

UNIVERSITÉ DU QUÉBEC À MONTRÉAL

EFFET DE LA PRÉSENCE DE MÉTAUX SUR L'OZONATION CATALYTIQUE DE
L'ANTIBIOTIQUE NORFLOXACINE ET SON IMPACT ÉCOTOXICOLOGIQUE

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LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

$\equiv\text{Al-OH}$	Groupe ment hydroxyle aluminol de la montmorillonite
$\equiv\text{Si-OH}$,	Groupe ment hydroxyle silanol de la montmorillonite
A	Absorbance
C	Concentration
CAT	Catalase
CEC	Capacité d'échange cationique (Cation Exchange Capacity)
<i>Chl</i>	Chlorophylle
<i>Chl a</i>	Chlorophylle a
<i>Chl b</i>	Chlorophylle b
Co(II)	Ions cobalt
Co(II)Mt	Montmorillonite sodique échangée au cobalt
Cu(II)	Ions cuivre
Cu(II)Mt	Montmorillonite sodique échangée au cuivre
d001	Distance interréticulaire
DLS	Diffusion dynamique de la lumière
DRX	Diffraction des rayons X
EC50	Concentration efficace médiane
Fe(II)	Ions fer
Fe(II)Mt	Montmorillonite sodique échangée au fer
FQ	Fluoroquinolone
HPLC-UV	High-Performance Liquid Chromatography–Ultraviolet
I	Intensité de la lumière transmise par l'échantillon
I_0	Intensité de la lumière incidente sur l'échantillon
ICP-OES	Spectrométrie d'émission optique à plasma à couplage inductif
LC50	Concentration létale
LC-TOF-MS	Chromatographie en phase liquide couplée à la spectrométrie de masse à temps de vol
Mt	Montmorillonite
NaMt	Montmorillonite sodique
Ni(II)	Ions nickel
Ni(II)Mt	Montmorillonite sodique échangée au nickel
NOF	norfloxacin
OCDE	Organisation de Coopération et de Développement Économiques
OCH	Ozonation par catalyse hétérogène
POD	Peroxydase
PQ	Plastoquinone
PSII	Photosystème II
PZ	Potentiel zêta
QA	Quinone A
QB	Quinone B
ROS	Reactive Oxygen Species
S	Supporting Information
SIS	Swedish Institute for Standards

SOD	Superoxyde dismutase
UV-Vis	Ultraviolet-visible
ε	Coefficient d'absorption

LISTE DES SYMBOLES ET DES UNITÉS

%	Pourcentage
°C	Degré Celsius
µg	Microgramme
µg/g	Microgramme par gramme
µm	Micromètre
cm	Centimètre
CO ₂	Dioxyde de carbone
g	Gramme
g/L	Gramme par litre
g/mol	Gramme par mole
h	Heure
H ₂ O	Eau
K	Kelvin
L	Litre
L.mol ⁻¹ .cm ⁻¹	Litre par mole par centimètre
M	Molaire
M ² /g	Mètre carré par gramme
mA/cm ²	Milliampère par centimètre carré
mEq	Milléquivalent
mg	Milligramme
mg/h	Milligramme par heure
mg/L	Milligramme par litre
mg/min	Milligramme par minute
min	Minute
mL	Millilitre
ml/min	Millilitre par minute
mm	Millimètre
mol	Mole
mol/L	Mole par litre
nm	Nanomètre
O ₃	Ozone
ppm	Partie par million
rpm	Révolutions par minute
s	Seconde
ug/L	Microgramme par litre
ug/mg	Microgramme par milligramme
V	Volt
W/m ²	Watt par centimètre carré
λ	Longueur d'onde

RÉSUMÉ

Dans ce travail de recherche, la dégradation de la norfloxacin (NOF), un antibiotique de la famille des fluoroquinolones fréquemment détecté dans les milieux aquatiques, a été étudiée par ozonation catalytique en présence de minéraux argileux, afin de simuler et de mieux comprendre les processus d'oxydation naturelle accélérée dans les milieux riches en argiles. L'objectif principal de cette étude était d'élucider le rôle des cations métalliques et d'évaluer simultanément l'évolution de la dégradation et de la toxicité des mélanges réactionnels.

