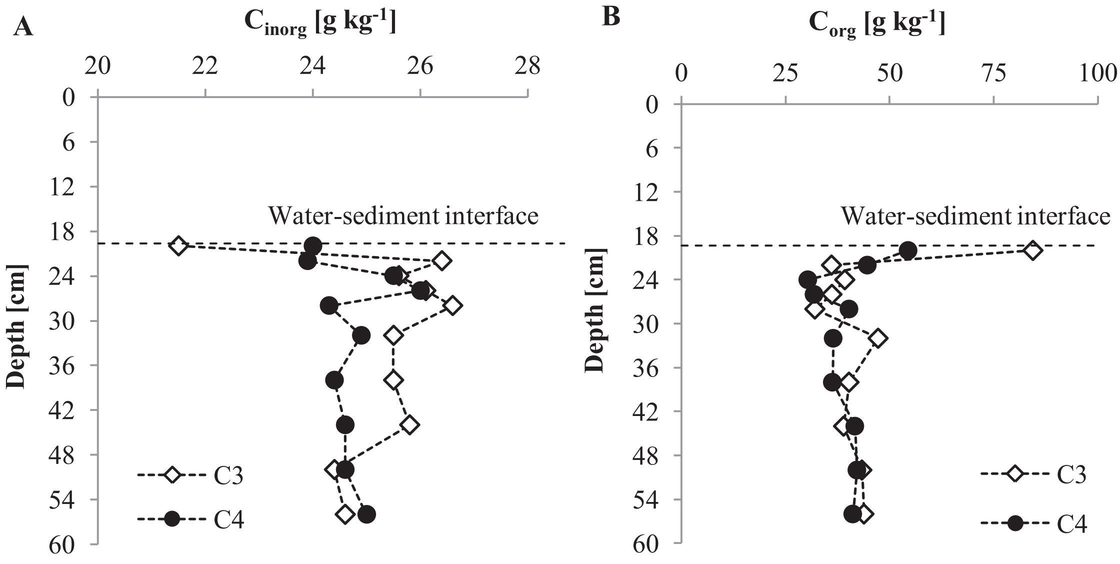

Figure V-2: Sediment-bound inorganic (A) and organic (B) carbon concentrations along the depth profile of columns C3 and C4.

The total nitrogen concentrations in the sediments correlated with SOM content ($r=0.97$, $p<0.001$, $n=20$), highlighting that organic nitrogen forms may predominate in the sediments (Table V-S1). Ammonium (NH_4^+) concentrations decreased upwards in both the porewater and the sediments, indicating algal consumption of NH_4^+ (Tables V-S1 and V-S2).[29] Sediment-bound NO_3^-concentrations were generally low (<3.5 mg kg^{-1}) and only increased in the surface sediment layers of C3, suggesting NO_3^- consumption by denitrifyers and Fe^{2+}-oxidizers reducing NO_3^- (Table V-S1).[30]

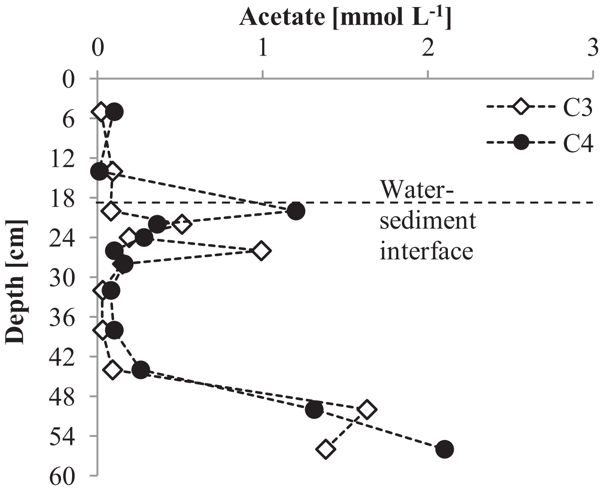

Figure V-3: *Acetate concentration profile in the porewater of the sediment layers in the columns C3 and C4. Acetate is considered as the most important fermentation acid produced by the degradation of organic matter under anaerobic conditions.*[28]

3.2. Algal oxygen production and redox gradients

Dissolved oxygen (DO) and redox potential (Eh) depth profiles in the final columns (C3 and C4) confirm the establishment of a redox gradient in the columns (Figures V-4). Dissolved oxygen was rapidly consumed in the 5-6 cm of the sediment layers, while up to 23.2 (C3) and 32.4 mg L^{-1} (C4) were observed in the overlying water (Figure V-4). Larger DO concentrations in C4 may be related to the higher algal biomass in the overlying water. Eh values showed similar trends and decreased from >100 mV in the overlying water to <-400 mV in the sediment layers (Figure V-4).

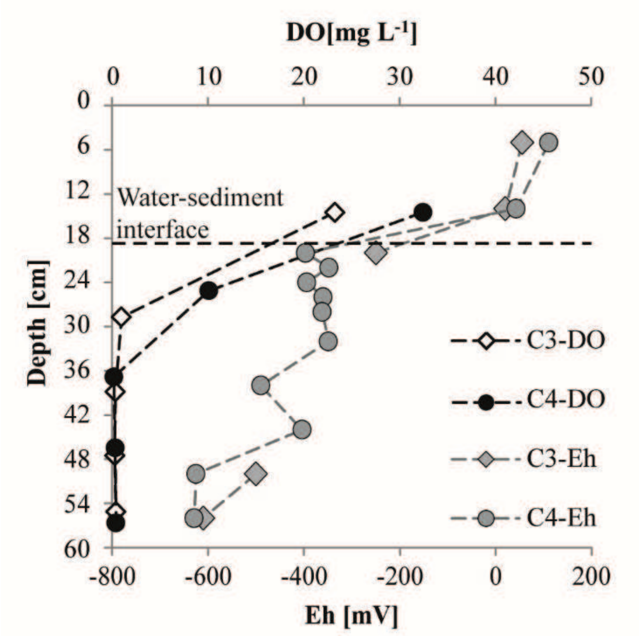

Figure V-4: *Dissolved oxygen (DO) concentration and the redox potential (Eh) in the columns C3 and C4 (due to inconsistent values in the C3 Eh is not displayed between 19 and 48 cm depth).*

Higher algal concentrations prevailed in C4 with 232 083 cells mL^{-1} and a biovolume (volumetric biomass concentration) of 30 337 062 µm^{3} mL^{-1} compared to 124 541 cells mL^{-1} and 13 048 069 µm^{3} mL^{-1} in C3. Green algae (Chlorophyta) were the unique algal group observed in both columns. *Tetracystis*/*Chlorococcum* sp. (9 261 762 µm^{3} mL^{-1}) of 5.0-14.5 µm diameter were the dominant genera present in unicellular and colonial forms (Figure V-5). Both *Tetracystis* and *Chlorococcum* are most commonly found in soils or subaerial habitats and are difficult to distinguish. The second most abundant taxa were morphologically similar to the dominant *Tetracystis*/*Chlorococcum* sp. but cells were only 2.5-5.8 µm in diameter (Figure V-6).

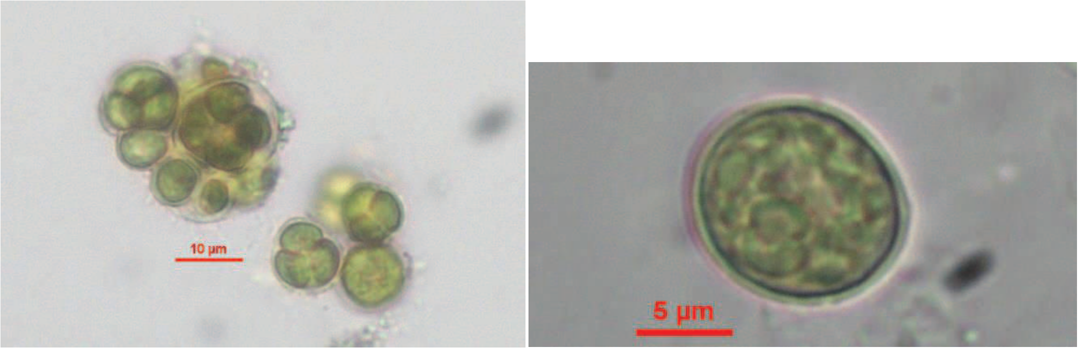

Figure V-5: *Colonial and unicellular forms of Tetracystis/Chlorococcum sp.*

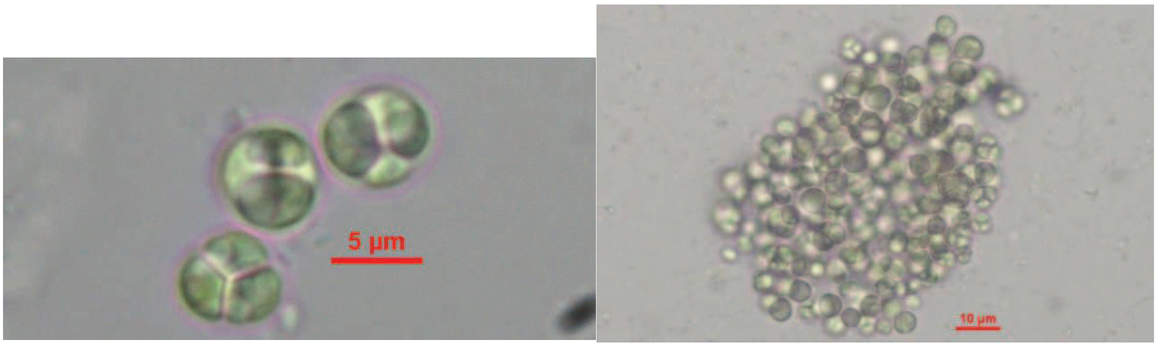

Figure V-6: *The second unidentified chlorophyte sp. smaller in size than the identified Tetracystis/Chlorococcum sp.*

Algae also colonized the first 5 cm of the sediment layers at the column wall-sediment interface (exposed to light). Consequently, oxygen was also produced into the upper sediment layers (Figure V-4), although it was most probably depleted radially as the oxygen demand for the aerobic heterotrophic bacteria is expected to exceed diffusive oxygen supply.[31]

3.3. Iron cycle

Iron (Fe) is a major redox-sensitive metal whose cycle in anoxic surface sediments is often controlled by microbially-mediated redox reactions. The sediment-bound Fe was re-distributed over time as the redox gradient established along the columns (Figure V-7). XRD analysis revealed chlorite and hematite as the main Fe-bearing minerals (Table V-1), whereas hematite was only detected in C4 at day 520. This indicates that hematite was formed over time in C4, whereas it might be present but non-detectable in the initial sediments and in C3.

Depth profile analysis with the Thorium (Th) normalization was used to rule out dilution/concentration effects, as Th is considered as an immobile element.[32] The Th normalized depth profile revealed Fe accumulation between 4 and 8 cm below the WSI in C4, and between 8 and 10 cm in C3, whereas Fe depletion occurred underneath (Figure V-7). The total Fe and Fe^{2+} concentrations increased with depth in the porewater of both columns, which indicates microbial Fe^{3+} reduction in the anoxic sediment layers (Figure V-7 and V-8).[33] Organic matter and reduced sulfur species may also reduce Fe^{3+} under reducing conditions, however at circumneutral pH that prevailed in our columns, microbial processes are suggested to be responsible for the Fe^{2+} production.[28]

Extractable Fe was mainly found in the acid-soluble fraction (Figure V-9), suggesting that extractable Fe was bound to carbonates (i.e., co-precipitated with carbonates or in the form of siderite and green rust). Although acetic acid used for extracting the acid-soluble fraction can extract specifically sorbed metals, it may also partly attack silicates in the sediments, such as Fe-rich phyllosilicates (i.e., chlorite).[34] Acid-soluble Fe fraction was followed by the reducible Fe fraction, but the largest Fe pool was found in the non-extractable residual fraction (≥90%) (Figure V-9).

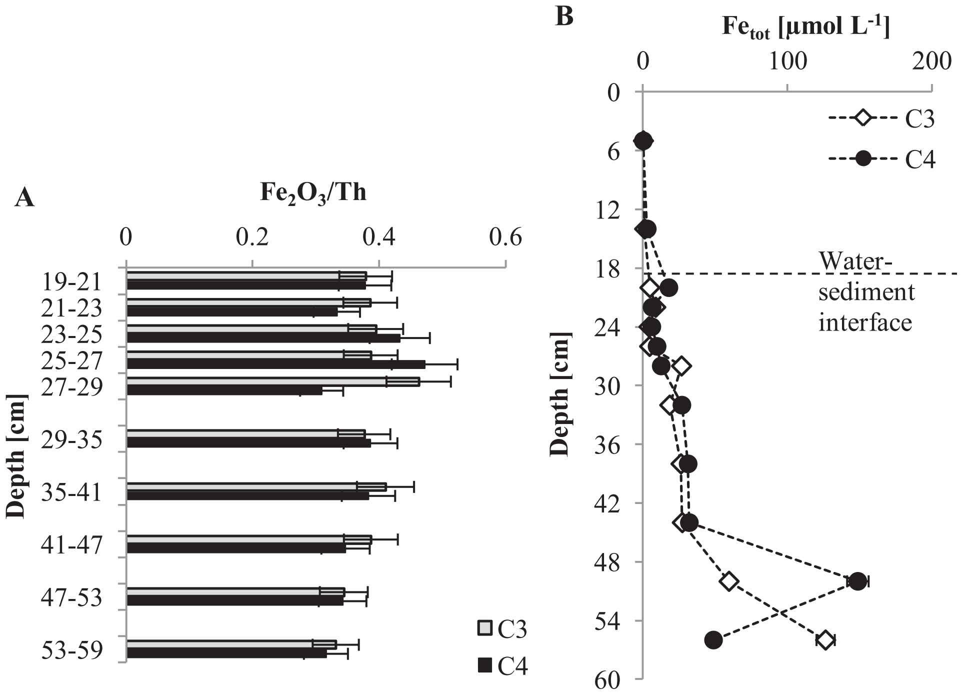

Figure V-7: *The Th normalized Fe concentrations in the sediment layers (A) and Fe_{tot} concentrations in the overlying and the porewater (B) of the columns C3 and C4.*

Samples		**Phases detected by XRD**
	Depth in the whole column	
Initial sediments		Quartz, calcite, dolomite, feldspars, clay minerals (illite, chlorite and/or kaolinite)
C3	27-29 cm	Quartz, calcite, dolomite, feldspars (albite, microcline), chlorapatite, clay minerals (illite, chlorite and/or kaolinite)
	53-59 cm	Quartz, calcite, dolomite, feldspars (albite, microcline), clay minerals (illite, chlorite and/or kaolinite)
C4	25-27 cm	Quartz, calcite, dolomite, feldspars (albite, microcline), apatite (chlorapatite and/or fluorapatite), hematite, clay minerals (illite, chlorite and/or kaolinite)
	53-59 cm	Quartz, calcite, dolomite, feldspars (albite, microcline), chlorapatite, hematite, clay minerals (illite, chlorite and/or kaolinite)

***Table V-1 :** Mineral phases detected by XRD analysis in the initial sediment and in two sediment layers in the columns C3 and C4.*

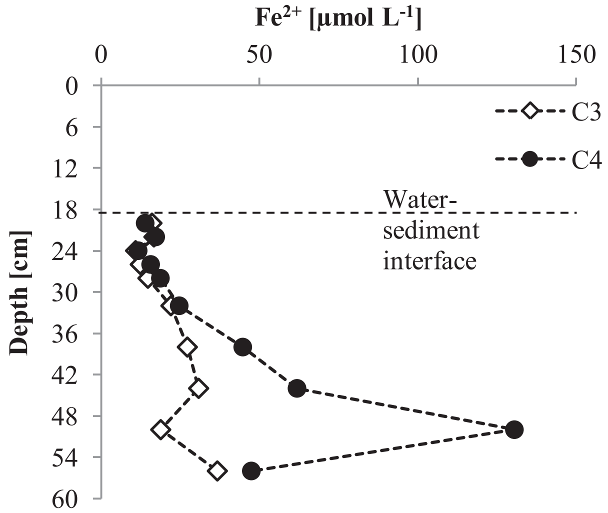

***Figure V-8:** Ferrous iron (Fe^{2+}) concentrations in the porewater of the sediment layers in the columns C3 and C4. Fe^{2+} is produced by the reductive dissolution of Fe^{3+}. Fe^{2+} was not analyzed in the overlying water.*

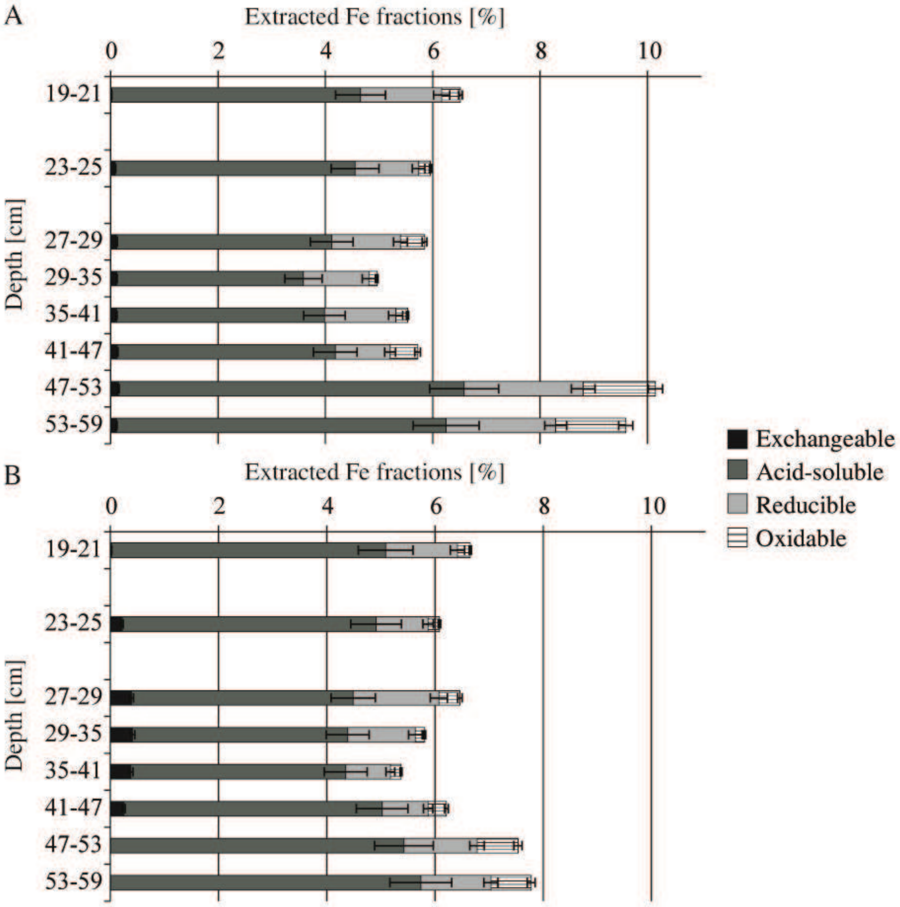

Figure V-9: *Partitioning of extractable Fe phases (exchangeable, acid-soluble, reducible, oxidable) in the sediment layers of the columns C3 (A) and C4 (B) the residual Fe fraction accounts for the difference between the sum of all extractable Fe fractions and total Fe in the sediment and is not represented.*

3.4. Sulfur cycle

Higher sulfide (S^{2-}) concentrations in the porewater from the bottom of the columns highlighted sulfate reduction in both columns and indicate reducing conditions in the bottom layers of the sediments (Figure V-10A). Sulfate is utilized by heterotrophic bacteria as a TEA to degrade the SOM, preferentially thriving on intermediate fermentation products (such as acetate).[35] The Fe^{2+} and S^{2+} accumulation in the poreawater of the bottom layers indicate that Fe^{3+}-reducing microorganisms did not significantly inhibit sulfate-reducers in metabolizing SOM, which may occur when SOM is less bioavailable in the sediments.[28] Sulfide likely diffused upwards in the column and could be oxidized in the top sediment layers chemically or by sulfur/sulfide-oxidizing bacteria into oxidized sulfur species, such as sulfate, thiosulfate.[35]

In contrast, the sulfate concentration profile in the sediment porewater showed decreasing sulfate concentration upwards (Figure V-11). The dissolution of the Na_2SO_4 nutrient added to the initial sediment most probably supplied the dissolved sulfate in the porewater

(Figure V-10B). Sulfate depletion in the porewater was observed from the 7th layer (from 16 cm below the WSI) as indicated by the higher sodium (Na) mole concentration in the porewater compared to the stoichiometric 2:1 mole ratio expected for Na_2SO_4 dissolution without sulfate depletion (Figure V-10B). Sulfate depletion occurs by reduction, in agreement with higher S^{2-} concentration in the porewater in the bottom layers (Figure V-10A).