L'ozonation de la norfloxacin a été réalisée en milieu aqueux en présence de montmorillonites échangées avec différents cations métalliques de transition (Na^+ , Fe^{2+} , Ni^{2+} , Co^{2+} et Cu^{2+}), ainsi que de leurs homologues sous forme zéro-valente (Fe^0 , Ni^0 , Co^0 et Cu^0). La toxicité intrinsèque de la norfloxacin et des mélanges réactionnels issus de l'ozonation vis-à-vis de la plante aquatique *Lemna minor* a été mise en évidence à travers plusieurs critères de toxicité, incluant l'inhibition du nombre de frondes, la variation du poids frais, la teneur en chlorophylle, la production d'espèces réactives de l'oxygène (ROS) et la fluorescence de la chlorophylle *a*.

La première partie de ce travail a porté sur la dégradation partielle du NOF en présence des matériaux argileux. Les résultats ont montré que cette toxicité est principalement induite par un stress oxydatif et qu'elle évolue significativement au cours de l'ozonation en fonction de la nature du cation échangeable et du degré d'avancement de l'oxydation.

La seconde partie de cette recherche a été consacrée à l'étude de l'adsorption et de l'ozonation de la norfloxacin en présence de cations métalliques libres et de leurs homologues supportés sur montmorillonite. L'évolution conjointe de la dégradation de la NOF et de la toxicité envers *L. minor* a été discutée en lien avec les interactions cation:argile:NOF, la dispersion du catalyseur dans le milieu aqueux et la décroissance du pH au cours de l'ozonation. Les interactions d'échange ionique et les interactions acide-base de Lewis ont été identifiées comme des mécanismes clés gouvernant l'adsorption de la NOF, la réactivité catalytique des argiles et la formation progressive d'espèces acides dans le milieu réactionnel.

La troisième partie de ce travail a porté sur l'étude de l'influence de l'état de valence du métal sur les propriétés de surface des minéraux argileux chargés en métaux. L'évaluation écotoxicologique réalisée sur *Lemna minor* a montré que les cations métalliques réduisent la biodisponibilité de la norfloxacin, tandis que les métaux zéro-valents induisent une toxicité plus élevée.

Cette recherche doctorale met en évidence l'impact potentiel de la co-présence de NOF et des métaux sur la toxicité des milieux aquatiques. Les résultats apportent des connaissances fondamentales sur le rôle des minéraux argileux et de la nature chimique des métaux dans les processus de dégradation oxydative de NOF. Ils démontrent que les milieux argileux, tels que les sols et les suspensions argileuses des rivières et des lacs, possèdent une capacité intrinsèque à moduler la transformation et l'impact environnemental des polluants organiques.

Cette approche intégrée, combinant la chimie de l'environnement et l'écotoxicologie, a permis d'aborder simultanément les aspects physico-chimiques et biologiques liés au devenir des polluants dans les milieux aquatiques, et a conduit à la publication de trois articles scientifiques dans des revues internationales.

Mots clés : Norfloxacin ; Catalyseurs argileux ; Montmorillonite ; Cations métalliques ; Ozonation ; *Lemna minor* ; Métaux à valence zéro ; Toxicité.

ABSTRACT

In this research work, the degradation of norfloxacin (NOF), a fluoroquinolone antibiotic frequently detected in aquatic environments, was investigated through catalytic ozonation in the presence of clay minerals, in order to simulate and better understand accelerated natural oxidation processes in clay-rich media. The main objective of this study was to elucidate the role of metal cations and to simultaneously assess the evolution of both degradation efficiency and toxicity of the resulting reaction mixtures.

Norfloxacin ozonation was carried out in aqueous media in the presence of montmorillonites exchanged with various transition metal cations (Na^+ , Fe^{2+} , Ni^{2+} , Co^{2+} , and Cu^{2+}), as well as their zero-valent counterparts (Fe^0 , Ni^0 , Co^0 , and Cu^0). The intrinsic toxicity of norfloxacin and of the ozonized reaction mixtures toward *Lemna minor* was evaluated using several toxicity endpoints, including inhibition of frond number, changes in fresh weight, chlorophyll content, production of reactive oxygen species (ROS), and chlorophyll a fluorescence.

The first part of this work focused on the partial degradation of NOF in the presence of clay materials. The results showed that toxicity is mainly induced by oxidative stress and that it evolves significantly during ozonation depending on the nature of the exchangeable cation and the degree of oxidation advancement.

The second part of this research was devoted to the study of norfloxacin adsorption and ozonation in the presence of free metal cations and their montmorillonite-supported counterparts. The concomitant evolution of NOF degradation and toxicity toward *Lemna minor* was discussed in relation to cation:clay:NOF interactions, catalyst dispersion in the aqueous medium, and pH decrease during ozonation. Ion-exchange processes and Lewis acid–base interactions were identified as key mechanisms governing NOF adsorption, the catalytic reactivity of clay minerals, and the progressive formation of acidic species in the reaction medium.

The third part of this work addressed the influence of the metal valence state on the surface properties of metal-loaded clay minerals. Ecotoxicological assessment using *Lemna minor* showed that metal cations reduce the bioavailability of norfloxacin, whereas zero-valent metals induce higher toxicity.

This doctoral research highlights the potential impact of the co-occurrence of norfloxacin and metals on the toxicity of aquatic environments. The results provide fundamental insights into the role of clay minerals and metal chemical nature in the oxidative degradation of NOF and demonstrate that clay-rich environments, such as soils and clay suspensions in rivers and lakes, possess an intrinsic capacity to modulate the transformation and environmental impact of organic pollutants.

This integrated approach, combining environmental chemistry and ecotoxicology, enabled the simultaneous investigation of the physicochemical and biological aspects governing the fate of pollutants in aquatic environments, and resulted in three scientific publications in international journals.

Keywords : Norfloxacin ; Clay catalysts ; Montmorillonite ; Metal cations ; Ozonation ; *Lemna minor* ; zero valent metals ; Toxicity.

INTRODUCTION

1. Mise en contexte

L'essor rapide et souvent incontrôlé des activités humaines libère continuellement dans la nature une grande diversité de polluants dont des métaux lourds, des gaz toxiques, des plastifiants, des déchets inorganiques, des composés organiques volatils ou non ainsi que des agents biologiques. Cette pollution généralisée entraîne des conséquences écologiques majeures, et l'accumulation de telles substances dans l'atmosphère, les eaux et les sols mettent en péril les écosystèmes (Stosic and Zholobenko 2025). Ces composés peuvent atteindre les sols par infiltration, ruissellement, irrigation ou à travers les boues issues des stations d'épuration (Baral, Bhattarai and Chaudhary 2024).

Leur présence dans l'eau potable est particulièrement préoccupante car une exposition prolongée aux résidus pharmaceutiques peut entraîner de graves problèmes de santé tels que des allergies, des maladies ou des tumeurs (Fraiha, Hadoudi et al. 2024). Une fois dans l'eau ou dans le sol, ces composés peuvent subir divers processus de dégradation naturelle (**Schéma 0.1**). Ces derniers regroupent la biodégradation, la photolyse, l'adsorption, l'hydrolyse ou même l'ozonation, sur lequel se base le présent travail.