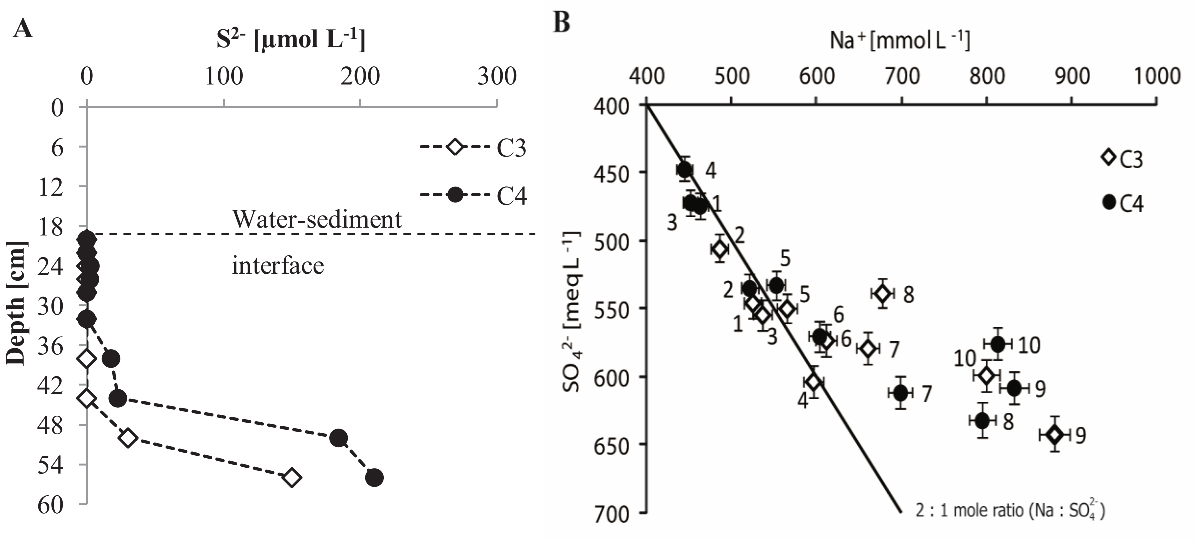

Figure V-10: *Evolution of the dissolved S^{2-} concentration (A) and the SO_4^{2-} vs. Na concentrations (B) in the porewater of the sediment layers in columns C3 and C4.*

Higher total sulfur proportions with depth in the solid phase of the sediment layers indicating the formation of reduced and precipitated metal sulfides further confirmed more sulfate consumption in the porewater at the bottom (Figure V-11A and B). Moreover, Th normalized S_{tot} concentration in the sediment layers increased with depth, highlighting the spatial re-distribution of sediment-bound sulfur over time (Figure V-11C).

The ^{32}S isotope enrichment in the sediment phase compared to the bulk sulfur isotope signature in the initial sediment indicated that sulfate was reduced by bacteria in the columns (Figure V-11E). Sulfate reducing bacteria produce sulfide depleted in ^{34}S during their metabolism yielding negative isotope values ($\delta^{34}S$) in the precipitated sulfides in the sediment.[36] The extent of isotope fractionation is within the range (between 4‰ to 46‰) found for bacterial sulphate reduction in case of abundant sulfate.[36] In our columns the apparent fractionation between the initial sediment and the final column sediments ranged from 9.1 to 19.4‰. The isotope fractionation between the sediment-bound sulfur species and the remaining, non-reduced sulfate fraction in the porewater should be even higher, as non-reduced sulfate in the porewater phase is expected to get enriched in ^{34}S compared to the initial value of the total sulfur (i.e., the value in the initial sediment).

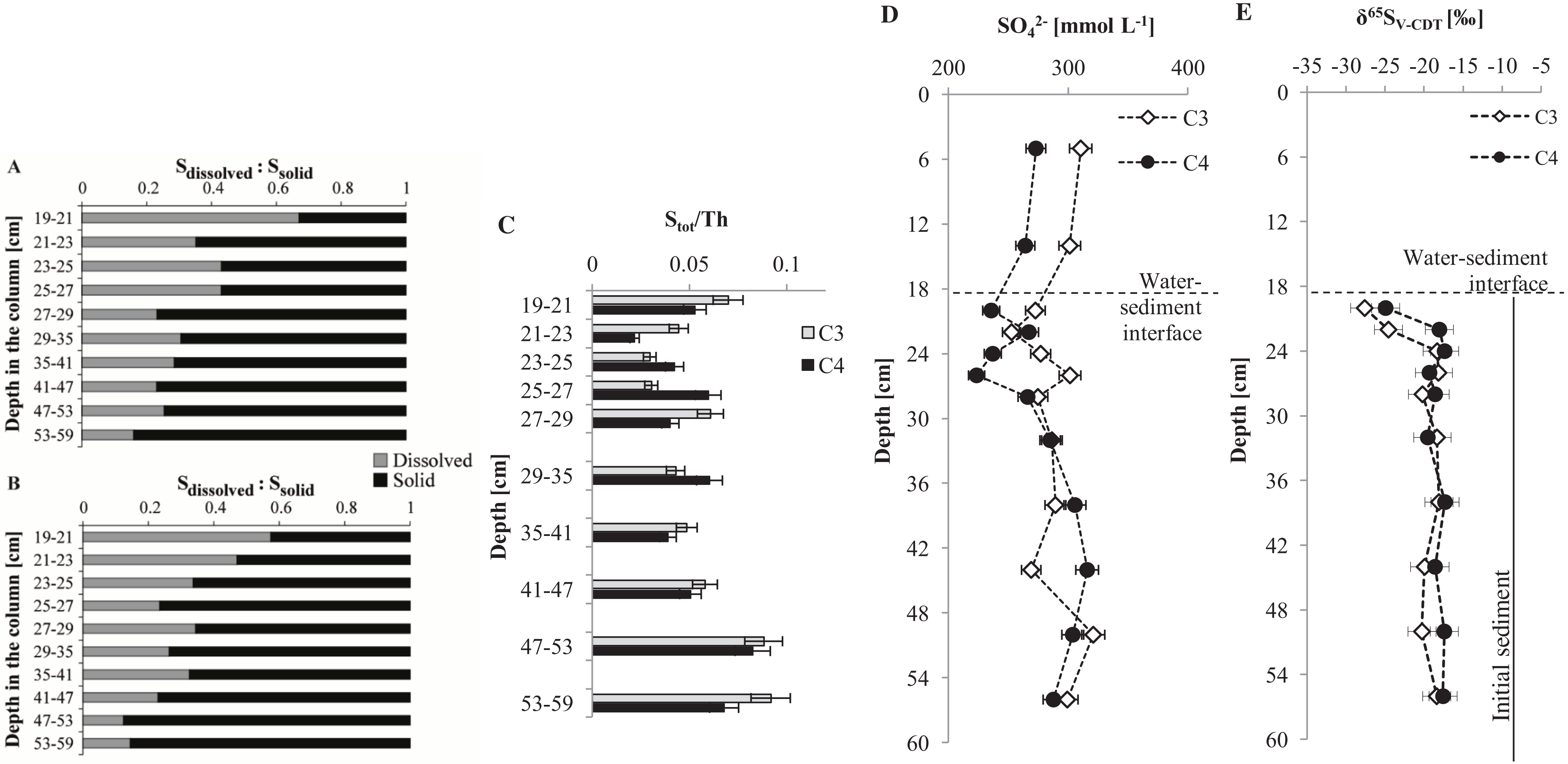

Figure V-11: *Total sulfur partitioning between the porewater and the sediment phases in C3 (A) and C4 (B), Th normalized S_{tot} concentrations in the sediment layers (C), and SO_4^{2-} concentrations in the overlying water and the porewater (D), sulfur isotope ratios in the sediment phase compared to the sulfur isotope ratio in the initial sediment (E).*

3.5. Geochemical evolution of the sediments

3.5.1. Silicon release during mineral dissolution

The biogeochemical changes previously described also induced significant changes in the geochemical composition of the sediment layers.

The silicon (Si) depth profiles (SiO_2/Th) revealed well-defined zones of Si dissolution in both columns, suggesting enhanced weathering of primary minerals as the main Si-bearing compartment of the sediments (Figure V-12). XRD analysis detected Si-bearing minerals such as quartz, feldspars and clays (i.e., kaolinite, illite and chlorite) (Table V-1). Silicate dissolution was observed through the high Si concentrations in the porewater (Figure V-12). The enhanced silicate dissolution at 6-8 cm depth below the WSI in C4 and at 8-10 cm depth in C3 may be due to bacterially-induced alkaline leaching of quartz (quartz dissolution can be produced at $pH \geq 9$).[37] The dissolution of quartz can be presumed as depletion in K or Al that would be expected in the case of dissolution of feldspars and clay minerals could not be observed in the Si depletion zone. At alkaline pH (> 6-7), the dissolution rate of quartz can increase in the presence of alkali and alkaline earth cations in the porewater. In our case, higher concentrations of Ca prevailed in the porewater of upper sediment layers, which may be involved in the quartz dissolution (Figure V-13).[38] In addition, the porewater Si might have been taken up by the algae and diffused to deeper layers as highlighted by the higher Si concentrations 16 cm below the WSI (Figure V-12).[39]

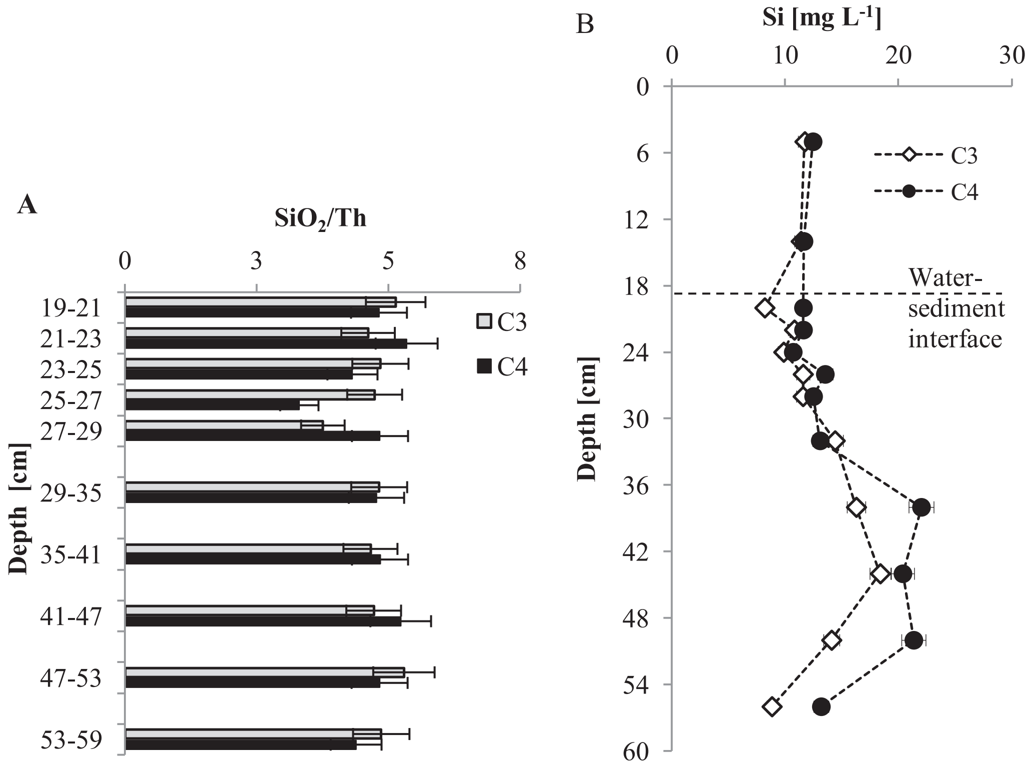

Figure V-12: *The Th normalized Si concentrations in the sediment layers (A) and Si concentrations in the overlying and the porewater (B) of the columns C3 and C4.*

3.5.2. Dissolution of Ca-bearing phases

Ca significantly varied in the sediment layers indicating the dissolution of Ca-bearing calcite and dolomite minerals (the major Ca-bearing minerals detected by XRD) in the sediments (Figure V-13 and Table V-1). Dissolution of carbonates at 6-8 cm depth below the WSI can be related to pH decrease caused by the release of H^+ during pyrite oxidation or by the production of CO_2 during the degradation of SOM by heterotrophic microorganisms.[40] Noteworthy, it has been shown recently that photosynthesis can also produce CO_2 due to the use of HCO_3^- as a mobile H^+ acceptor that leads to the transient formation of H_2CO_3 prior to decay into H_2O and CO_2.[41] The molar ratios between Ca, Mg and HCO_3^- enable distinguishing processes of calcite dissolution in sediments (i.e., pH decrease or CO_2), assuming stoichiometric dissolution of carbonates.[40] Molar ratios of ~0.3 to ~0.4 (Ca+Mg over HCO_3^-) in the upper layers characterized by significant carbonate dissolution point to the production of CO_2 (Table V-S2). Molar ratios below 0.5 (as for CO_2 driven calcite and dolomite dissolution)[40] suggest that Na_2CO_3 dissolution contributes to the porewater carbonate load. The high Ca concentrations observed in the porewater of the top sediment layers, decreased with depth highlighting alkalinity production during more pronounced sulfate-reduction in the bottom layers likely leading to calcite precipitation (Figure V-13).[40] However, precipitation of gypsum cannot be excluded due to the high sulfate concentrations.

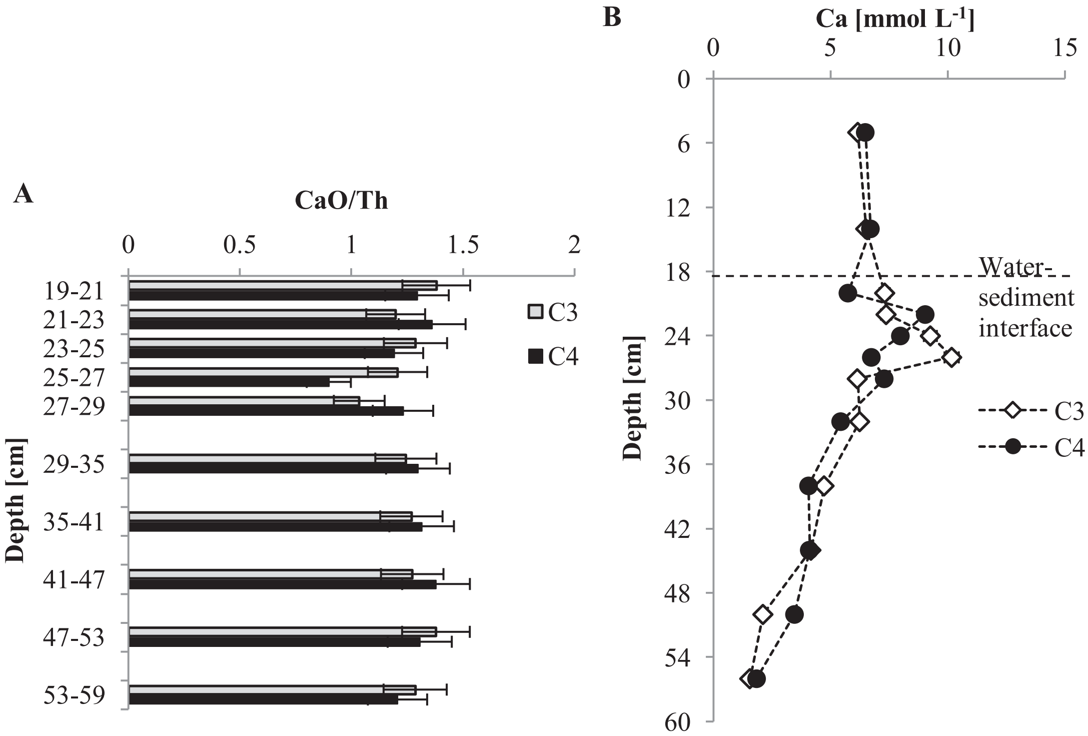

Figure V-13: Th normalized Ca concentrations in the sediment (A) and Ca concentrations in the overlying and the porewater (B) of the columns C3 and C4.

3.6. Cu distribution and isotope fractionation in the sediment

The depth profiles of Th normalized Cu concentration revealed changes in the Cu distribution over time (Figure V-14B). Cu accumulated in the sediment columns from 4 to 8 cm below the WSI in C4, and from 8 to 10 cm in C3 (Figure V-14B). These zones of the sediment corresponded to those with the largest biogeochemical changes, indicating that Cu accumulation may be related to microbial activity. Cu stable isotope ratios in the sediment bulk did not significantly change in the column C3, whereas a small but significant change was observed in the column C4 at the zone of Cu accumulation ($\delta^{65}Cu_{initial}$ - $\delta^{65}Cu_{C4,6\text{-}8cm}$= -0.19‰) (Figure V-14C and D). The difference between the two columns may be related to the larger Cu accumulation in C4 compared to C3. Change in the Cu isotope ratios between the initial sediment and the Cu-enriched layer in the C4 also indicates that the Cu re-distribution in the sediment resulted in Cu isotope fractionation.

Cu partitioning also changed over time in the column, as revealed by the sequential extraction (Figure V-15). In particular, the amount of extractable Cu decreased over time: 61%

in the initial sediment compared to 33%, 29% and 9% after 40, 80 and 520 days of sediment aging (Figure V-15). No acid-soluble Cu fraction was observed after 40 days. Meanwhile, the non-extractable Cu (residual Cu) was the only fraction that increased, suggesting Cu transformation from more mobile to more strongly bound Cu (and less available) (Figure V-15). In aged sediments (after 520 days), no systematic spatial pattern was noticed, except for an increase of the reducible Cu fractions in sediment layers below the Cu accumulation zone, which was more marked in the C4 (Figure V-16).