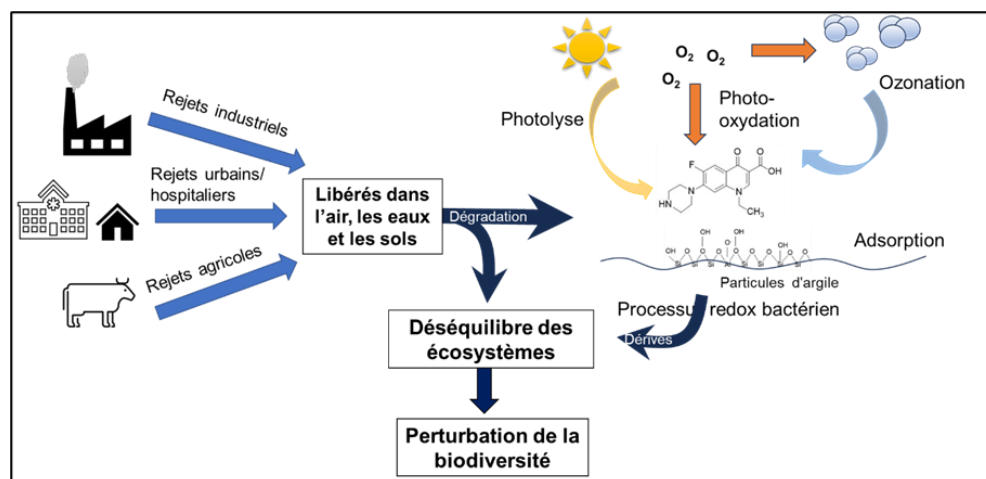


Schéma 0.1. Voies de dégradation d'un polluant organique dans l'environnement (Conception propre).

Dans cette recherche, un accent particulier est mis sur les polluants organiques et plus particulièrement les agents antibiotiques, qui relèvent d'un enjeu socio-économique majeur. Ce dernier confronte des exigences adverses liées à l'économie, la santé humaine et l'environnement. En amont, l'utilisation de ce genre de médicaments répond aux deux premières exigences (l'économie souvent avant même la santé humaine) au détriment des deux dernières (santé humaine et l'environnement et inversement) dans la plupart des cas. La simple présence de ces molécules organiques dans les milieux récepteurs (tous genres d'effluents pollués) induit des effets directs et indirects au contact des écosystèmes et leur biodiversité (**schéma 0.1**).

Les milieux récepteurs peuvent être des effluents solides, liquides et/ou gazeux, qui vont imposer des échanges de matière et d'énergie, qui vont, à leur tour, perturber les équilibres des milieux naturels. De plus, leur persistance dans ces milieux pollués, en cas d'absence de traitements immédiats appropriés, entraîne des changements dans les propriétés physico-chimiques de ces milieux qui vont accentuer ces perturbations et affecter les échanges hydriques et nutritifs essentiels et la survie et au développement de la biodiversité.

2. Problématique

Parmi l'ensemble des polluants organiques détectés dans les milieux naturels, les antibiotiques sont des composés qui résistent aux procédés classiques de traitements des eaux. Leur stabilité chimique et leurs potentiels de bioaccumulation en fait une menace continue pour l'écosystème. L'exposition prolongée des rejets d'antibiotiques aux facteurs ambiants peut entraîner à long terme souvent une contamination d'écosystèmes adjacents, et disperser non seulement la toxicité intrinsèque de l'antibiotique polluant et de celles de ces dérivés de dégradation inévitable.

Les antibiotiques sont de par leur nature nocifs pour les espèces vivantes. En grande concentration dans les effluents aqueux, ils peuvent détruire des bactéries en équilibre dans un environnement sain. En faibles quantités, ou bien même à la sortie de traitement des eaux insuffisant, elles peuvent permettre à certaines bactéries, le plus souvent pathogènes, d'acquérir une résistance aux antibiotiques, qui peut être nocive et pour la faune, la flore et même la santé humaine. À cela, il faudrait ajouter qu'une dégradation partielle produit inévitablement des intermédiaires, pouvant présenter des toxicités individuelles qui peuvent contribuer à l'impact négatif sur la biodiversité.

Ces molécules sont caractérisées par une forte polarité et plusieurs fonctions ionisables, ce qui les rend très solubles dans l'eau, sensibles aux variations de pH et favorise leur dispersion dans les milieux aquatiques (Kümmerer 2009). Ces mêmes propriétés modifient leurs interactions avec différents composants du milieu, notamment les espèces métalliques et leurs affinités envers les surfaces adsorbantes telles que les aluminosilicates dans les sols et les suspensions d'argiles en milieux aquatiques. Ces suspensions peuvent également influencer le devenir et les trajets de ces molécules. Elles favorisent non seulement leur adsorption mais aussi leur dégradation lorsqu'elles sont exposées à des facteurs favorisant l'oxydation naturelle. Ces surfaces argileuses servent de supports catalytiques et de surface de rencontre entre polluant et les agents oxydants.

Parallèlement, les métaux potentiellement présents dans les rejets industriels et agricoles peuvent interagir avec les antibiotiques entraînant la formation de complexes ou/et modifiant leur biodisponibilité. Ces interactions dépendent non seulement de la concentration du métal, mais aussi de sa valence, sa biodisponibilité et le pH du milieu (Sazykin, Khmelevtsova et al. 2023). Les données restent limitées quant à la toxicité de ses complexes métal-polluant organique chez les espèces non ciblées (Baral, Bhattarai and Chaudhary 2024).

Une fois dans le sol, le comportement physico-chimique d'un antibiotique dépend de nombreux facteurs dont la composition minéralogique du sol et en particulier sa fraction argileuse. Les argiles du fait de leur grande surface spécifique, une forte capacité d'adsorption, leur pouvoir de gonflement et leur importante capacité d'échange cationique jouent un rôle clé dans la rétention de ces polluants (Mishra, Mehta and Basu 2018, Wołejko, Jabłońska-Trypuć et al. 2020). Ces composés peuvent être adsorbés sur les surfaces argileuses ou piégés entre les feuillets limitant ainsi leur mobilité, mais prolongeant leur persistance. De plus, les suspensions argileuses peuvent contenir des métaux qui interagissent avec les polluants organiques par des mécanismes de complexation ou de chélation. Ces interactions modifient non seulement la mobilité et la biodisponibilité des polluants, mais aussi leur toxicité et leur comportement en milieu naturel.

Ainsi, la dégradation des antibiotiques dépend non seulement de leurs propriétés physicochimiques, mais également de la composition du milieu dans lequel ils sont dispersés. C'est dans ce contexte qu'il devient nécessaire de comprendre les mécanismes de dégradation et de transformation de ces

polluants dans des milieux complexes, ainsi que leur toxicité pour développer des stratégies de remédiation efficaces et durables.

3. Stratégie et objectifs

Une des voies de dégradation naturelle possible dans un environnement exposé aux facteurs atmosphériques figure la photo-oxydation. Celle-ci produit entre autres et inévitablement de l’ozone par la conversion de l’oxygène sous les rayonnements UV du soleil (Rowland 2006). Ainsi l’ozonation est un processus induit par photo-oxydation qui concentre l’intérêt de cette recherche. L’efficacité de cette oxydation peut toutefois être fortement modulée par la présence d’argiles et de métaux, surtout dans les sols aérés et les surfaces et interfaces des milieux aqueux. Ces derniers peuvent catalyser la décomposition de l’ozone et accélérer la formation d’espèces oxydantes encore plus réactives. L’utilisation de l’ozone en milieux contenant des matériaux argileux cationiques est une stratégie judicieuse pour reproduire à l’échelle de laboratoire la lente dégradation oxydative d’un polluant organique dans la nature en des temps permettant de corréler l’influence du milieu sur son écotoxicité. L’application de cette stratégie à la Norfloxacin (NOF) est l’objectif majeur dans cette recherche.

En effet, la NOF altère les interactions bénéfiques essentielles au maintien de l’équilibre écologique entre les organismes aquatiques et leurs communautés bactériennes associées (Lin, Chang et al. 2025). L’eau, grâce à ses propriétés de solvant, peut dissoudre une large gamme de composés ioniques et polaires facilitant ainsi leurs transferts aux plantes et aux animaux. En conditions aérobies, les cations métalliques à la surface des sédiments et particules minérales jouent un rôle déterminant dans les processus d’oxydation. De même, en profondeur de ces matrices, en absence d’oxygène, les cations peuvent se convertir en métaux sous formes réduites, qui peuvent aussi interagir avec le polluant organique, modifiant à la fois sa biodisponibilité et son écotoxicité vis-à-vis des organismes vivants. L’étude des effets de ces interactions sur la dégradation du polluant organique et la toxicité résultante sur une espèce vivante représentant la biodiversité constitue l’un des objectifs de cette recherche (**Schéma 0.2**).