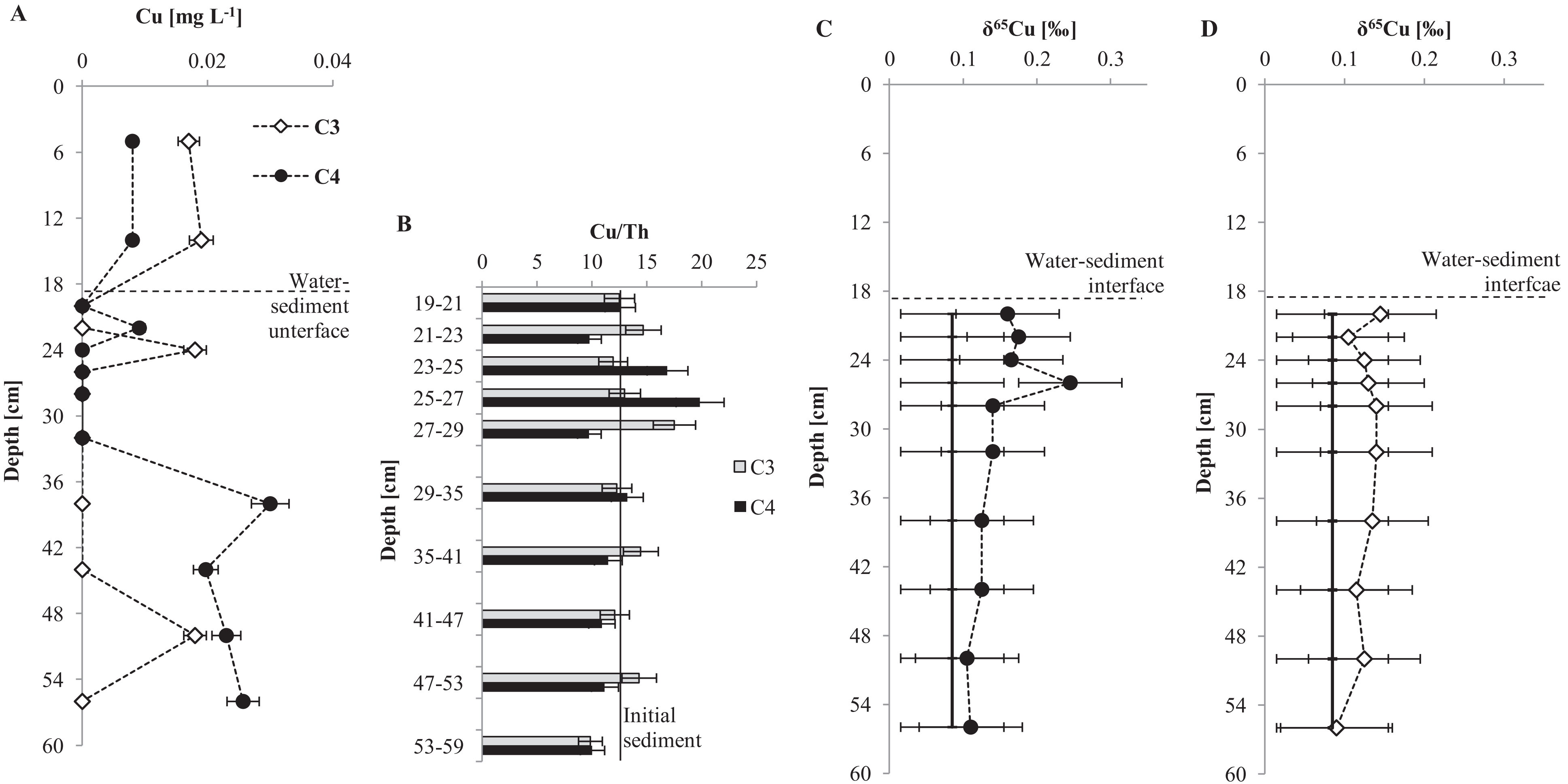

Figure V-14: *Depth profiles of Cu concentrations in the porewater of the columns C3 and C4 (A), Th normalized Cu concentrations (B), and the δ⁶⁵Cu of the sediment-bound Cu in the columns C4 (C) and C3 (D) compared to δ⁶⁵Cu of the initial sediment. Error bars denote the 2s of the Cu isotope measurements of the geological standards (±0.07‰, n=15).*

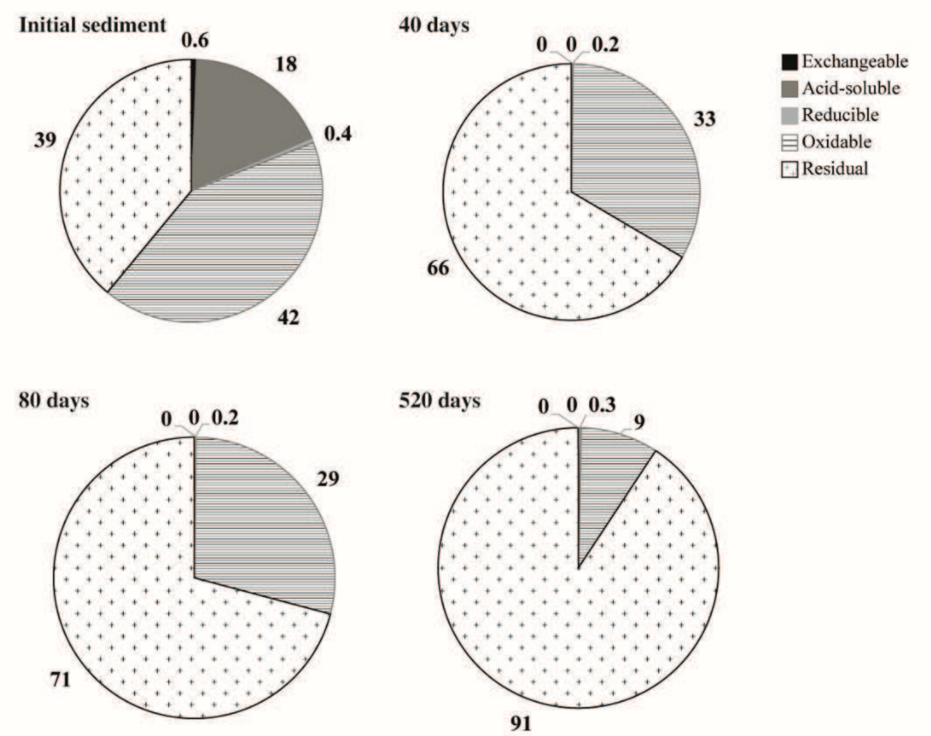

Figure V-15: *Temporal changes in the Cu partitioning using sequential chemical extractions of the initial sediment, after 40 days, 80 days and 520 days.*

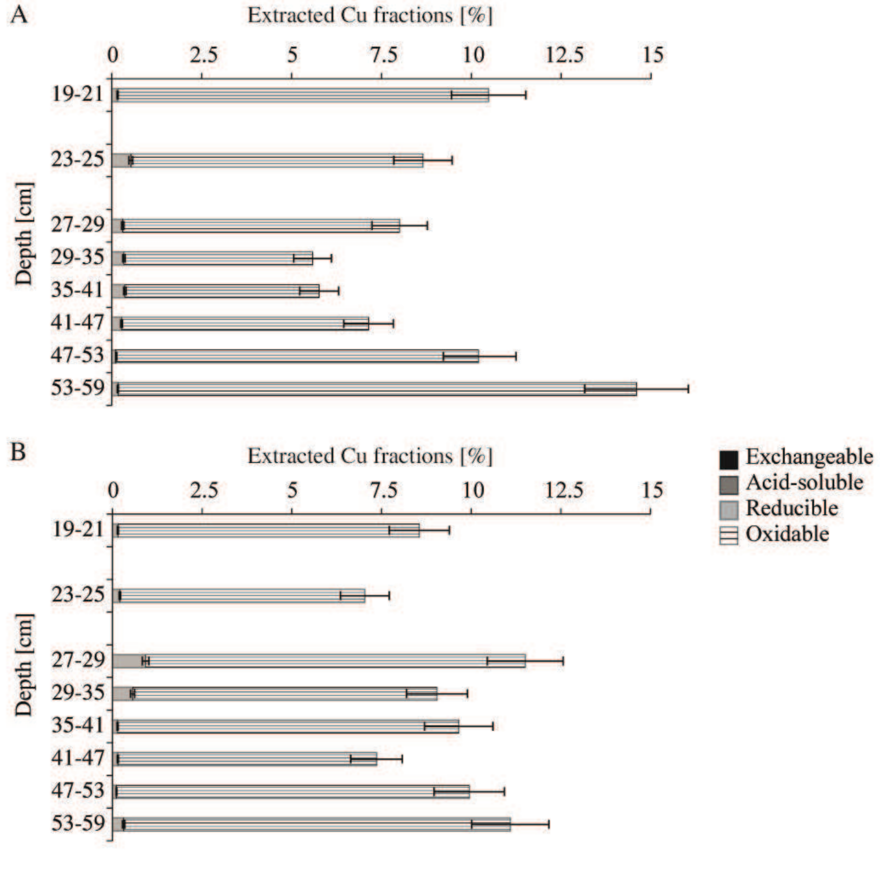

Figure V-16: *Spatial changes in the geochemical partitioning of Cu using sequential chemical extractions in the columns C3 (A) and C4 (B). The residual Cu fraction accounts for the difference between the sum of all extractable Cu fractions and total Cu in the sediment and is not represented.*

4. Discussion

4.1. Biogeochemical conditions and redox gradients in the Winogradsky columns

Although high DO concentrations were observed in the overlying water produced by the photosynthetic activity of the algae, the redox-indicative species (i.e., DO, Fe^{2+}, S^{2-}) indicated reducing conditions below the sediment surface with increasing rates of Fe^{3+} reduction and sulfate reduction with depth. Based on the initial sulfate load added to the sediments and the estimated remaining sulfate in the different sediment layers (assuming that all sulfate was in dissolved form in the porewater) after 520 days, estimates of sulfate consumption rates in each layers range from 0.30 μmol SO_4^{2-} cm^{-3} d^{-1} in top layers to 0.49 μmol SO_4^{2-} cm^{-3} d^{-1} in the bottom layers. These rates are comparable to those observed in sulfate enriched lake sediments (≤0.30 μmol SO_4^{2-} cm^{-3} d^{-1}).[35] Such high rates are rarely found in natural lake sediments, however, in seawater sediments even higher rates are regularly observed.[42] Gypsum precipitation might also contribute to the sulfate consumption, as the porewater was saturated with regards to gypsum (K_{sp}= $2.5*10^{-5}$) (Table V-S2). However, XRD analysis did not reveal any precipitated sulfate minerals (such as gypsum).

The higher isotopic shift found in the top sediment layers ($\delta^{34}S_{initial\ sed}$- $\delta^{34}S_{final\ sed}$ = 16.3 to 19.4‰) in the columns compared to the initial sediment also suggests lower rates of sulfate reduction compared to deeper layers ($\delta^{34}S_{initial\ sed}$- $\delta^{34}S_{final\ sed}$ = 9.1 to 12.0‰). The extent of the sulfur isotope fractionation is suggested to reflect the rate of bacterial sulfate reduction.[42] Other processes affecting sulfur partitioning between porewater and sediment phases, such as bacterial and abiotic oxidation of sulfides (notably pyrite) are not expected to cause isotope fractionation in the same extent as sulfate reduction, and generally fall within the range of −4‰ to 3.5‰ relative to the sulfide.[43,44] These results support the idea that the lower S isotope fractionation occurring in the bottom layers corresponds to higher sulfate reducing rates (Figure V-11E).[42] Therefore, the Rayleigh isotope fractionation model was applied to evaluate the rates of sulfate reduction in the sediment layers (1):

$$\delta^{34}S_{sulfide} = (\delta^{34}S_{initial} + 1000) \times \frac{1 - f^{\alpha}}{1 - f} - 1000 \qquad (1)$$

where, $\delta^{34}S_{sulfide}$ is the sulfur isotope ratio of the accumulated sulfide in the sediment layers and $\delta^{34}S_{initial}$ is the initial sulfur isotope ratio of the sulfates. In our case we assumed that the $\delta^{34}S_{initial} \approx \delta^{34}S_{initial\ sediment}$, which is a rough assumption as the initial sediment probably contained other sulfur forms than sulfate species. f in the equation stands for the fraction of the residual, non-reduced fraction of sulfate in the porewater and α is the fractionation factor between the sulfate and the sulfide phases. In order to estimate which sulfate-reduction rates can result in the measured $\delta^{34}S_{sulfide}$ observed in the sediment layers, the sulfur isotope fractionation factors observed in organic rich sediments (from two sites: Solar Lake, Egypt, Logten Lagoon, Denmark) in which sulfate was not limited (as in our case) for high (>10 µmol cm^{-3} d^{-1}) and lower sulfate-reduction rates were used.[42] Lower reduction rates typically induce a sulfur isotope fractionation of 30 - 40‰, whereas at higher rates it ranges from 10 - 20‰.[42,45,46]

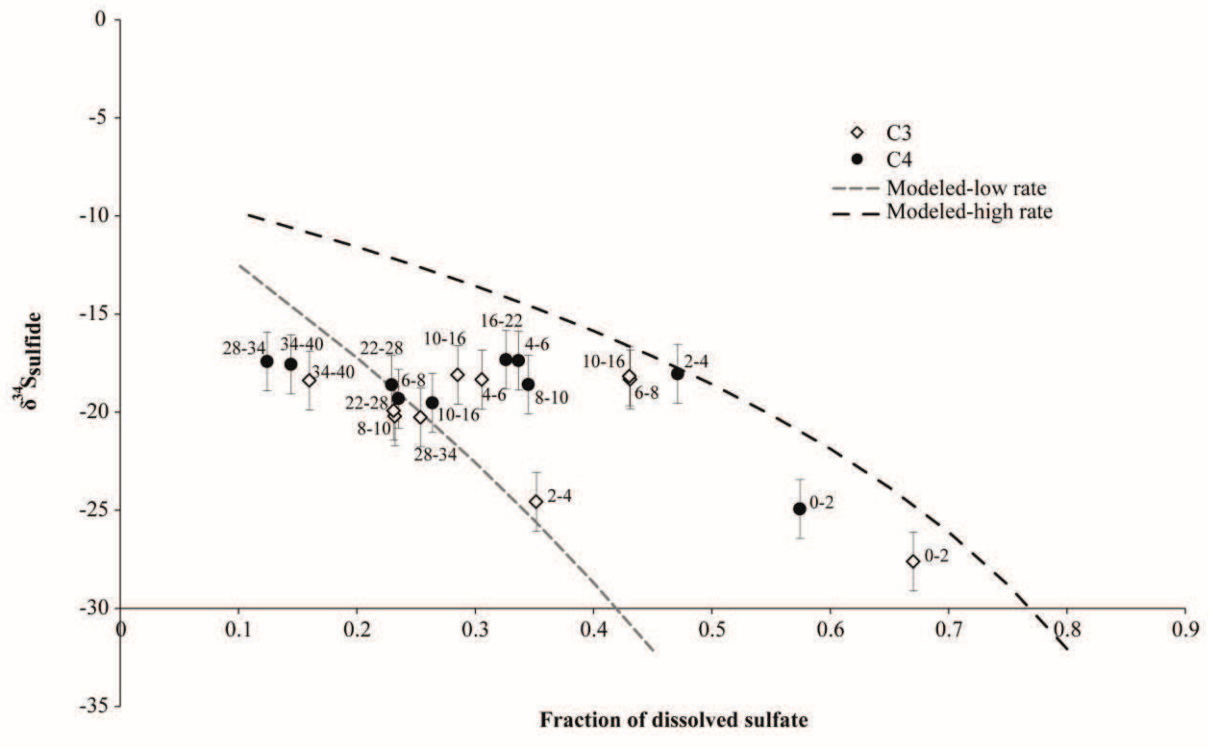

Figure V-17: Theoretical and observed $\delta^{34}S$ values of reduced sulfides in the column sediments for low and high rates of sulfate reduction. The depth of the layers is displayed close to the symbol (in cm below the WSI).

The modeled $\delta^{34}S_{sulfide}$ values suggest higher sulfate-reduction rates in the top sediment layers (Figure V-17).[42] Hence, the sulfate reduction rates based on S isotope data or on the sulfate consumption in the different layers during the 520 days of sediment aging are contradictory. However, recent findings suggest that sulfur isotope fractionation rates may not be directly related to the sulfate reduction rates but can highlight different metabolic pathways of sulfate reduction.[47] Sulfate reducers that oxidize the carbon source completely to CO_2 showed greater fractionations than sulfate reducers that produced acetate as a final product of carbon metabolism.[47] Therefore, the apparently lower isotopic shift between the initial and aged sediment in the bottom layers might be due to the incomplete oxidation of the SOM, which corroborates with higher acetate concentrations found in these layers (Figure V-3).

Sulfate reduction thus prevailed in the whole sediment column with probably higher rates at the bottom. Methanogenesis was probably not a major SOM-metabolizing process, even at the bottom layers, as high sulfate concentrations remained in the porewater (>200 mM L^{-1}) after 520 days of evolution that can suppress methanogenic activity.[48]

Besides, the intense cycle of S by microbially mediated processes, Fe re-distribution in sediments during sediment aging may also be explained by the microbial activity. In the columns, the heterogeneous re-distribution of Fe along the sediment depth can be related to the oxidation of Fe^{2+} or Fe^{2+}-bearing phases, such as pyrite in the top sediment layers, as well as Fe^{3+} reduction in the bottom layers.

In intertidal sediments, Fe enrichment was observed at the redox boundary as a result of the oscillating hydrological and redox conditions.[49] This suggests that oxidative dissolution of Fe sulfides with subsequent precipitation of Fe oxy(hydr)oxides resulted in trace metal sorption and co-precipitation within the Fe-enriched zone. In our case, oxidation of Fe sulfide species might have occurred by microbial Fe sulfide oxidation as the whole sediment was anoxic except for the sediment periphery exposed to light, where algae developed. Anaerobic Fe sulfide oxidation is coupled to nitrate reduction as sulfides can serve as an electron donor in nitrate reduction processes.[50] Although nitrate could not be quantified in the porewater, nitrate-N was detected in the top sediment layers in C3 that supports the idea of nitrate reduction in the columns (Table V-1). In soils, sulfide has been shown to promote dissimilatory reduction of nitrate to ammonium rather than denitrification, thereby explaining ammonium increase with depth.[51]

However, no significant Fe depletion was observed in the top sediment layers and Fe might be mobilized from the bottom sediment layers (Figure V-8) during dissimilatory Fe^{3+}-reduction that uses Fe^{3+} as a TEA and fermentation products as a carbon source.[28] The use of Fe^{3+} as a TEA by the SOM fermenting microorganisms may also explain why higher concentrations of acetate and ferrous iron (Fe^{2+}) co-occurred in the porewater of the bottom sediment layers (Figure V-2 and V-9).[28] At circumneutral pH Fe oxides and other Fe-bearing minerals are known to be insoluble, however, Fe^{3+}-reducing bacteria use pili and flagella as an electron shuttle to transfer electrons to crystalline solid-phase Fe^{3+} in Fe oxides, but also in clay minerals.[33,52] The produced Fe^{2+} can be stabilized by dissolved OM, thereby maintaining Fe^{2+} in solution.[53] Upwards Fe^{2+} diffusion might have occurred in the column, followed by re-oxidation in the upper sediments driven either by Fe^{2+} oxidizing bacteria using Fe^{2+} as an electron donor or chemically by DO or Mn(IV).[33] Under circumneutral pH, phototrophic and nitrate-dependent Fe^{2+}-oxidizing bacteria can oxidize Fe^{2+} in the absence of DO to produce Fe-oxides.[33] Nitrate-dependent Fe^{2+} oxidation may prevail in the Winogradsky columns, as phototrophic bacterial activity is strictly limited to the peripheral sediment layer that is exposed to light. The microbially driven Fe^{2+} re-oxidation produces Fe^{3+} oxy(hydr)oxides or mixed Fe^{2+}/Fe^{3+} phases (such as green rust) in the sediments.[33]

Slightly higher inorganic carbon concentrations and Fe_{tot} enrichment were observed in the Fe-enriched layers in the Winogradsky columns, corresponding to conditions for green rust formation (Figures V-2 and V-7). Green rust is a mineral with a mixed Fe^{2+}/Fe^{3+} valence formed as a product of anaerobic Fe^{2+} oxidation at the Fe^{2+}/ Fe^{3+} redox boundary with carbonates and sulfates at slightly alkaline pH.[33,54] Green rust is a highly reactive mineral subject to fast oxidation when exposed to oxygen.[54] Fast oxidation of green rust may explain why it could not be detected with XRD analysis in the layers characterized by higher Fe and C_{inorg} concentrations.

On the other hand, the hematite detected in the column C4 might have been produced as a result of microbial Fe^{2+} re-oxidation. Although less crystalline Fe oxy(hydr)oxides, such as ferrihydrite, may be the main product of microbial Fe^{2+} oxidation,[55] poorly crystalline Fe oxy(hydr)oxide phases could not be detected by the XRD analysis. However, hydrous Fe oxides can be transformed into more crystalline hematite even under natural conditions within a reasonable timeframe (estimated half-life: ~300 days at 25°C).[55,56] In addition, Fe oxides might not have been efficiently extracted during the sequential extraction. Well-crystallized Fe oxides, such as hematite, are hardly dissolved at room temperature with acidified hydroxylamine as used in our protocol.[34] Extraction recoveries for Fe oxide phases in the reducible fraction may thus be underestimated. Altogether, more than 90% of Fe was in the residual phase accounting for hematite, pyrite-Fe, chlorite-Fe and Fe in primary minerals, as pyrite (Fe sulfide) is not expected neither to be extracted by the applied extraction protocol as nitric acid is necessary to dissolve pyrites.[57]

In addition to Fe oxides, Fe sulfide species may thus also contribute to the sequestration of Fe^{2+}. The pyritization of the reduced Fe may be an important process of Fe sequestration in anoxic sediments. The presence of Fe sulfides was not evidenced in the sediments by XRD, but Fe sulfides can be re-oxidized rapidly exposed to air.[58]

Overall, the results highlight that the Fe and S redox cycle in the sediments subjected to biogeochemical processes significantly influenced the distribution of trace metals, such as Cu.

4.2. Copper aging at biogeochemical gradients

The re-distribution of Cu along the sediment depth profile suggests Cu mobilization by re-oxidation of reduced Cu species (mainly sulfides) and/or the release of Fe oxy(hydr)oxide-bound Cu.

In anoxic sediments, Cu distribution and speciation can be affected by the degradation of SOM and the release of SOM-bound Cu, the formation/dissolution of oxy(hydr)oxides of Fe, Al as well as the redox-induced changes of sulfur forms and the subsequent sequestration/mobilization of reduced sulfides.[59,60] In addition, microorganisms and algae may also take up some Cu, although biological Cu uptake cannot be identified as the biomass was part of the SOM pool of sediment. The processes involved in Cu transformation and re-distribution amongst the sediment layers are discussed below based on the isotopic and geochemical data.

The isotopically heavier Cu-enriched zone in C4 suggests that either Cu isotope fractionation took place during Cu mobilization or that isotopically heavier Cu-sorbing phases were dissolved (i.e., SOM, Fe/Al oxy(hydr)oxides). Cu was found to be mainly associated with the residual phases in the sediments after 520 days, indicating that Cu was likely associated with sulfides, silicate minerals and crystalline Fe oxide phases not extracted by the applied extracting reagents.

The decreasing Cu proportions in the oxidable fractions of the sediments during sediment aging suggest that Cu could be mobilized in the sediments due to SOM degradation.[61] This idea is supported by the isotopic data as SOM-bound Cu is expected to be isotopically heavier.[16,17] In contrast, the largest SOM mineralization occurred in the sediment layers enriched in Cu which does not confirm Cu mobilization during SOM degradation. The geochemical data rather suggest that Cu dynamics within the sediments are linked to the Fe biogeochemical cycling.

Secondary minerals, such as Fe-bearing clay minerals and Fe oxy(hydr)oxides sorb significant amounts of Cu.[60] Microbially mediated Fe redox processes may thus cause the co-mobilization of Fe and Cu in the bottom sediment layers and Fe enrichment in the upper sediment layers where Fe likely re-precipitated.[49] Clay minerals are expected to sorb isotopically lighter Cu,[62] which does not support the hypothesis of Cu mobilization during microbial Fe-bearing clay dissolution in the bottom layers, as the mobilized Cu was isotopically heavier. Instead, Fe and Al oxy(hydr)oxides were major sorbing phases of Cu in runoff in stormwater wetland sediments (see Chapter IV). Fe and Al oxy(hydr)oxides sorb isotopically heavier Cu, and reductive dissolution of Fe oxy(hydr)oxides are expected to release isotopically heavier Cu into the porewater that may re-precipitate in the upper layers.[14,15] Al oxy(hydr)oxides are not affected by the changing redox conditions. Cu diffusing upwards can be subsequently entrapped into precipitating Fe oxy(hydr)oxides following microbially-mediated Fe oxidation in upper layers or by the forming metal sulfides.

The increasing residual Cu fraction during sediment aging may also suggest the formation of Cu sulfides in the sediments.[63] The dissolution of reduced sulfur species in the sediment surface and at the sediment periphery colonized by algae (the top 5 -6 cm) may also cause Cu re-distribution within the sediment columns. Isotopically heavier Cu is expected to be released during re-oxidation of the Cu^+ sulfides.[18,19] On the other hand, the formation of soluble bisulfide and polysulfide complexes with Cu at high sulfide concentrations (>30 µmole L^{-1}) prevailing in the porewater in the bottom layers can increase dissolved Cu concentrations.[57,64] Hence, dissolved Cu bisulfide and polysulfide complexes may diffuse upwards and can be subsequently entrapped into the precipitating Fe and/or sulfide phases. Our results do not support Cu mobilization as colloidal reduced Cu^+ and Cu^0 as previously observed,[8] because the reduced Cu^+ sulfide phases are expected to be isotopically lighter.[18]

The relative increase over time of Cu associated with the residual fraction also highlights the aging of Cu in the Winogradsky columns (Figure V-15). An increase in the residual Cu fraction may be due to Cu binding to higher energy binding sites during aging, thereby reducing Cu mobility and availability as showed previously in soils.[11,60] Cu may undergo aging following its rapid sorption to vineyard soils at Rouffach, as previously hypothesized (see Chapter III). If diffusion processes are involved, ageing should favor the stronger binding of isotopically lighter Cu into the sediment, leaving the mobile Cu pool heavier. This phenomenon would explain the shift towards ^{65}Cu- enrichment (mobilized from other layers) in the Cu accumulating layer in C4. However, Cu isotope fractionation upon Cu diffusion into micropores has not been investigated yet.

The lack of significant ^{65}Cu depletion in the sediment layers where Cu depletion occurred did not enable to distinguish Cu release upon Fe^{3+} reduction in the bottom layers from oxidation of Cu sulfides in the top layers. Although minor Cu amounts were mobilized in individual layers, it might be insufficient to result in a significant isotope fractionation in the Cu-depleted layers.

5. Conclusion and perspectives

Overall, the long-term fate of Cu in wetland sediments is intimately linked to the redox cycle of Fe and S. Our results suggest that Cu can be released from the sediments into the porewater under both Fe-reducing and sulfate-reducing conditions, but also through the oxidation of sulfides. Based on the sequential extraction and Cu isotope analysis in the solid phase the prevailing process by which Cu was released could not be identified.

Changes in the chemical composition of the initial sediments were highlighted in the Winogradsky columns following 520 days of aging. These changes can be attributed to the TEAPs responsible for the Fe re-distribution amongst the sediment layers, as well as the SOM mineralization by the heterotrophic organisms potentially causing the dissolution of carbonates. Higher rates of sulfates reduction at the bottom layers and active sulfate reduction in the whole sediment column underscored the sulfur dynamics in the columns. The silicate mineral dissolution in some layers of the sediments, although surprising, may be explained by the alkaline pH conditions and the presence of algae for which Si is an important nutrient.

Our results suggest that the isotopically heavier Cu^{2+} species are more mobile in anoxic sediments upon reducing conditions and Cu can be released in the reducing sediments following the reductive dissolution of Fe^{3+} oxy(hydr)oxides and/or Cu complexation with polysulfides. Although the isotopic data do not support the mobilization of reduced Cu species, the re-oxidation of Cu sulfides by oxygen produced by algae or sulfur-oxidizing bacteria may also be a Cu mobilizing process in the upper sediment layers. Sequential chemical extractions indicate increasing proportions of Cu in the residual phases with Cu aging in the sediments. These findings suggest that Cu is increasingly associated with more strongly binding phases, such as crystalline Fe oxides, as well as Cu sulfides.

Cu isotope measurements in environmental settings are often useful in deciphering the processes that control the transformation of Cu. In our study, Cu isotope ratios have not been analyzed yet in the porewater due to analytical difficulties. In the Winogradsky experiments small isotopic differentiation was produced within the sediment layers showing the limited interest of using Cu isotopes in bulk and static systems. However, Cu isotope ratios in the porewater phase may confirm or reject the hypothesis that Cu mobilized in the sediments would be isotopically heavier. In contrast, $\delta^{65}Cu<0$ in the porewater samples would indicate the mobilization of reduced colloidal Cu species.

DNA extraction and the characterization of the microbial communities using high-throughput sequencing techniques (16S rRNA of Bacteria and Archaea) in the sediment layers of the Winogradsky columns are planned in the future and may provide relevant information on biogeochemical gradients established in the columns. The microbial community characterization may also confirm that the Fe redox cycle is microbially driven in the sediments. Presence of sulfate reducing and sulfur oxidizing bacterial species would also help in interpreting how the microbial sulfate/sulfur-transforming processes controlled the sulfur distribution in the sediment columns and affected Cu cycling.

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SUPPORTING INFORMATION

	Depth	pH_{H2O}	OM	N_{tot}	$N\text{-}NO_3^-$	$N\text{-}NH_4^+$	SiO_2	Al_2O_3	MgO	CaO	Fe_2O_3	MnO	TiO_2	Na_2O	K_2O	P_2O_5	S_{tot}	Cu	Zn	Ni	Th
	[cm]	[-]	[g kg^{-1}]		[mg kg^{-1}]		[%]											[mg kg^{-1}]			
Column 3	19-21	n.a.	146.0	9.20	n.a.	n.a.	46.50	7.50	1.40	12.50	3.43	0.090	0.43	2.47	1.93	0.81	0.63	113.1	122	28.7	9.05
	21-23	9.05	62.4	2.36	3.53	60	45.40	8.60	1.46	11.80	3.80	0.071	0.44	2.47	2.14	0.55	0.44	144.4	137	29.7	9.84
	23-25	8.91	68.0	2.50	1.52	121	46.30	8.70	1.50	12.30	3.77	0.078	0.44	2.85	2.16	0.71	0.28	113.9	125	29.5	9.55
	25-27	8.88	62.7	2.26	<1.53	211	46.30	8.60	1.46	11.80	3.78	0.081	0.45	2.70	2.13	0.73	0.30	126.8	139	28.6	9.77
	27-29	9.06	55.4	2.09	<1.27	253	38.80	10.10	1.60	10.70	4.78	0.098	0.44	3.28	2.43	0.93	0.63	180.8	182	36.3	10.33
	29-35	9.22	81.9	3.06	<1.22	582	46.90	8.40	1.43	12.10	3.66	0.081	0.44	2.61	2.09	0.75	0.42	119.2	129	28.5	9.72
	35-41	9.55	69.7	2.66	<1.16	714	44.40	8.80	1.50	12.10	3.91	0.086	0.44	2.93	2.16	0.85	0.46	137.6	137	30.4	9.53
	41-47	9.77	67.4	2.57	<1.4	929	45.60	8.50	1.47	12.30	3.74	0.083	0.44	2.93	2.13	0.81	0.56	116.6	128	29.0	9.66
	47-53	9.81	75.1	2.88	<1.39	1140	49.50	7.70	1.39	12.90	3.22	0.075	0.44	2.56	1.94	0.68	0.82	133.6	105	26.3	9.35
	53-59	9.86	76.0	2.89	<1.27	1020	48.40	7.80	1.39	12.80	3.30	0.077	0.43	2.54	1.95	0.73	0.91	98.0	110	26.0	9.95
Column 4	19-21	9.37	94.3	4.76	n.a.	n.a.	45.80	8.00	1.43	12.30	3.59	0.079	0.44	2.41	2.03	0.64	0.50	119.4	128	27.7	9.50
	21-23	9.04	77.3	2.85	<1.66	131	51.00	7.90	1.47	13.00	3.18	0.066	0.43	2.11	1.99	0.49	0.21	93.2	102	25.5	9.54
	23-25	9.00	52.6	1.80	<1.5	169	42.40	9.20	1.52	11.70	4.25	0.087	0.44	2.56	2.23	0.74	0.42	165.7	158	33.3	9.82
	25-27	8.91	55.1	1.87	<1.55	294	36.80	11.00	1.69	10.00	5.25	0.106	0.45	3.25	2.63	1.00	0.66	220.6	205	41.1	11.12
	27-29	9.01	69.7	2.43	<1.46	426	49.90	7.60	1.34	12.70	3.19	0.074	0.44	2.24	1.89	0.60	0.41	100.6	107	25.1	10.31
	29-35	9.16	63.1	2.25	<1.4	775	46.30	8.40	1.46	12.60	3.75	0.084	0.44	2.47	2.03	0.78	0.58	128.1	134	29.6	9.70
	35-41	9.55	62.8	2.22	<1.27	836	47.10	8.20	1.43	12.80	3.73	0.081	0.45	2.55	2.03	0.76	0.38	111.7	122	44.7	9.73
	41-47	9.77	72.1	2.65	<1.47	1140	49.30	7.70	1.39	13.00	3.27	0.076	0.44	2.39	1.91	0.70	0.48	102.7	110	26.2	9.42
	47-53	9.86	72.9	2.78	<1.29	1040	47.70	7.90	1.43	12.90	3.38	0.078	0.45	2.73	1.98	0.75	0.81	110.3	120	27.2	9.87
	53-59	9.87	71.2	2.73	<1.25	897	47.60	8.00	1.43	13.10	3.43	0.079	0.45	2.66	1.98	0.74	0.74	108.9	118	27.0	10.85
	Initial sediment	7.85	77	n.a.	n.a.	n.a.	50.26	7.59	1.57	13.09	3.21	0.074	0.44	1.17	2.20	0.31	0.15	120.0	114	26.0	9.83

Table V-S1: *The physico-chemical characteristics and the major and some trace element compositions of the sediment layers of C3 and C4, as well as the initial sediment.*

	Depth [cm]	pH	NH_4^+	Na^+	K^+	Mg^{2+}	Ca^{2+}	Si	Acetates	CL^-	SO_4^{2-}	PO_4^{3-}	DOC	CO_3^{2-}	HCO_3^-	Alkalinity
		[-]	[mmol L^{-1}]										[mg L^{-1}]	[meq L^{-1}]		
Column 3	0-9	8.80	0.00	584.2	32.61	1.91	6.17	0.42	0.02	0.61	310.5	0.67	89	3.8	20.0	24.7
	9-19	8.72	0.00	571.2	31.71	1.93	6.51	0.41	0.09	0.94	301.4	0.69	89	2.8	20.5	24.2
	19-21	7.31	1.39	524.8	25.71	1.99	7.32	0.29	0.08	0.66	272.7	0.82	5200	0.5	29.3	31.4
	21-23	8.23	1.46	486.1	25.62	1.90	7.38	0.39	0.51	0.73	252.8	1.16	5300	1.5	27.0	29.5
	23-25	8.32	4.34	536.3	27.65	2.28	9.27	0.35	0.19	0.60	277.1	1.02	2610	3.8	39.0	43.9
	25-27	8.17	9.05	596.2	31.11	2.72	10.18	0.41	0.99	0.56	301.6	1.05	2750	1.7	58.8	62.2
	27-29	8.40	11.88	565.2	28.69	2.44	6.15	0.41	0.15	0.57	274.8	0.95	1460	5.8	60.0	66.6
	29-35	8.17	22.00	611.4	31.00	2.96	6.25	0.51	0.03	0.65	286.5	1.36	1300	6.5	105.0	112.7
	35-41	8.22	32.58	660.2	33.39	2.80	4.73	0.58	0.03	0.62	289.4	1.70	3550	11.8	153.0	166.5
	41-47	8.35	36.72	677.3	34.10	2.57	4.17	0.66	0.09	0.63	269.2	1.88	3900	20.0	192.0	213.8
	47-53	9.06	17.46	879.5	47.02	1.42	2.12	0.50	1.63	0.65	321.0	2.89	925	76.0	244.0	321.0
	53-59	8.47	43.75	799.4	39.97	1.46	1.56	0.31	1.38	0.68	299.3	2.80	4400	32.0	262.0	294.0
Column 4	0-9	7.97	0.00	517.3	30.18	1.87	6.49	0.44	0.10	0.57	273.1	0.60	84	5.3	19.8	25.8
	9-19	8.70	0.00	501.7	29.64	1.88	6.71	0.42	0.01	0.64	264.3	0.60	85	3.3	21.0	24.7
	19-21	8.30	1.47	451.7	24.17	2.29	5.75	0.41	1.20	0.68	235.8	1.05	567	0.8	25.0	27.4
	21-23	7.40	2.64	521.1	29.11	2.37	9.05	0.41	0.36	0.56	267.3	1.11	680	2.8	31.9	34.7
	23-25	8.29	6.02	462.9	25.77	2.22	7.99	0.38	0.28	1.08	237.1	1.05	971	3.6	38.9	43.4
	25-27	8.35	9.70	444.7	23.95	2.27	6.75	0.48	0.10	0.66	223.6	1.09	943	3.5	47.4	53.3
	27-29	8.29	17.59	552.5	29.73	2.75	7.30	0.45	0.16	0.71	266.2	1.03	1076	6.0	70.6	77.4
	29-35	8.28	26.64	603.3	32.36	3.09	5.44	0.47	0.08	0.70	285.0	1.40	3690	8.8	104.0	113.2
	35-41	8.43	32.11	698.5	37.88	3.08	4.07	0.78	0.10	0.69	305.7	2.33	1390	18.5	154.6	173.2
	41-47	8.40	44.00	794.4	43.24	2.65	4.10	0.73	0.26	0.66	315.9	3.12	2180	30.0	238.0	268.2
	47-53	8.55	40.11	832.0	45.11	1.87	3.47	0.76	1.31	0.68	303.9	2.81	1700	42.0	282.0	325.4
	53-59	8.46	46.42	812.8	43.58	1.45	1.85	0.47	2.10	1.58	287.8	3.25	2350	36.0	300.0	336.7

Table V-S2: *Hydrochemical parameters and major chemical composition of the water samples in columns C3 and C4.*

VI. Chapter: General discussion and perspectives

The adoption of a new geological age, the Anthropocene, is currently under debate in the scientific community. The Anthropocene is the slice of Earth's history during which people have become a driving geological force.[1] Mining activities alone move more sediment than all the world's rivers, and the consequences of human activities significantly impact the global climate, the oceans (i.e., sea level rising, acidification), thereby justifying adopting the concept of Anthropocene.[1]

Cu is one of the earth crust's elements whose global cycle has been deeply modified by human activity through mining, and its ubiquitous use in industry and agriculture.[2] Since more than 10 000 years, humans have been increasingly using Cu that progressively led to the dominance of anthropogenic over natural Cu flows at a global scale. Approximately 6% of all anthropogenic Cu inputs to the soils originate from agricultural Cu applications. Cu inputs in the environment has potential short and long term impacts on large portions of the agro-ecosystems, and resulting in diffuse pollution of soils by Cu. Cu use in agriculture started at the end of the 19th century and Cu remains extensively used today, mainly as fungicide formulae in vineyards and orchards. Cu has been accumulating in some agricultural soils since more than a century, and the risks of these chronic applications of Cu on ecosystem functioning and services are still not well understood. Evaluating how agricultural Cu use impacts on the ecosystem is complex and requires both analytical effort and appropriate means for the thorough and quantitative investigation. Investigating processes of Cu accumulation, distribution in soils and mobilization by rainfall is a prerequisite to better understand the risks related to the long-term application of Cu fungicides and to adopt strategies for limiting detrimental effects on soils and aquatic ecosystems ultimate receptors of the Cu contamination.

In this context, the present Ph.D. evaluated the fate of Cu fungicides in a vineyard system and a hydrologically connected wetland. Despite a multitude of studies on Cu speciation, Cu bioavailability and Cu transport, most investigations focused on the point characterization of Cu in soils and fail to provide insight into the processes that control the retention and the distribution of freshly added Cu at different scales of the ecosystems. We hypothesized that Cu stable isotopes may help capturing processes involved in the Cu partitioning through isotope fractionation, thereby providing an integrative framework for investigating the environmental fate of Cu. Cu stable isotopes were therefore used to trace processes of retention and distribution of Cu fungicides in vineyard soils, mobilization by rainfall and retention in wetlands under fluctuating hydrological conditions. Prior to the application of the Cu isotopic tool in field studies, we performed methodological adaptations to precisely measure the Cu isotope ratios in diverse environmental samples (i.e., soils, sediments, plants, water) (Chapter II). Combining Cu isotope analysis with other more traditional approaches (i.e., particle-size separations, sequential chemical extractions, XRD analysis, chemical composition analysis), we studied the contrasting behavior of Cu under oxic conditions prevailing in vineyard soils (Chapter III) and under fluctuating oxic-anoxic conditions occurring in wetlands (Chapter IV), with emphasis on the processes in anoxic sediments (Chapter V).

In this discussion chapter, we review the main outcomes and some transversal aspects of the different studies of the Ph.D. thesis, namely:

i) The Cu behavior under contrasting biogeochemical conditions in soils and wetland sediments,
ii) the Cu binding and distribution in soils and sediments,
iii) the contribution of Cu stable isotopes to investigate the environmental transport and reaction that anthropogenic Cu undergoes in an agricultural context,
iv) the sustainability of Cu use in agriculture for soil and aquatic ecosystems.

Some direct research perspectives to evaluate the transformation of Cu in soils and sediments by using Cu stable isotopes combined with other approaches (e.g. source tracing, microcosm experiments, extractions targeting specific phases, DNA analysis) are also presented below. As an example of longer-term perspective, evaluating the co-application of synthetic pesticides and Cu in agriculture, that may impact their environmental behavior and the biodegradation rates of the synthetic pesticides, is finally presented.