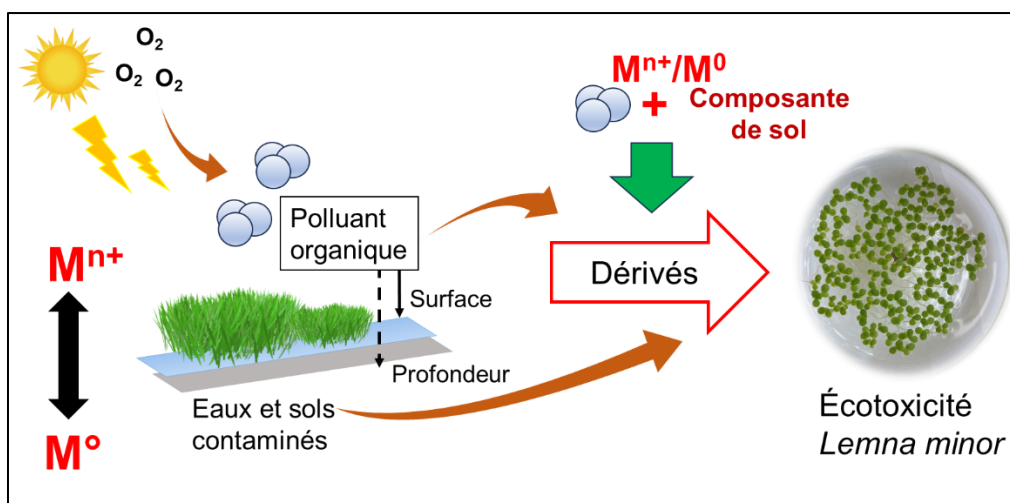


Schéma 0.2. L'approche de la thèse

La décomposition et la rétention du polluant et de ses dérivés dans le milieu récepteur sont influencées par de nombreux facteurs tels que la surface spécifique et taille de particules de l'adsorbant, le temps de contact, le pH du milieu, la température, la dose d'adsorbant et la concentration initiale en polluant organique (Shahidi, Moheb et al. 2015, Benghaffour, Dewez and Azzouz 2023). Ces paramètres déterminent non seulement le niveau de rétention du polluant, mais aussi la cinétique de sa transformation et la persistance de ses effets dans l'environnement. Parmi les objectifs spécifiques figurent la corrélation entre l'état de dispersion de la suspension argileuse sur ses performances adsorptives et catalytiques, l'effet du pH sur les échanges des différentes formes métalliques entre le milieu liquide, la surface argileuse et la NOF, l'étude des interactions entre l'antibiotique et les métaux à différents états de valence ainsi que l'évaluation de l'impact de la progression de l'oxydation du polluant organique sur la plante aquatique *Lemna minor*. Un macrophyte très sensible aux polluants et elle fait partie des espèces de référence dans les lignes directrices de l'Organisation de coopération et de développement économiques (OCDE) pour évaluer la toxicité des substances chimiques en milieu aquatique. L'évaluation de la toxicité sur *L. minor* est réalisée en déterminant l'inhibition de croissance telle que le nombre de frondes et la biomasse fraîche, le changement de la teneur en pigments photosynthétiques et l'activité photosynthétique par des mesures de fluorescence de la chlorophylle *a*. Ces étapes sont à la base

de l'élaboration de la méthodologie de recherche pour atteindre les objectifs visés. Ceci impose une structure spécifique à cette thèse de doctorat.

4. Structure de la thèse

Afin d'atteindre les objectifs visés, ce travail a été organisé selon une structure impliquant la succession logique de six chapitres principaux. Les descriptions des différents chapitres sont présentées ci-dessous accompagnées d'un schéma récapitulatif (**Schéma 03**).