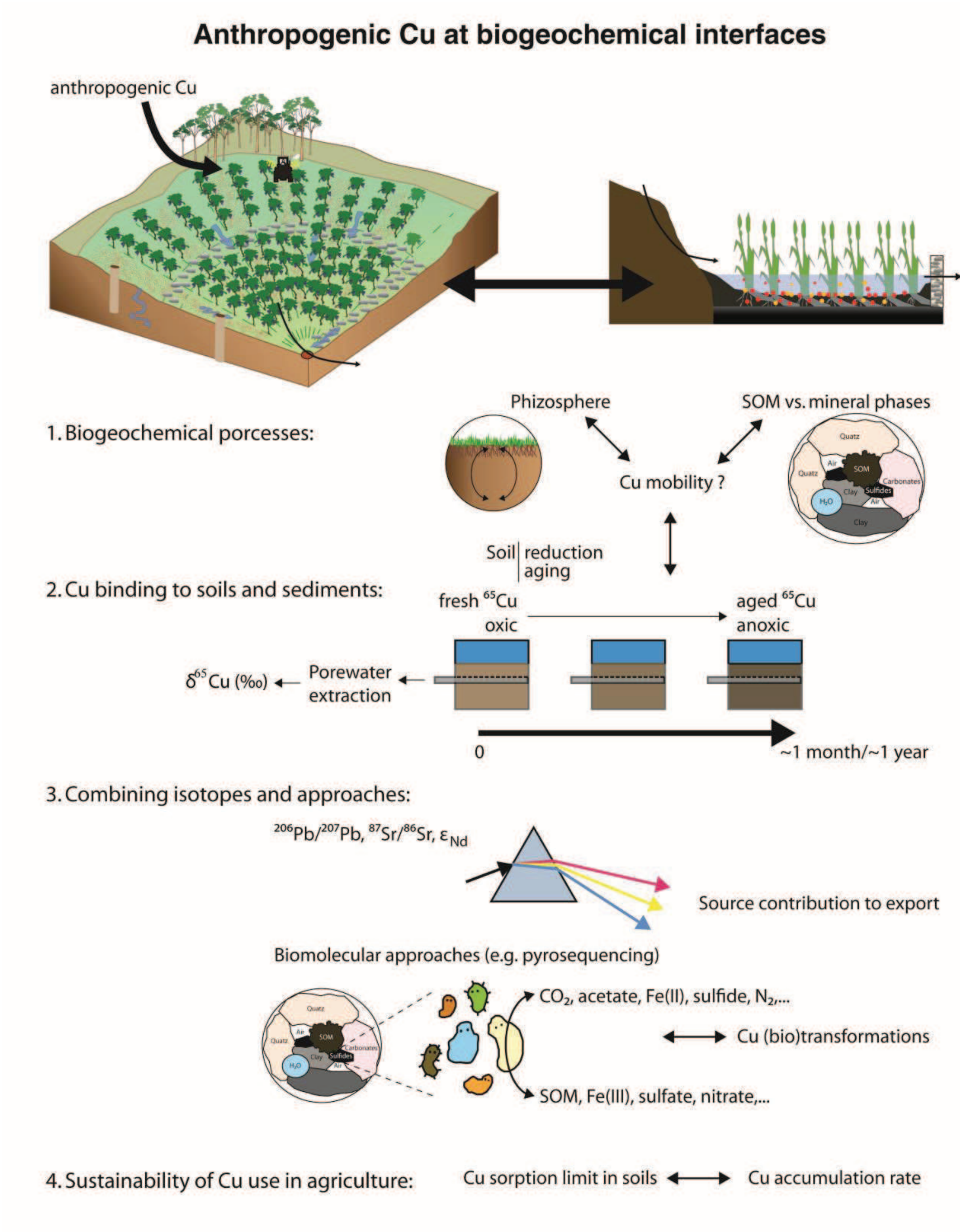

Figure VI-1: Graphical outline of the discussion and the perspectives.

1. Cu behavior under contrasted biogeochemical conditions

Cu is a versatile metal that can undergo a multitude of processes and has a contrasted behavior in different biogeochemical conditions. Cu has been shown to display contrasting behavior notably in oxic soils and hydromorphic soils.[3] Therefore, we have chosen to evaluate the impact of the diverse biogeochemical conditions on Cu behavior in well-drained (i.e., oxic) vineyard soils, in oxic-anoxic wetlands and predominantly anoxic wetland sediments. In our studies, we can highlight the contrasting behavior of Cu between the soils and the sediments that underscores the redox control on Cu speciation. Our studies also highlight how Cu transformations in oxic-anoxic sediments can alter Cu mobility.

We have shown based on Cu isotopic data that in the vineyard soils Cu was mainly controlled by sorption to clay minerals and probably complexation with soil organic matter (SOM). In contrast, in the wetlands, Cu(II) was likely reduced to Cu(I) species following Cu sorption to Al-bearing minerals. These results further confirm that Cu under oxic conditions is dominated by the Cu(II) oxidation state, whereas under anoxic conditions Cu(II) reduction occurs. Cu(II) species are suggested to be more mobile, whereas Cu reduction can reduce Cu mobility as Cu(I) species are less soluble.[4] Cu was mobilized in the vineyard soils, mainly associated with clays by soil erosion. Isotopically heavier Cu in the dissolved phase of runoff suggests also mobilization of organically-bound Cu in the soils by rainfall. In the wetland study, based on Cu isotopic data, we proposed that isotopically lighter reduced Cu species were mobilized under reducing and high-flow conditions. Recent studies have also shown Cu mobilization in soils under flooded conditions due to the formation of colloidal Cu(0) and Cu(I) sulfides.[5] However, the higher mobility of Cu(I)S colloids was not confirmed by the Winogradsky columns. Instead, we hypothesized the mobilization of isotopically heavier Cu(II) species that subsequently accumulated in Fe-enriched layers of the columns. These discrepancies may be explained by the kinetics of Cu sulfide oxidation that can be slow enough to enable the release of reduced Cu species from the wetland sediments upon high-flow conditions.

Overall, studies about the oxidation kinetics of Cu sulfides reveal contradicting results. While Caetano et al.[6] observed fast re-oxidation of CuS (re-oxidation occurring within 1 hour of aeration) in which Fe(III) may be involved, others argue that Cu sulfides are kinetically stable over periods of several hours due to their lower solubility compared to other metals sulfides.[7,8] Therefore, we suggest that the hydrological conditions may significantly impact the mobilization processes of Cu in anoxic sediments due to the slower re-oxidation kinetics of CuS. While the fast water flow may mobilize reduced Cu species without their re-oxidation in wetlands, in static anoxic sediments the TEAPs and photosynthesis likely mobilize Cu by the re-oxidation of reduced Cu species and/or dissolution of Cu-sorbing minerals (Fe oxides).

1.1. Cu behavior under soil reduction

However, the higher mobility of reduced Cu species in colloidal forms under soil reduction is still not completely understood. We still lack knowledge on how the eroded soil-bound Cu transported by runoff from the vineyard soils to the wetland changes its speciation and mobility in the submerged wetland sediments.

Perspective: The hypothesis of a higher mobility of reduced Cu species under soil reduction (changing from oxic soil-bound Cu(II) to reduced Cu(I) in wetland sediments) could be examined by subjecting an oxic vineyard soil to flooding conditions and following Cu concentrations in the porewater and its isotope ratio. To tackle this question, one can set up a series of microcosm experiments. In the experimental set-up, air-dried vineyard soils can be submerged with synthetic runoff water for a period of 1 month and investigated by regularly extracting some porewater volume for the eventual Cu release in the dissolved and colloidal phases. Before submerging the soils, they should be equilibrated with the applied synthetic water and centrifuged out as desorption/adsorption of nutrients can occur in the early stage.[9] The volume of the water for the soil submersion should be carefully evaluated to ensure that enough water remains in the microcosm until the end of the experiment (with a remaining overlying water) accounting for the porewater extractions. A suction cup previously installed in the microcosm can be used to regularly (e.g. daily during the first week and weekly thereafter) withdraw soil porewater and evidence temporal changes in Cu concentrations. The material of the suction cup should be beforehand tested to avoid Cu sorption on the cup material and eventual Cu isotope fractionation during porewater extraction. The soil porewater can then be subjected to ultracentrifugation to separate the colloidal from the truly dissolved phases. The influence of specific TEAPs on Cu mobility could also be tested by the addition to the soil of organic compounds, and/or sulfate (to favor sulfate-reduction), iron, etc. before the microcosm experiment.[10] Understanding the influence of soil reduction on Cu mobility is essential to better understand Cu transport at oxic-anoxic interfacial environments, such as wetlands.

1.2. The role of plants

Besides the redox conditions, plants may also impact Cu speciation in soils and sediments. In particular, they can enhance Cu mobility by changing pH and secreting metal-complexing organic agents in their rhizosphere.[11,12] On the other hand, they can sequester Cu in their biomass.[12,13] In our studies the direct role of the vegetation uptake was not assessed in the vineyard soils. We have studied Cu uptake by plants in the stormwater wetland and found a limited role of biomass uptake in Cu immobilization accounting for less than 4% of the total Cu load within the wetland. Based on these findings and previous studies about Cu uptake by grapevines,[14] we hypothesized that plant uptake may not play a significant role in the vineyard soils. However, we have found lower Cu concentrations in the top layers under grass-covered inter-rows, compared to the weeded inter-rows that may be due to local heterogeneities or to the impact of the grass cover on Cu mobility in soils. Cu leaching may occur in the rhizosphere by the formation of Cu-chelate complexes as has been previously observed for lead (Pb).[12] The direct (i.e., Cu uptake in the biomass) and the indirect role (i.e., influencing Cu mobility in soils) of the vegetation on Cu behavior in soils and sediments is hence a relevant research topic. For instance, Cu(II) complexation with root-secreted organic agents may enhance Cu mobility in soils. In wetlands, the mycorrhizal fungi associated with plants roots may also affect Cu mobility by the formation of Cu(0) nanoparticles.[15] pH is another parameter significantly affecting Cu sorption properties in the rhizosphere, hence its bioavailability.[16] Cu is most readily available at pH values below 6.[16] However, plants are able to regulate to a certain extent the pH in their rhizosphere, as shown previously for wheat (*Triticum turgidum durum* L.).[11]

Perspective: The influence of plants (i.e., grass, wetland plants) on Cu mobility in particular in the rhizopshere (the microenvironment influenced by roots) in soils and sediments should therefore be assessed. To tackle experimentally this issue, one could use RHIZOtests.[11] The principle of this experimental set-up has been detailed previously.[17] During the experiment a continuous contact is established between the roots and a thin layer of soil previously disposed on a disk.[11] The device enables an easy recovery of the plant roots and soil at the end of the bioassay owing to the thin mesh (30 µm, polyamide) that separates the roots from the soil.[11] To test the influence of the grass cover on Cu mobility in soils, *Lolium perenne L.* can be a good grass model as it is often used in vineyards to limit soil erosion. Four series of tests can be set up each with several replicates (3-5): i) acidic soils with grass, ii) acidic soil without grass, iii) alkaline soil with grass and iv) alkaline soil without grass. The soils should be in contact with the plants roots in the RHIZOtest set-up for at least 8 days to induce changes in the soil due to the presence of roots. The soil solution can then be extracted from all test soils by centrifugation and analyzed for Cu and Cu isotope composition. The grass can also be harvested and separated into roots and shoots to quantify Cu uptake. RHIZOtests could be carried out using soils with and without Cu spikes. Cu addition to soils before the test could stimulate the protection mechanisms of the grass against the Cu stress to mimic the field conditions of vineyards with the regular Cu fungicide additions. Similarly, wetland plants could also be used to study the direct (i.e., Cu uptake in the biomass) and indirect (i.e., changes of Cu mobility in the rhizosphere) impact of plants on Cu mobility. Water saturated conditions should be achieved when performing these tests with sediments to avoid changes in Cu speciation when exposing sediments to air by the re-oxidation of the reduced Cu species. These experiments could shed light on the Cu behavior in the rhizosphere zone that can significantly differ to what happens to Cu in the bulk soil. The altered Cu mobility in the vicinity of roots is an important factor to account for when evaluating Cu transport processes in both oxic soils and anoxic wetland sediments.

2. Cu binding and distribution in soils and sediments

Besides the influence of various biogeochemical conditions, other important aspects controlling the fate of Cu in diverse environments are the longer-term transformations of soil/sediment-bound Cu following its rapid sorption and also the respective role of mineral and organic phases present in soils and sediments in Cu binding.

2.1. Cu aging

Cu aging in soils and sediments is a process by which following the initial rapid sorption of soluble Cu, Cu undergoes a slower reaction that reduces its mobility and availability over time.[18-20] Understanding Cu aging in soils that receive repeated Cu applications is essential when studying Cu mobility and transport. The aging effect on Cu solid-phase speciation in agricultural soils spiked with Cu has recently been highlighted and was explained by a redistribution of a weakly-bound Cu to a stronger binding in SOM and goethite-humate complexes.[21] The aging studies hypothesized the role of precipitation/nucleation of Cu, hydrolysis of Cu(II) and diffusion into micropores, and Cu occlusion within SOM and mineral-SOM associations (i.e., goethite-humate complexes) in Cu aging in soils.[21,22] Under anoxic conditions, Cu transformation mainly consist of formation of reduced Cu(I) species from the initially SOM-bound or clay-sorbed Cu(II).[9,10] Therefore, aging processes may significantly change the Cu isotope partitioning between the different Cu pools (freshly added sorbed Cu and aged Cu) in the soil, in particular if diffusion, precipitation and reduction processes are involved.

In our studies, we suggest that the aging processes in oxic soils and anoxic sediments significantly differ. Whereas the clay-sized particles (enriched in clay minerals and SOM) were suggested to entrap aged Cu in oxic soils, in wetland sediments Cu aging was attributed to Cu sulfide precipitation and/or co-precipitation with Fe oxides. In the vineyard soils, we hypothesized that Cu aging may explain the isotopically lighter Cu enrichment in the clay fractions that could be attributed to a kinetic isotope fractionation upon Cu diffusion into micropores. However, Cu isotope fractionation upon Cu aging in soils has not been investigates yet. It has been shown that upon soil reduction, the initially SOM-bound or clay-sorbed Cu(II) may be transformed into reduced Cu(I)S species. Indeed, in anoxic sediments the produced sulfides (upon sulfate-reduction) outcompete SOM for the binding of Cu.[4,10] Therefore, during the sediment aging, the transformation of the acid-soluble Cu (i.e., carbonate-bound and sorbed) initially present in the sediments to residual Cu forms suggests the conversion of sorbed Cu forms into sulfide-bound and/or Fe oxide-occluded Cu. Even though, micropore diffusion into silicate minerals cannot be excluded neither.

Perspective: A possible experiment to study Cu aging and the associated Cu isotope fractionation (i.e. to confirm if Cu aging in soils favor the binding of isotopically lighter Cu) could be a microcosm set-up with pH-contrasted natural soils.[23] pH has been identified as a crucial factor for Cu sorption to soils and aging and should also be tested.[22,24,25] Cu slightly enriched in ^{65}Cu could be spiked into soils (to reach different Cu concentrations) to differentiate between the old Cu in the soil and the freshly added Cu. Before spiking, the soils should be thoroughly characterized (pH, SOM content, mineralogy, elemental composition, Cu distribution in the different particle-size fractions and their respective δ^{65}Cu, etc.). The spiked soils should be mixed and then leached with synthetic rainwater.[18] An aliquot of the soil should be collected for separation into different particle-size soil fractions to study the distribution of the freshly added Cu in soils. By leaching the soils shortly after Cu addition, the Cu quantities and isotopic ratios of the non-sorbed Cu following rapid solution-solid partitioning could be determined.

A critical aspect is that sorption equilibrium should be reached to avoid kinetic isotope effects (some hours may be needed for that). After leaching, the soil samples can be incubated and left to age. Periodically, soil samples should be collected from the microcosms over a period of several months (with decreasing sampling frequency: from weekly to monthly), and subject to leaching with the synthetic rainwater solution. All samples should be processed identically: leaching and centrifugation, drying and then particle-size separation. By isotopic mass balance approaches, we could deduce the Cu isotope ratio of the spiked Cu in each soil fraction over time (based on the Cu amounts and Cu isotope ratios in the non-spiked soil fractions and in the spiked soil fractions at time t). Thereby, we could show the heterogeneous Cu isotope partitioning in the soil fractions during Cu distribution in the soil during aging. If aging favors ^{63}Cu binding to the soils, we should observe relative increase of the ^{63}Cu enrichment in the fine soil fractions over time as hypothesized previously. However, two major assumptions are made during this experiment that should be kept in mind: i) no significant changes occur in the proportions of the particle-size soil fractions due notably to SOM degradation during the experiment period, and ii) the distribution of the old Cu initially present in the soils do not change significantly during the course of the experiment. However, the errors resulting from the latter can be minimized by increasing the amounts of the spiked Cu so that the initial soil Cu can be considered negligible.

2.2. SOM control on Cu behavior

Several studies highlighted the predominant role of SOM in Cu binding in agricultural soils.[26-28] In the studied soils and sediments, SOM may govern the sorption of Cu fungicides and the mobilization of dissolved Cu under oxic conditions in the vineyard soils, but might not play a significant role in anoxic sediments.

We could not clearly evidence the role of SOM in controlling Cu behavior in the vineyard soils based on the Cu isotope data. Cu sorption to SOM is expected to enrich the OM-bound Cu in ^{65}Cu, with fractionation factors depending on the quality of the OM.[29] If SOM controlled the Cu sorption to the studied vineyard soils, we should have observed ^{65}Cu enrichment in the clay-sized particles enriched in SOM and in Cu. However, we cannot exclude that the eventual role of SOM in the retention of Cu fungicides in the coarser soil fractions (i.e., silts) bearing isotopically heavier Cu. The relative importance of SOM and secondary minerals (clay and Fe oxides) in controlling Cu sorption in diverse agricultural soils is still not well understood. Cu sorption to mineral and/or organic phases can be controlled by the quality and the composition of the soils (i.e., SOM content and quality, pH, mineral composition, etc.).[16] On the other hand, SOM desorption may be responsible for the dissolved Cu release during rainfall in the vineyard soils that was supported by the isotopically heavier dissolved in the runoff. Further study is therefore needed for elucidating the exact role of SOM in Cu retention/release in vineyard soils. Even if Cu isotope fractionation during Cu mobilization from the soils cannot be excluded, there might exist a Cu subpool within the soils displaying high δ^{65}Cu found in the dissolved phase of runoff that has not yet been identified.

Similarly, we could not confirm that SOM dynamics controlled Cu re-distribution in the sediment depth profile in the Winogradsky columns as suggested elsewhere.[30,31] It has been proposed that pH-induced SOM desorption was responsible for Cu release in sediments during the reductive dissolution of Fe(III) oxy(hydr)oxides and the ensuing pH increase.[32,33] In the pH buffered sediments in the Winogradsky columns, no clear correlation could be observed between the dissolved organic carbon (DOC) and dissolved Cu in the sediment porewater. We therefore hypothesized that mineral phases, such as sulfides and oxy(hydr)oxides controlled Cu behavior in the anoxic sediments of the columns.

Perspective: To confirm our hypothesis about Cu sorption to SOM in the soils in the coarser fractions, SOM could be eliminated in each fraction of the vineyard soils following particle-size separation. SOM destruction could be done by doing H_2O_2 treatment, followed by several washing with distilled water (until no more Cu is found in the washing solution). Then, all solutions can be combined and analyzed for Cu and δ^{65}Cu, as well as the leached soil fractions. This way we could confirm if Cu was predominantly bound to the SOM in the coarser fractions but also in the other soil fractions as well as their isotopic signatures. This could clearly highlight the role of SOM in Cu binding and distribution in the different particle-size soil fractions. We may also eventually point at the origins of the isotopically heavy dissolved Cu observed in the runoff in our field study that was hypothesized to be organically bound. This could shed light on the Cu leaching processes in alkaline soils.

Overall, in our studies Cu isotope data allowed to better understand the processes that controlled Cu retention and release in both oxic soils and anoxic sediments. However, in the future Cu isotope analysis could be coupled to additional approaches, such as microcosm experiments to better understand the processes that govern Cu isotope fractionation in the field conditions.

3. Combining Cu stable isotopes and biogeochemical approaches at different scales of the agricultural landscape

Most studies focusing on Cu contamination of agricultural soils rely on Cu concentration analysis in bulk soil and the geochemical partitioning of Cu (i.e., the different Cu binding forms in the soil).[16] Several approaches can be today applied and combined to evaluate the behavior of Cu in different soil types (Cu isotopes, XAS techniques, etc.). Among them, Cu stable isotope analysis is an approach with the potential to trace the biogeochemical processes affecting Cu. This tool is based on the Cu isotope fractionation produced by the different physico-chemical and biological processes that affect Cu in natural environments. However, our current understanding of Cu isotope systematics is still incomplete and a multitude of questions remain open concerning the Cu isotope fractionation rates observed in field studies.

3.1. Cu isotopes to trace Cu retention and transport in soils

At the catchment scale, we investigated the retention of Cu fungicides in the vineyard soils and the impact of Cu fungicide applications and hydro-climatic conditions on Cu mobilization and transport by runoff (Chapter III). Our results highlight that the δ^{65}Cu in the bulk soil may not be efficient in tracing anthropogenic Cu in soils if Cu fungicide signatures are in the same range than those of background Cu. As hypothesized in our study, the vertical transport of anthropogenic Cu can also overprint the natural variations of Cu isotopes in the soil profiles produced by pedogenetic processes.[34] In contrast, the δ^{65}Cu in particle-size fractions of vineyard surface soils revealed precious information about the heterogeneous distribution of Cu among soil fractions and the processes by which fungicide-derived Cu is retained in the vineyard soils. This underscores the need for combining isotope approaches sequential extractions and size fractionation to gain a better insight into isotopic differences between various Cu pools in natural samples.[23,35]

However, the potential of Cu stable isotopes to understand processes governing Cu in soils remains moderate due to the missing fractionation factors for numerous processes that commonly occur in a water-soil-plant system (e.g., Cu fractionation by sorption to different minerals and mineral-OM associations, influence of the Cu speciation in the applied Cu solution on the fractionation during Cu sorption and desorption). Cu isotope fractionation have been characterized so far for a limited number of plant and bacterial species whose spectrum could be enlarged also by involving fungal species that may significantly alter the Cu bioavailability, and uptake rate by plants.[36] So far, only one clay mineral, the kaolinite was used to evaluate Cu isotope fractionation upon Cu sorption to clays.[37] Further experiments are needed to assess Cu isotope fractionation by diverse clay minerals (illite, smectite, etc.) as well as SOM-clay associations that most often co-occur in soils and can bind significant amounts of Cu. We expect to retrieve fractionation factors for Cu partitioning between solution and solid phases, i.e., clay and clay-SOM phases. For this purpose, pure clay minerals could be used and reacted with Cu solution at different Cu concentrations, contact time (e.g. ranging from 10 min to 360 min) to reach equilibrium at the end and at different pH (e.g. from 4 to 7). An experimental design for investigating Cu isotope fractionation upon sorption to clays has been described previously.[37]

Our results also suggest that following Cu-fungicide applications, Cu reaching the soil may rapidly react with soil constituents, hence hindering the original isotope signatures of Cu fungicides. Therefore, the δ^{65}Cu in runoff could not be related directly to the fungicide treatments. Indeed, in such complex environments source tracing is often difficult due to various sources and overlapping isotope signatures of anthropogenic and natural Cu sources. In general, if Cu isotope fractionation does not mask the original δ^{65}Cu of the sources and the δ^{65}Cu significantly different in the diverse Cu sources, Cu isotopes may be used as source tracer.

Perspective: Combining different isotope systems may also help elucidating the fate of Cu in agrosystems. For instance, Cu isotopes could be combined with Pb, Sr or Nb isotopes to trace the sources and the contribution of the different Cu fungicides and natural sources in the Cu export from agricultural land. Pb, Sr, and Nd isotope ratios are appropriate tools to identify different sources of pollution in soil, water and atmospheric dust.[38-40] Pb combined with the Cu isotope system was useful to trace Cu mineralization processes and sources of sedimentary Cu in a geological Cu deposit.[41] For applying the Pb, Sr, Nb isotope systems in our case, they should be analyzed in all external sources (i.e., pesticides, atmospheric input) and in the vineyard soils, as well as in a non-treated reference soil. This would enable source characterization, while isotope analysis in runoff at the outlet of the catchment would enable to identify the relative mobilization of the above-mentioned sources. This way we could assess the origins of the exported chemical loads.

3.2. Cu isotopes to asses Cu behavior at biogeochemical gradients

At the wetland scale, Cu isotopes could be successfully used as a biogeochemical tracer of Cu behavior in a natural redox-dynamic environment (Chapter IV). The δ^{65}Cu in the water phase assessed between the wetland inlet and outlet suggested Cu adsorption to and/or co-precipitation with Al-bearing minerals in the wetland sediments as well as Cu(II) reduction. Cu isotopes were able to capture the dynamic behavior of Cu in the wetland under variable flow conditions, thereby highlighting the release of reduced Cu species from the sediments during high-flow conditions. In the latter study, Cu isotopes were coupled with mass balance approaches, including those of redox-indicative species (nitrate, Fe, sulfate) to delineate the biogeochemical evolution of the wetland. As in field conditions multiple processes may affect Cu isotope fractionation, a thorough characterization of the system (hydrology, mass balance approaches, hydrochemical, geochemical analyses, extractions, mineralogy, etc.) can considerably help in the interpretation of the isotope data.[23]

The sediment-bound Cu transformations that can occur in wetlands were investigated in the Winogradsky columns (Chapter V). At the column scale, we could study Cu aging in a sediment column at an expanded biogeochemical gradient, formed by microbial activity. However, based on the geochemical and Cu isotope data we could not clearly identify the exact processes by which Cu mobilization took place in the Cu-depleted sediment layers under the contrasted biogeochemical conditions. The applied sequential extraction protocol also failed to give clear indications about the major Cu phases in the sediment layers. Therefore, in the future the sediment layers should be subjected to extractions that target specifically sulfide phases, such as the acid volatile sulfide (AVS) and chromous reducible sulfide (CRS) as explained previously.[42,43] Pyrite can be specifically determined by dissolution with concentrated HNO_3 of the residue obtained after AVS extraction, following silicate dissolution with HF digestion.[43] This way the different sulfide species present in the sediment layers and the metals bound to these sulfides could be identified. This identification phase is a prerequisite to confirm the role of sulfide formation in Cu aging in the anoxic sediments. Whereas, applying extracting reagents specifically targeting amorphous and crystalline Fe oxide phases in the sediments, could confirm the role of Fe redox processes in the sediment-bound Cu transformations. Several methods exist for Fe oxide extraction in soils and therefore, tests should be made before choosing the most appropriate one.[44] Extractions could thus indicate the changes in the sediment-bound Fe and S phases in the sediment depth profile and reveal the predominant processes affecting Cu partitioning in the sediments.

Perspective: Characterization of the microbial communities in the sediment depth profile is also a major perspective to understand Cu behavior with regards to TEAPs. The initially homogeneous sediment evolved during 520 days due to the microbial communities established along the sediment depth profile. Microbial activity modified the geochemical composition of the sediments layers by creating contrasting biogeochemical conditions by TEAPs, and thus could be used as bioindicators of *in situ* conditions. For example, the role of microorganisms in the dissolution of Fe-bearing minerals, as well as in the diverse sulfur transformations that commonly occur in wetland sediments and govern the fate of Cu in such redox-dynamic environments could be addressed. Currently, only few studies combined microbiology and isotope approaches to study the direct influence of microbial activity on Cu behavior.[45,46] Molecular tools allow for the identification of microbial populations that may help to have a better characterization of a system in which the microbes are responsible for establishing diverse biogeochemical conditions. DNA fingerprinting techniques (e.g. DGGE and T-RFLP) and more recently high-throughput sequencing techniques provide a characterization of the profile of a given microbial community and can be used to reveal the dominant bacterial phylotypes even in high-diversity environments (e.g. agricultural and wetland soils).[47] In addition, microbial characterization of Cu contaminated soils and sediments have the potential of evaluating the impact of Cu contamination on the microbial populations and develop ecotoxicological microbial indicators.

4. Sustainability of agricultural systems with regards to Cu applications

Field observations and experimental results suggest that repeated Cu applications create agro-ecosystems, where nutrient cycling may be significantly impacted by the toxicity of Cu towards microorganisms and other organisms (such as earthworms) that are responsible for the turnover of SOM.[16] These impacts may ultimately lead to reduced soil fertility, which raises the question about the sustainability of these agricultural systems. An environmentally-sound sustainable viticulture "should sustain the health of soil, plant animal, human and planet".[48] Translating this idea in the context of Cu application in agriculture means that Cu applications should not affect adversely soil biota, while the Cu export from agricultural areas should be limited to avoid exceeding ecological limits in the neighboring water bodies. Organic farming

(OF) is commonly considered as a promising solution for reducing environmental burdens related to intensive agricultural management practices. A fundamental issue is whether OF really reduces the environmental impacts once all the changes in farming methods have been taken into consideration.[48] Downy mildew is one of the most destructive diseases of grapevine, particularly in warm and wet climate. Currently Cu-based fungicides are applied in both conventional and in organic farming, however, in OF, the control of fungal diseases is based almost exclusively on Cu fungicides due to the lack of alternatives.[49]

European legislation restricts the annual dose of applied Cu to 6 kg Cu ha^{-1} $year^{-1}$, which would correspond to an annual accumulation of about 5 mg Cu kg^{-1} soil in the top 10 cm of a vineyard soil assuming no losses.[50] Such a sustained application may increase in 150 years of Cu application concentrations up to 750 mg Cu kg^{-1} in unplowed vineyard top- soils.[50] At Rouffach, the Cu fungicide application rates ranged from 1.8 to 2.6 kg ha^{-1} $year^{-1}$ (from 2008 to 2011), however the majority of vineyards were not managed in OF, and hence other synthetic fungicides were also applied on the grape-vines (such as kresoxim methyl, cyazofamid). However, at earlier periods we can hypothesize that higher Cu application doses were commonly applied (20 – 40 kg ha^{-1} $year^{-1}$).[51] Historically, vine growing started at Rouffach in 1962 and was extended in 1973 to former grasslands that were converted to vine-growing. Today, Cu concentrations in the top 20 cm range from 40 mg kg^{-1} to 231 mg kg^{-1} compared to a background concentration of 11 mg kg^{-1}. On average during the 50 years of vine-growing, Cu loads in the first 5 cm of the vineyard soils in the whole catchment increased from ~200 kg to ~1670 kg Cu. These values reported to the total land surface dedicated to wine-production reflect a Cu accumulation rate of ~1.2 kg ha^{-1} $year^{-1}$ on average in the vineyard top-soils. In the vineyard soils of Rouffach the total Cu sorbing capacity should be evaluated to estimate the time period necessary to exceed the Cu sorbing capacity of the soils with the current Cu accumulation rates. For that, batch experiments could be set up using the vineyard soil from Rouffach and repeated additions of Cu solutions should be done and drying the soil at room temperature between Cu additions. Following the additions of the solution to the soil and mixing (until equilibrium is reached), an aliquot of the solution could be retrieved and filtered to check the decrease in the Cu concentration. At the point when no more Cu is depleted from the added solution (indicating Cu sorption to the soil), the Cu sorbing capacity of the soil is assumedly reached.

Evaluating the potential adverse effects on the soil biota of Cu accumulation in the vineyard soils was out of the scope of the present thesis and would mean focusing on Cu availability in soils and sediments. Besides the increasing Cu concentrations in agricultural soils, the bioavailability of Cu is another key aspect in assessing the risks of Cu (i.e., Cu toxicity) use in agriculture. Bioavailability of Cu depends largely on soil properties (such as pH, SOM content, clay content, etc.).[16] Therefore, Cu bioavailability should be evaluated at each site, in particular where Cu concentrations exceed the warning and critical legislative limits valid in the EU set at 50 and 140 mg kg^{-1} in agricultural soils.[16]

Currently, even if there is no treatment that is as effective as Cu for controlling grapevine downy mildew in organic vine-growing, some promising candidates are under investigation for reducing doses or partially substituting Cu fungicides.[52] Model-driven decision support systems (e.g. Coptimizer) are currently developed to help vine-growers to optimize and track the use of Cu-based fungicides against grapevine downy mildew in European organic viticulture. By using Coptimizer growers could be able to maintain the same level of protection as that gained by traditional application schedule while applying only half the amount of the Cu fungicides.[53] Reducing the external Cu sources is essential for maintaining the good health of vineyards, therefore, the Cu concentration in the vineyard top-soils should be regularly controlled (every ~10 years).

Runoff is regarded as a major contributor to pesticide entry in surface waters where slopes of more than 2% are common in the vineyard areas.[54] Steep slopes often characterize vineyards: in the Pyrénées Mountains (25 to 50%),[35] in Galicia in Spain (~20%),[55] in the Mediterranean region of Slovenia (≥30%).[56] At Rouffach, the mean slope is of 15% that favors Cu export from the vineyard catchment by rainfall-runoff mainly in the particulate form (i.e., by soil erosion). During the study period that covered much of the Cu fungicide application period (May to July, 2011), we observed a Cu export that represented ~1% of the applied Cu that year. This Cu export is approximately 1‰ of the Cu stock present in the 5 cm of the vineyard top-soils in the whole catchment. However, as explained above, we could not differentiate between the freshly added and the "older" Cu in runoff based on Cu isotopic ratios. In the runoff the dissolved Cu concentration (7.7 - 32.0 µg L^{-1}) exceeded in 90% of samples the US Environmental Protection Agency (EPA) eco-toxicological recommendations for aquatic environments (acute level: 13 µg L^{-1}; chronic level: 9 µg L^{-1}). This means that Cu fungicide applications at the Rouffach site may be harmful to the downstream aquatic ecosystems. Therefore, the stormwater wetland that collects the Cu-contaminated runoff from the vineyards in Rouffach has an essential role as a risk mitigation measure. The wetland decreased the dissolved Cu concentration in the runoff below the eco-toxicological recommendations. Cu accumulated in the wetland sediments, but could be re-mobilized during high-flow conditions. Therefore, these systems should be carefully engineered in order to limit the remobilization of accumulated Cu.

Overall, until real alternatives are available to substitute for Cu in vine-growing, remediation strategies should be applied to limit the ecological impacts of Cu use by the implementation of grassed field paths, buffer strips, vegetated ditches or wetlands is necessary to limit Cu export.[54]

5. Example of a longer-term research perspective: characterization of multi-contaminated agro-ecosystem using modern isotope approaches

In agricultural systems the co-occurrence of Cu and other synthetic pesticides may influence the mobility of each other by forming Cu-pesticide complexes and Cu-pesticides-soil ternary complexes. Recently, an increasing number of studies have been dedicated to evaluate the impact of the high Cu concentrations found in vineyard soils on the sorption and biodegradation of synthetic pesticides as well as their combined toxicity towards soil biota.[57-61] For instance, it has been shown that increasing Cu concentrations reduce glyphosate leaching through calcareous soils, and conversely, increase glyphosate leaching through granitic acid soils.[58] Cu can also impact the sorption of lambda-cyhalothrin and cypermethrin to soils, greatly influenced by the soil properties (e.g. OM content, surface area).[62] Competition between Cu and the synthetic pesticides for the same sorption sites may explain the reduced sorption of synthetic pesticides in the soils.[62]

On the other hand, Cu may restrain the biodegradation of synthetic pesticides. Cu significantly reduced the degradation of the pesticides cypermethrin and cyhalothrin in soil by its toxicity towards microorganisms, whereas it had catalyzing effect on the photodegradation of these pesticides in water.[63] Cu may also inhibit to a certain extent the biodegradation of the herbicide atrazine and the insecticide indoxacarb by affecting the degradation of their secondary metabolites like hydroxyatrazine in soil.[57] The results were explained by a reduction in the soil microbial biomass and enzyme activities with increasing Cu concentrations.

To experimentally tackle those issues, we may combine Cu isotopes with traditional stable of isotope systems (e.g. $^{13}C/^{12}C$, $^{2}H/^{1}H$) to quantify the effect of Cu on the rate of biodegradation of diverse synthetic pesticides. Cu isotope fractionation upon Cu complexation with synthetic pesticides that are frequently used in vineyards (e.g., glyphosate) and during the formation of ternary complexes between Cu-pesticides and different soil constituents could be evaluated.

In parallel, compound specific isotope analysis of organic compounds ($^{13C}/^{12}C$ and $^{2}H/^{1}H$) can indicate *in situ* degradation and can also allow for the quantification of the degradation of organic compounds in field conditions.[64] Since microorganisms preferentially degrade isotopically lighter organic molecules, the residual or undegraded fraction of the organic compound becomes isotopically enriched in the heavy isotopes of the constituent elements (^{13}C, ^{2}H).[65,66] Compound-specific isotope measurements do not only offer the possibility to qualitatively detect, but also to quantitatively estimate the extent of contaminant degradation using the Rayleigh distillation model.[65] Therefore, we could evaluate the effect of the increasing Cu concentrations in agricultural soils on the *in situ* biodegradation rate of synthetic pesticides by measuring their C or H isotope ratios by extracting them from soils. These aspects could be investigated in microcosm experiments incubating Cu-spiked soils (at different concentrations), with added synthetic pesticides. The synthetic pesticide could be extracted from the soils after different time periods of soil incubation to follow the degradation patterns by concentration and compound-specific isotope analysis.

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VII. Annexes

EXTENDED SUMMARY FOR THE WINE-GROWERS AND STAKEHOLDERS (IN FRENCH)

LE CUIVRE VITICOLE

Le cuivre (Cu) est un métal omniprésent dans l'environnement. Il est utilisé par l'Homme depuis ~10 000 ans et joue encore aujourd'hui un rôle important dans la société. À très faible dose, un oligo-élément indispensable à la vie, à des doses plus élevées il peut modifier profondément les structures et le fonctionnement de différentes écosystèmes, en particulier les milieux aquatiques où les concentrations atteignent régulièrement les seuils recommandés par l'USEPA pour la protection des écosystèmes aquatiques (9 µg/L en exposition chronique, 13 µg/L en exposition aiguë).[1] Depuis la fin du 19ème siècle le cuivre est largement utilisé dans les vignobles français, mais aussi dans d'autres régions viticoles du monde. Les traitements de plants de vignes par le cuivre ont engendré des teneurs élevées du cuivre dans les sols viticoles dues à son accumulation, excédant souvent 100 $mg.kg^{-1}$ comparées à la moyenne des sols sans ajouts du cuivre anthropique de 30 $mg.kg^{-1}$.[2] Cependant, l'état moyen des teneurs en cuivre total des sols du vignoble alsacien demeure peu connu.

Des teneurs élevées du cuivre dans les sols peuvent avoir de effets toxiques vis-à-vis des organismes vivants du sol, notamment des vers de terre et la microflore du sol et peut à la longue réduire sa fertilité.[3] La toxicité du cuivre dépend non seulement de sa concentration, mais aussi de sa spéciation dans le sol. Le cuivre peut être complexé avec de la matière organique, adsorbé aux oxydes de fer, manganèse et d'aluminium, aux carbonates, ainsi qu'aux minéraux argileux.[2] Le cuivre s'accumule dans le sol via des processus de sorption, notamment à la matière organique et aux phases minérales et/ou par sa (co-)précipitation avec des carbonates et des hydroxydes. Alors que certaines études ont démontré que le cuivre était principalement lié à la matière organique,[4,5] d'autres études prônent le rôle dominant de la précipitation du cuivre sous formes carbonate et hydroxyde dans les sols calciques (le type de sol à Rouffach).[6,7] Ainsi le rôle de la matière organique et des carbonates dans la rétention du cuivre reste à être clarifié dans le cas des sols calciques.

L'existence de sols nus ou faiblement enherbés et de pentes élevées (10-15%), caractéristiques dans les vignobles, favorisent l'érosion et le ruissellement surfacique mobilisant ainsi le cuivre. Dans les lames ruisselantes, le cuivre est principalement transporté sous forme de matière en suspension, introduisant des quantités importantes de cuivre dans les écosystèmes aquatiques en aval. La plupart du cuivre est mobilisé sous forme solide via des phénomènes d'érosion.[8] Par conséquent, la distribution du cuivre dans des différentes fractions granulométriques doit être également étudiée. Le cuivre dissous en solution se trouve alors sous forme ionique libre (Cu^{2+}), inorganique ($CuOH^+$, $CuOH_2$, etc.) et organique (cuivre complexé à des substances humiques).

Les lames ruisselantes provenant de bassins versant agricoles peuvent être interceptées par des zones tampons humides artificielles ou basins d'orage, avant d'être transférées vers des écosystèmes aquatiques en aval. Initialement conçus pour la protection contre les inondations, ces bassins retiennent les matières solides, et peuvent influer positivement sur la qualité de l'eau en combinant des processus physiques, chimiques et biologiques. Ces systèmes sont envisagés depuis peu comme une alternative opérationnelle et peu coûteuse au traitement des eaux de ruissellement chargées en pesticides, notamment en cuivre, mais reste encore en phase d'évaluation. Des performances variables révèlent notamment des lacunes dans la compréhension du devenir, du transfert et de la rétention du cuivre au sein de ces milieux dynamiques et hétérogènes vis-à-vis des processus biogéochimiques. De par leur ubiquité dans la région du Rhin supérieur et leur capacité de traitement, les bassins de rétention hydrologiquement connectés à des bassins versants viticoles constituent des interfaces stratégiques lors du transfert de contaminants et représentent des zones préférentielles pour l'étude de la mobilisation et du transfert du cuivre dans les systèmes eau-sol-plantes-microorganismes.

COMPORTEMENT DU CUIVRE DANS L'ENVIRONNEMENT

Dans ce milieu, le cuivre va subir des processus physique tels que la sorption aux sédiments et aux plantes submergées, des processus chimiques, tels que la (co-)précipitation avec des phases minérales nouvellement formées ((hydr)oxydes de fer), ainsi que des processus biologiques, tel que le prélèvement par les plantes aquatiques.[9] A bas potentiel redox les changements de spéciation du cuivre sont notamment liés à l'activité microbienne qui vont dissoudre certaines phases de sorbant de cuivre (e.g. (hydr)oxyde de fer) ainsi le libérant en solution et former d'autres phases minérales tels que les sulfures de fer et du cuivre, induisant sa séquestration dans le milieu.[10] La formation de colloïde de cuivre élémentaire (Cu^0) et de sulfure de cuivre contrôle également la dynamique du cuivre et sa mobilité.[11] La matière organique joue également un rôle prépondérant dans la mobilité et le transfert du cuivre qui peut elle aussi subir des changements de mobilité dus à la variation du pH dans le milieu.[12] La mobilité du cuivre séquestré dans les sédiments de ces milieux aquatiques peut également être affectée par des processus à long-terme (« vieillissement » du cuivre).[13] Cependant, les changements des conditions ambiantes, notamment par des événements hydrologiques peuvent considérablement altérer la performance du système vis-à-vis de la séquestration du cuivre et peuvent même devenir des sources secondaires de cuivre en le remobilisant.[14] Par conséquent, il est important d'étudier les processus contrôlant le devenir du cuivre dans les milieux humides en particulier en fonction des conditions hydrologiques et des changements de potentiel redox.

Ainsi, la modification des cycles biogéochimiques dans les agro-écosystèmes viticoles affecte les patrons de séquestration et de mobilisation du cuivre, contrôlant l'accumulation et le transport du cuivre dans les écosystèmes terrestres et aquatiques. Cependant, les processus régissant la mobilisation du cuivre au niveau des sols viticoles et son transfert par ruissellement sont peu connus.

POTENTIEL DE L'ANALYSE ISOTOPIQUE DU CUIVRE

De plus, le fonctionnement des bassins de rétention collectant le ruissellement chargé en cuivre et les phénomènes de rétention du cuivre dans ces systèmes restent à évaluer en lien avec les pratiques agricoles et la dynamique des processus biogéochimiques contrôlant la mobilité du cuivre.

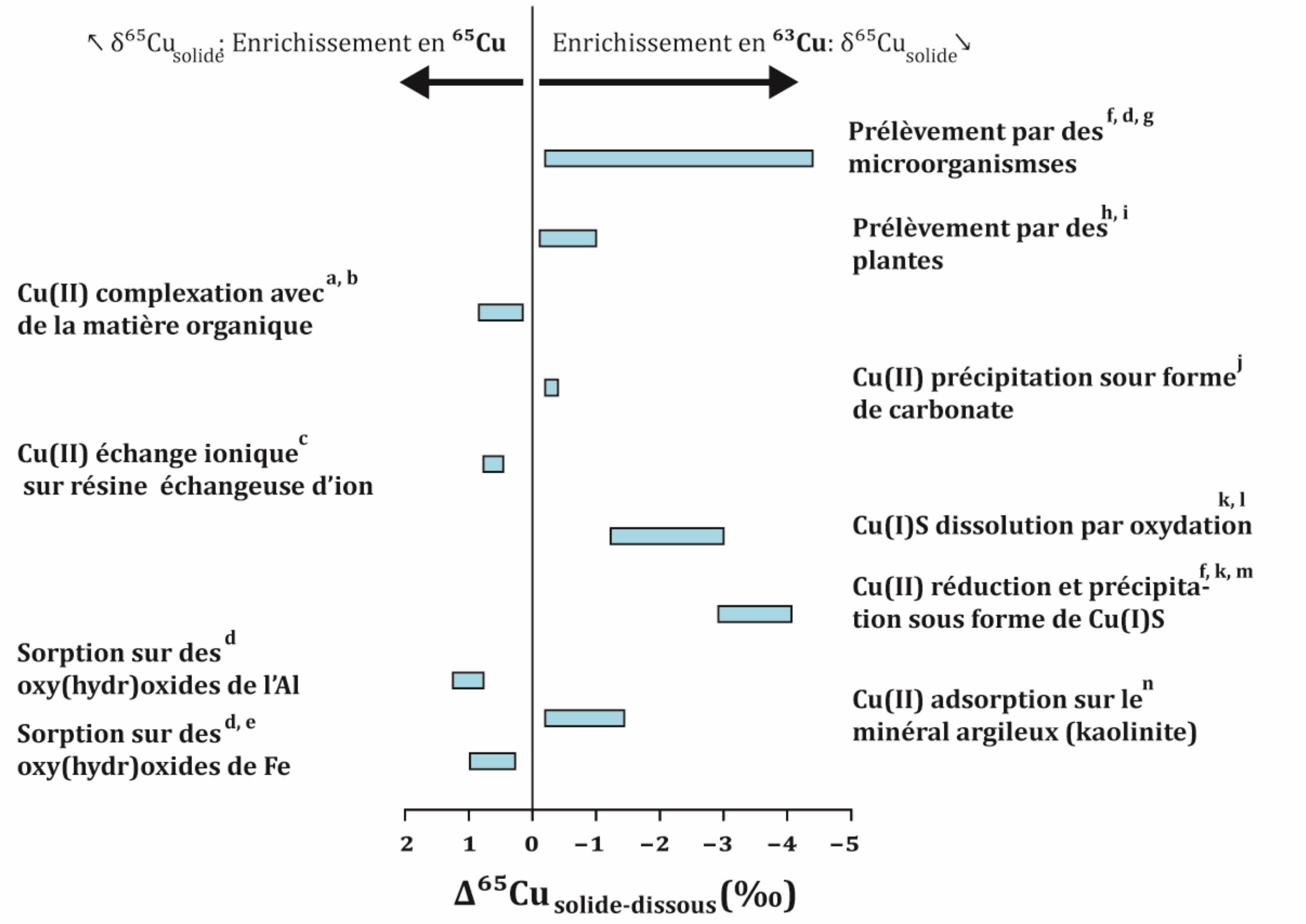
En complément des outils traditionnels, tels que la mesure de concentration des métaux, les extractions chimiques et des analyses minéralogiques donnent tous des informations ponctuelles dans le temps, les isotopes stables des métaux apportent potentiellement de précieux renseignements sur les processus qui régissent le comportement de ces derniers dans l'environnement. Très prometteuse, l'étude du fractionnement isotopique d'isotopes de métaux de transitions, tel que **^{65}Cu/^{63}Cu,** est **désormais possible grâce au développement du spectromètre de masse à source à plasma et à multi-collection (MC-ICPMS). Ainsi, l**'étude du rapport des isotopes stables du cuivre peut être adaptée au suivi intégratif du cycle biogéochimique du cuivre à l'échelle des agro-systèmes et des bassins de rétention afin de mieux comprendre les mécanismes régissant sa mobilisation et son transfert.

Les isotopes d'un élément donné contiennent le même nombre de protons et d'électrons, par contre, leur nombre de neutron est différent et ainsi leur masse atomique varie. On distingue entre les isotopes stables et radioactifs. L'isotope carbone 14, ou ^{14}C est un isotope radioactif, et sa radioactivité décroît au fil du temps à un rythme régulier. Dans les études scientifiques on s'en sert donc comme «chronomètre» pour estimer l'âge d'objets très variés : œuvres d'art, roches, fossiles, etc. En revanche, les isotopes stables ne se désintègrent pas et sont utilisés comme traceurs de processus biogéochimiques et/ou de source. Les isotopes stables du carbone (^{13}C/^{12}C) sont notamment utilisés pour tracer la biodégradation des contaminants organiques dans l'environnement.[15] Les isotopes d'oxygène (^{18}O/^{16}O) sont utilisés dans la climatologie pour tracer notamment les sources d'évaporation de l'eau de pluie et de tracer les trajectoires de différentes masses d'air.[16]

Le cuivre possède deux isotopes stables le ^{63}Cu et le ^{65}Cu (qui ont chacun 29 protons et 29 électrons, par contre possèdent 34 (^{63}Cu) et 36 neutrons (^{65}Cu) dans leur noyau), dont l'abondance moyenne dans la nature est 69,17% (^{63}Cu) et 30,83% (^{65}Cu). L'inégalité de masse entraîne l'existence de différences dans certaines propriétés physico-chimiques des isotopes : notamment la température de fusion, la viscosité, la vitesse de réaction, ainsi que la constante d'équilibre. Ces différences de propriétés sont à l'origine d'une répartition différente des isotopes entre les réactifs et les produits lors des réactions physico-chimiques produisant ainsi un fractionnement isotopique ($^{65}\mathrm{Cu}/^{63}\mathrm{Cu}_{\text{réactif}} \neq {}^{65}\mathrm{Cu}/^{63}\mathrm{Cu}_{\text{produit}}$). Les rapports isotopiques du cuivre sont exprimés en termes de δ^{65}Cu (‰) relatif à un standard isotopique international, le NIST 976 (dont le rapport ^{65}Cu/^{63}Cu est de 0,4456):

$$\boldsymbol{\delta^{65}\mathrm{Cu}} = \left(\frac{\mathbf{Cu^{65}}/\mathbf{Cu^{63}}_{\text{échantillon}}}{\mathbf{Cu^{65}}/\mathbf{Cu^{63}}_{\textbf{standard}}} - \mathbf{1} \right) \times \mathbf{1000}\ (‰)$$

Plusieurs études ont montré que la réduction/oxidation du cuivre produit un fractionnement distinct, que la sorption du cuivre sur des (hydr)oxydes métalliques et sur des argiles ou des substances humiques produit un fractionnement significatif, de même que la transformation biotique du cuivre (absorption par des racines, translocation dans les plantes) (**Figure 1**).[17-20] Le fractionnement isotopique du cuivre entre réservoir **a** et **b** est exprimé en $\Delta^{65}\mathrm{Cu}_{a\text{-}b} = \delta^{65}\mathrm{Cu}_a - \delta^{65}\mathrm{Cu}_b$. Notre hypothèse de travail est que mesurer le rapport isotopique du cuivre dans des échantillons environnementaux (tels que les sols, la lame ruisselante, la végétation) nous permetterait de d'indentifier les processus principales qui régissent le tranport et le transformation du cuivre.



a: Ryan et al., 2014, **b**: Bigalke et al., 2010, **c**: Maréchal et Albarède, 2002, **d**: Pokrovsky et al., 2008,
e: Balistrieri et al, 2008, **f**: Zhu et al., 2002, **g**: Navarrete et al., 2011, **h**: Jouvin et al., 2012, **i**: Ryan et al., 2013,
j: Maréchal et Sheppard, 2002 , **k**: Mathur et al., 2005, **l**: Kimball et al., 2009, **m**: Ehrlich et al., 2004,
n: Li et al., 2015

Figure 1 : Le fractionnement isotopique du cuivre lors des processus physico-chimiques et biologique compilé de la littérature

Bien qu'à la base cet outil a surtout servit à l'étude du comportant géologique du cuivre, récemment **un nombre croissant d'étude ont pour objet de regarder de plus près le devenir du cuivre anthropique dans l'environnement**.[21]

QUESTIONS DE RECHERCHE ET OBJECTIFS DE L'ETUDE

En partant du constat que les bassins de rétention collectent par défaut les lames ruisselantes chargées en cuivre provenant de bassins versants viticoles, plusieurs questions se posent et constituent autant de verrous à lever pour comprendre et prédire le transfert du cuivre en réponse au forçage hydrologique et aux perturbations anthropiques (climat, occupation du sol, épandage de fongicides cupriques) :

Comment et dans quelle mesure les différents processus biogéochimiques dans les systèmes en série bassin versant viticole-bassin de rétention affectent la mobilisation et le transfert du cuivre?

Quelle est l'apport de la géochimie isotopique du cuivre pour identifier et caractériser les transferts tant à l'échelle de bassins versants viticoles que dans les bassins de rétention?

L'objectif général de cette thèse est donc d'approfondir la compréhension du devenir du cuivre anthropique à l'échelle d'un bassin versant viticoles et dans un bassin de rétention associé où les processus biogéochimiques régissent le transport réactif du cuivre. Notre hypothèse principale était que les rapports isotopiques du cuivre pourraient constituer un outil efficace à l'étude du comportement du cuivre. Ceci permettrait de répondre aux objectifs suivants :

- tracer l'accumulation et la distribution des fongicides cupriques dans des sols viticoles et leurs mobilisation via la lame ruisselante ;
- étudier les processus de rétention et de relargage éventuel du cuivre dans un bassin de rétention, récepteur du ruissellement chargé en cuivre, en fonction du forçage hydrologique et de changement de condition redox ;
- évaluer les processus de transformation du cuivre dans des sédiments à long terme (le « vieillissement ») le long d'un gradient de potentiel redox.

En réponse à ces questions, comment est-il possible d'optimiser, voire de maîtriser l'éco-service rendu par ces zones humides artificielles? Ce projet qui s'inscrit dans les objectifs de la directive cadre sur l'eau (DCE 2000/60/CE) veut contribuer à apporter des réponses à ces questions fondamentales. L'originalité de l'approche repose sur un développement analytique pour l'étude du fractionnement isotopique du cuivre, et l'application de ce développement à des expériences en laboratoire (mésocosmes) et de terrain (système en série bassin versant viticole-milieux humides artificiels).

METHODOLOGIE

Développement méthodologique et analytique de la géochimie isotopique du cuivre. Des protocoles d'extraction et de purification du cuivre ont été développés à partir de différentes matrices (solutions cupriques, eaux, sols, sédiments et plantes). Des protocoles d'extraction séquentielle du cuivre ont servi à étudier la répartition du cuivre dans différentes fractions des matrices considérées et la distribution du cuivre dans différentes fractions granulométriques du sol a également été étudiée. En parallèle, l'incertitude et la précision de la mesure isotopique du cuivre par MC-ICPMS ont été évaluées par une série de tests, incluant notamment le matériel de référence NIST 976 (National Institute of Standards and Technology, Gaithersburg, MD).

Etude des processus hydro-biogéochimique régissant le transfert du cuivre dans un système en série bassin versant viticole - bassin de rétention planté. Caractérisation des sources du cuivre anthropique (fongicides), des profils de sols viticoles ayant une historique d'application différente, ainsi que des flux de cuivre en sortie du bassin versant a été accomplie pendant une période d'épandage de fongicide cuprique (en 2011).

Le bilan hydrique entrée-sortie au niveau du bassin de rétention, récepteur de la lame ruisselante, a été fait sur une base hebdomadaire pour permettre de dresser des bilans de masse des flux chimiques (cuivre, sulfate, fer, nitrate, etc.) entrée-sortie, afin d'évaluer la performance du système vis-à-vis de la rétention du cuivre dans des conditions hydro-biogéochimiques variables.

Etude du « vieillissement » du cuivre dans des sédiments de milieu humides où se forment des gradients biogéochimiques. Des *expérimentations en mésocosmes* de laboratoire (système fermé, colonnes en verre, conditions partiellement contrôlées) pour l'étude de la transformation du cuivre associée aux sédiments (provenant du bassin de rétention de Rouffach) ont été également menée.

PRINCIPAUX RÉSULTATS

Développement méthodologique et analytique

La méthode existante de la purification du cuivre (Maréchal *et al*., 1999)[22] a été adapté aux échantillons prélevés à Rouffach lors de la campagne d'échantillonnage en 2011 (Mai – Juillet). Plusieurs protocoles de purification ont été établis en fonction des échantillons (eaux, solides, végétaux). Ceci afin de s'assurer des bons rendements de cette étape qui est cruciale et indispensable pour des mesures précises du rapport isotopiques du cuivre. Le rapport isotopique du cuivre dans les échantillons est mesuré par un spectromètre de masse par torche à plasma à multi-collection (Neptune, Thermo Scientific). Les mesures brutes sont corrigées en utilisant un standard de nickel (NIST 986) et les résultats sont exprimés en ‰ (δ^{65}Cu) par rapport à un standard de référence de cuivre (NIST 976). Plusieurs tests ont été effectué afin d'évaluer l'impact de la présence des impuretés dans le cuivre purifié sur les mesures du rapport isotopique du cuivre dû aux phénomènes d'interférences sur les masse des isotopes de cuivre.

Suite aux résultats, la double purification (deux étapes successives de purification) des échantillons a été adoptée pour limiter toute interférence lors des mesures isotopiques. Notre protocole a été validé par l'analyse d'un standard géologique (BCR-2) et d'un standard de sol (SCL-7003).

À l'échelle du bassin versant

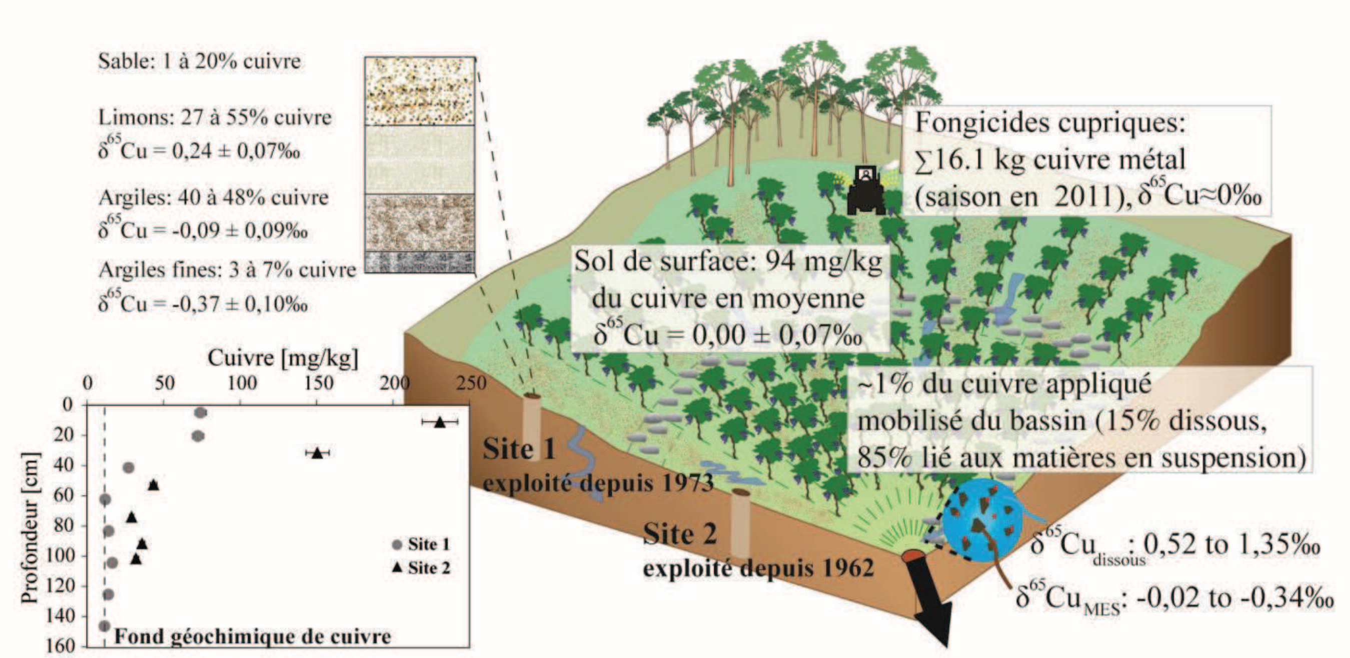


Figure 2 : Signatures isotopiques des sources, la répartition du cuivre dans les fractions granulométriques du sol, la signature moyenne des sols de surface et la répartition du cuivre dans la lame ruisselante à l'exutoire du bassin versant viticole à Rouffach

En premier lieu nous avons utilisé les isotopes stables du cuivre (δ^{65}Cu) pour étudier les sources, les processus de rétention du cuivre anthropique (fongicides cupriques) dans les sols viticoles et son transport via le ruissellement à l'échelle d'un bassin versant viticole (Rouffach, Alsace, France). Les rapports isotopiques du cuivre ont été mesurés dans les fongicides cupriques, dans deux profils de sols prélevés dans deux parcelles de vigne (avec des historiques d'applications du Cu différents), ainsi que dans la surface du sol de bassin versant prélevé le long de trois transects. Les eaux de ruissellement ont aussi été échantillonnées à l'exutoire du bassin versant durant la quasi-totalité d'une saison d'application des fongicides cupriques (Mai - Juillet 2011). Nous avons également effectué une séparation des fractions granulométriques dans les sols de surface, afin d'étudier la distribution du cuivre dans le sol. Le sol en surface du bassin versant viticole de Rouffach contient en moyen 92 mg kg^{-1} du cuivre comparé au fond géochimique de ~11 mg kg^{-1}.

Les résultats ont montré qu'il y a plus de cuivre accumulé dans la parcelle exploitée depuis plus longtemps, et la moyenne des signatures isotopiques des fongicides cupriques ($\delta^{65}Cu \approx 0‰$) correspondait à celles des sols de surface ($\delta^{65}Cu = 0{,}00 \pm 0{,}07‰$) des deux parcelles, montrant ainsi la prédominance du cuivre anthropique dans les deux sols de Rouffach (Figure 2). L'export du cuivre du bassin versant était de ~1% (~150 g) du cuivre appliqué ce qui renforce l'idée de l'accumulation de la quasi-totalité du Cu dans les sols du bassin versant. Les ratios isotopiques obtenus dans les différentes fractions du sol (i.e., limons, argiles, fines argiles) ont montré de larges variations ce qui implique que la distribution du Cu est très hétérogène dans le sol et que la rétention du Cu anthropique dans le sol se fait par sorption sur des minéraux argileux (dans les fractions fines du sol) et à la matière organique. Nos résultats ne confirment pas le rôle majeur de la précipitation du cuivre sous forme carbonate ce qui a été suggéré par d'autres études dans le cas des sols calciques.

La signature isotopique du cuivre lié aux solides en suspension correspond au cuivre dans les fractions fines des sols (argiles et argiles fines), montrant ainsi la mobilisation préférentielle de ces particules fines par la pluie. Le cuivre dissous portait une signature isotopiquement lourd, ce qui indiquerait que sa mobilisation se ferait par la complexation à la matière organique désorbée du sol (à pH alcalin). Cependant, la mobilisation du cuivre était principalement contrôlée par l'érosion du sol, le cuivre étant transporté en majeur partie sur la matière solide en suspension. L'intensité de pluie a fortement influencé l'export du cuivre, puisque de forts évènements pluvieux ont mobilisé davantage de limons « diluant » ainsi la concentration du cuivre dans les solides en suspension. En revanche, en termes de quantité, le plus important export de solides en suspension a résulté en une plus grande quantité de cuivre mobilisé lors des fortes pluies. Ainsi les isotopes du cuivre ont mis en évidence le comportement dynamique du cuivre anthropique lors de son accumulation dans les sols viticoles et nous ont donné un meilleur aperçu de sa mobilisation par la pluie.

Au niveau de la zone tampon humide artificielle

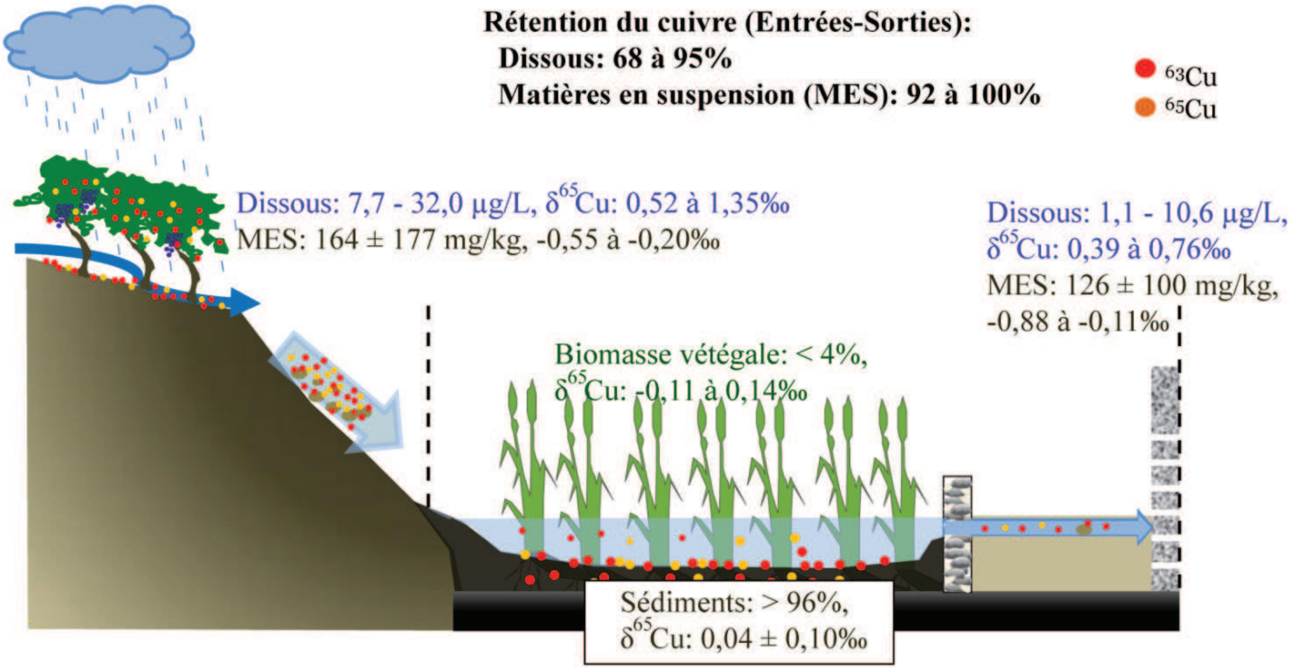


Figure 3 : Les concentrations et les rapports isotopiques du cuivre en entrée et en sortie du bassin de rétention, ainsi que la répartition du cuivre à l'intérieur du bassin à Rouffach

Dans un deuxième temps, nous avons utilisé les isotopes stables du cuivre pour étudier le comportement du cuivre dans le bassin d'orage qui collecte le ruissellement du bassin versant viticole (à Rouffach). La charge et les rapports isotopiques du cuivre ont été mesurés dans la phase dissoute, dans les solides en suspension, dans les sédiments ainsi que dans la végétation du bassin de rétention pendant une période d'applications des fongicides cupriques (Mai - Juillet 2011). Les résultats ont montré que plus de 68% du cuivre dissous et plus de 92% du cuivre liés aux solides en suspensions transportés par la lame ruisselante ont été retenu dans le bassin de rétention, et ce dernier représentait 85% du cuivre total dans le ruissellement. Le cuivre dissous a été appauvri en isotope lourd (^{65}Cu) lors de son passage dans le bassin reflétant ainsi le fractionnement isotopique du cuivre induit par des processus de transport dans le bassin de rétention (Figure 3).

Le taux de fractionnement observé, comparé à des valeurs de référence dans la littérature, impliquerait l'adsorption du cuivre à des hydroxydes d'aluminium et aux matières organiques présentes dans les sédiments. Le rapport isotopique du cuivre variait peu dans les sédiments qui accumulaient plus de 96% du cuivre pendant la période d'observation. En conditions de hauts débits transitoires dans le bassin, le cuivre en sortie devenait isotopiquement plus léger (enrichie en ^{63}Cu), suggérant la remobilisation du cuivre sous forme réduit (sulfure de Cu(I), Cu(0)) des sédiments du bassin (Figure 3). Nos résultats ont montré que l'utilisation des isotopes stables du cuivre permettrait de mieux comprendre le transport réactif du cuivre dans des environnements dynamiques d'un point de vue hydrologique et biogéochimique.

Colonnes de Winogradsky pour suivre l'évolution du cuivre dans les sédiments

Finalement, nous avons voulu regarder de plus près le devenir du cuivre dans les sédiments du bassin de rétention en utilisant les isotopes du cuivre. Pour cela nous avons réalisé une expérience en laboratoire en préparant des colonnes de Winogradsky. Ces colonnes sont des écosystèmes miniatures, qui permettent d'amplifier les processus naturels et notamment les gradients biogéochimiques qui se forment dans les sédiments des zones humides dus à l'activité microbienne. Nous avons construit quatre colonnes (65cm × 15cm) en prenant des sédiments du bassin de rétention à Rouffach dans lesquels des nutriments ont été rajoutés, fournissant des substrats supplémentaires aux microorganismes. Nous avons échantillonné deux colonnes au bout de 40 et 80 jours, et les deux derniers au bout de 520 jours d'évolution. Nous avons coupé les colonnes de sédiments en faisant des tranches de quelques cm pour mettre en évidence des variations spatiales. Nous avons également séparé le dissous (le liquide interstitiel) et les solides.

Les résultats des extractions séquentielles du cuivre dans les sédiments des colonnes ont montré que de plus en plus de cuivre se retrouvait dans la fraction dite résiduelle (i.e. dans la matrice des minéraux silicatés) ce qui indiquerait le « vieillissement » du cuivre dans les sédiments et une réduction de sa mobilité avec le temps (Figure 4). En outre ses changements de la répartition du cuivre entre les différentes phases dans les sédiments avec le temps, des zones d'accumulation du cuivre ont été mis en évidence dans les deux dernières colonnes. Les résultats isotopiques ont révélé un écart entre le sédiment initial (δ^{65}Cu = 0,09 ‰) et celui de certaines couches de sédiment (à 6-10 cm de profondeur) (δ^{65}Cu = 0,15 – 0,25, ±0,07 ‰), en particulier dans des zones où le cuivre s'est accumulé. Dans ces colonnes, un gradient d'oxydo-réduction s'est formé dû à la présence des algues en haut de colonnes produisant de l'oxygène et des conditions de plus en plus réductrices en descendant de les colonnes, créée par l'activité microbienne.

Ainsi la redistribution du cuivre le long des colonnes de sédiments sont peut être dû à la ré-oxydation des espèces du cuivre réduites dans la zone colonisée par des algues (conditions oxydées), entrainant la mobilisation du cuivre du haut de colonnes. Ceci indique la remobilisation possible du cuivre dans les sédiments des milieux aquatiques malgré une réduction générale de leur disponibilité lors du « vieillissement ». Ainsi les rapports isotopiques du cuivre permettent de souligner les transformations du cuivre en lien avec les gradients biogéochimiques qui s'établissent dans les sédiments des milieux aquatiques.

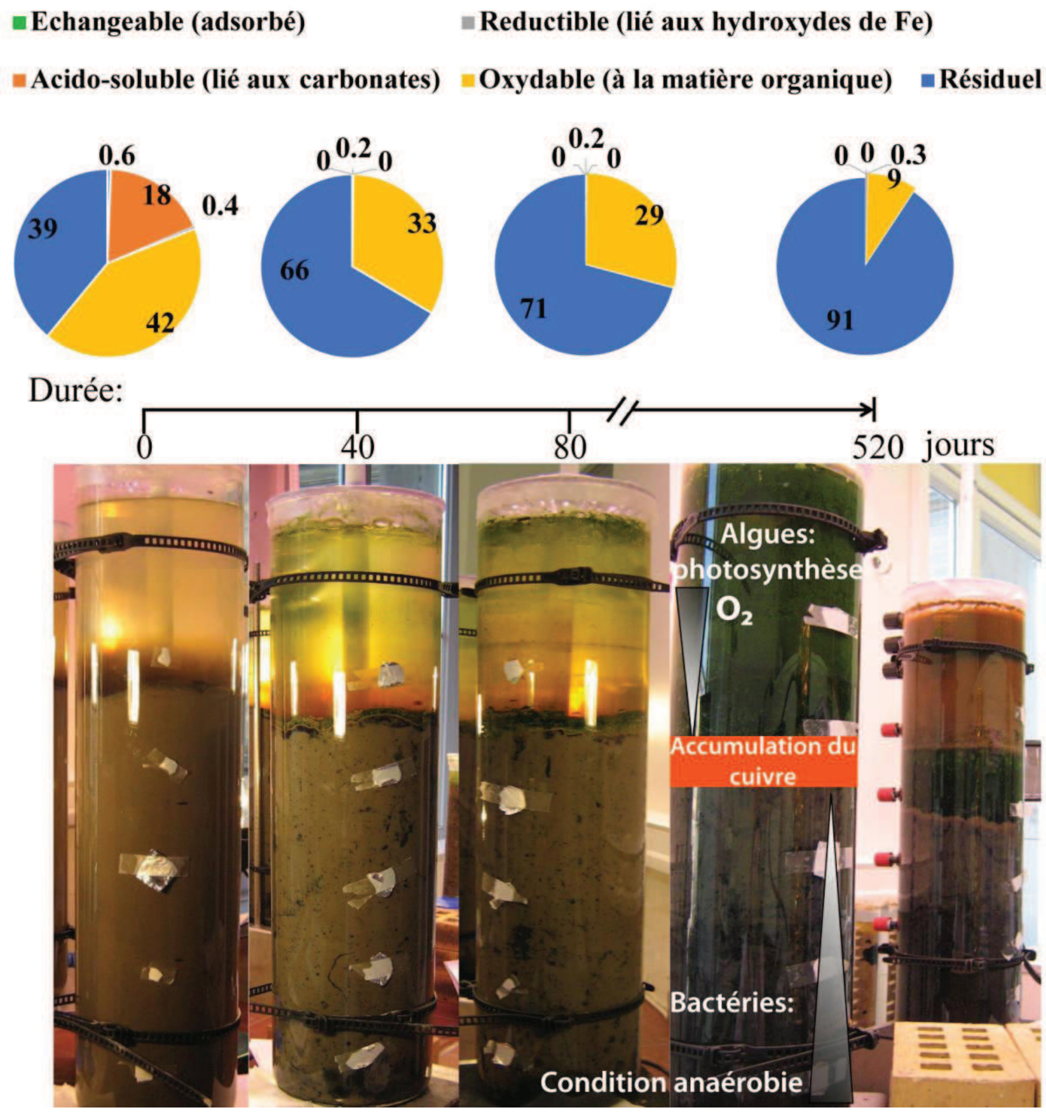


Figure 4 : L'évolution visuelle des colonnes de Winogradsky et le « vieillissement » du cuivre dans les sédiments mis en évidence par le changement de la répartition du cuivre entre les différentes phases dans les sédiments (i.e., carbonates, hydroxydes de fer, matière organique, minéraux silicatés)

Cette thèse a démontré que la combinaison de différentes échelles avec des mesures isotopiques permet une meilleure compréhension du cycle du cuivre ainsi qu'une meilleure appréhension des risques liés à son accumulation dans les agro-systèmes viticoles et des bassins de rétention associés.

IMPLICATIONS DE L'ETUDE:

Durant 4 à 5 décennies d'exploitation des parcelles viticoles, les sols en surface se sont enrichis en cuivre de 9 à 28 fois par rapport au fond géochimique.

Lors des évènements pluvieux, ~1% du cuivre appliqué (sous forme de fongicides cupriques) a été mobilisé pendant la saison, ce qui parait peu élevé. Cependant, la lame ruisselante transportait des concentrations en cuivre jusqu'à 3 fois plus élevées que le seuil écologique recommandé pour le bon fonctionnement des écosystèmes aquatiques.

Les zones tampons humide artificielle (i.e. bassin d'orage) récoltant les lames ruisselantes provenant de petits bassins versants viticoles peuvent retenir une grande partie du cuivre et réduire ainsi la charge du cuivre dans les lames ruisselantes. Cependant, lors des hauts débits ces ouvrages peuvent relarguer du cuivre préalablement accumulé dans leurs sédiments, et ainsi devenir des sources secondaires de cuivre.

Le « vieillissement » du cuivre dans les sédiments des zones humides a tendance à réduire sa mobilité et sa disponibilité.

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Articles in peer-reviewed journals:

Babcsányi I., Chabaux F., Duplay J., Imfeld G. Copper Stable Isotopes for Evaluating Copper in Soils and Runoff in Vineyards (Submitted to the journal *Environmental Science and Technology*)

Babcsányi I., Chabaux F., Granet M., and Imfeld G., Using Isotopic Analysis of Copper to Assess Copper Transport and Partitioning in Wetlands, *Environmental Science and Technology,* 2014. 48 : 5520-5529. DOI : 10.1021/es405688v.

Duplay, J.; Semhi, K.; Errais, E.; Imfeld, G.; Babcsanyi, I.; Perrone, T., Copper, zinc, lead and cadmium bioavailability and retention in vineyard soils (Rouffach, France): The impact of cultural practices. *Geoderma*, 2014. 230-231 : 318-328. DOI : 10.1016/j.geoderma.2014.04.022.

Conference contributions:

Babcsányi I., Chabaux F., Granet M., and Imfeld G., Copper isotope fractionation as a proxy for biogeochemical processes in wetlands receiving copper-contaminated runoff from a vineyard catchment. **Poster** at the « International Wetlands Conference » (June, 2012, Orlando, Florida, USA)

Babcsányi I., Chabaux F., Granet M., and Imfeld G., Copper isotope fractionation as a proxy for biogeochemical processes in wetlands receiving copper-contaminated runoff. **Poster** at the Eurosoil (July 2012, Bari, Italy)

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Babcsányi I., Chabaux F., Granet M., et Imfeld G., Étude du transport et du fractionnement isotopique du cuivre en contexte viticole. **Orale communication** at the Séminaire isotopes stables organized by Thermo Scientific (May 2013, St. Malo, France)

Izabella BABCSÁNYI

Le transport et le fractionnement isotopique du cuivre dans un agrosystème

Résumé

Depuis la fin du $19^{\text{ème}}$ siècle, le traitement des vignes par des fongicides cupriques a engendré une augmentation de la teneur en cuivre (Cu) dans les sols viticoles, ainsi que dans les écosystèmes aquatiques en aval. Cette thèse vise à mieux comprendre le devenir de ce Cu dans un agrosystème basé sur l'étude du fractionnement isotopique du ^{65}Cu/^{63}Cu.

Les résultats montrent que durant 4 à 5 décennies de culture de vignes, les sols en surface se sont enrichis en Cu de 9 à 28 fois par rapport au fond géochimique et que les minéraux argileux jouent un rôle important dans l'accumulation du Cu. Lors des événements pluvieux, ~1% du Cu appliqué est mobilisé, essentiellement lié à des argiles. Le bassin d'orage récoltant les lames ruisselantes retient en moyenne 68% du Cu dissous et plus de 92% du Cu particulaire. Les ratios isotopiques du Cu dans le bassin indiqueraient la sorption du Cu dissous dans les sédiments, ainsi que la réduction du Cu(II) *in situ* due à des processus biogéochimiques.

Mots-clés: fongicides cupriques, biogéochimie, isotopes stables, vignes, sol, bassin versant, zone humide

Résumé en anglais

Since the end of the 19th century, the use of copper (Cu)-based fungicides has resulted in increased Cu concentrations in vineyard soils, but also in downstream aquatic ecosystems. The aim of the thesis was to better understand the fate of this Cu in an agrosystem based on assessing Cu isotope fractionation (^{65}Cu/^{63}Cu).

The results have shown that the surface vineyard soils have become enriched in Cu from 9 to 28 times compared to the background level during 4 to 5 decades of vine-growing and that clay minerals were the major Cu sorbing phases in the soils. During rainfall, runoff mobilized ~1% of the applied Cu during the, mainly associated with clays. The stormwater wetland collecting the runoff retained in average 68% of the dissolved and more than 92% of particulate Cu. Cu isotope ratios measured in the wetland suggested dissolved Cu sorption to the sediments and *in situ* reduction of Cu(II) due to biogeochemical processes.

Keywords: copper fungicides, biogeochemistry, stable isotopes, vineyard, soil, catchment, wetland