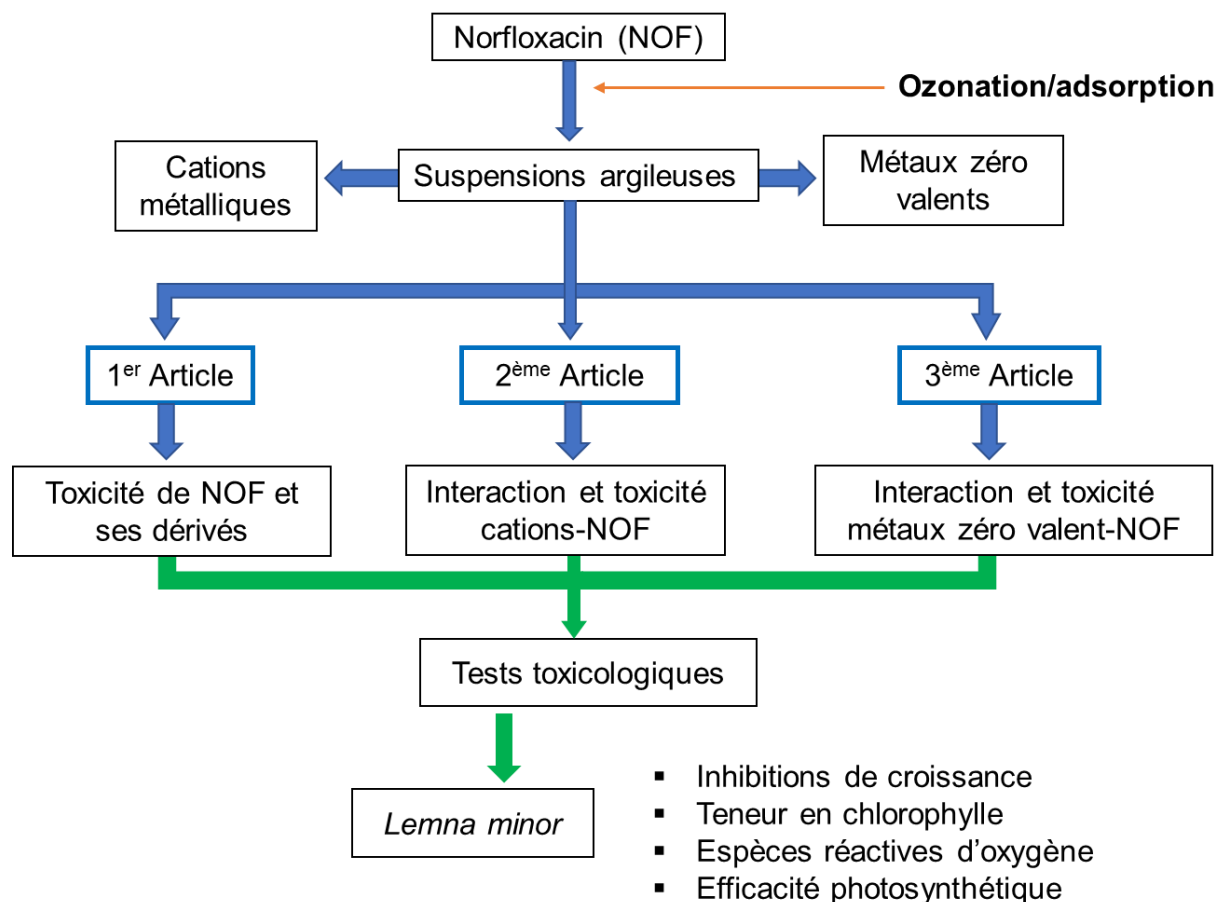


Schéma 03. Vue d'ensemble de la méthodologie utilisée en lien avec les objectifs visés.

1. Chapitre 1 : Cette partie est consacrée à une synthèse bibliographique qui a pour objectif une mise en contexte de l'étude et présente la thématique abordée. Il présente tout d'abord la

problématique de la pollution des milieux aquatiques. Après une présentation liée à la présence de polluants organiques, une revue sur le phénomène d'ozonation pouvant se produire spontanément et qui contribue à la dégradation des contaminants est proposée afin de justifier l'approche choisie. La seconde partie de ce chapitre est consacrée spécifiquement sur le rôle des métaux, qu'ils interviennent sous forme ionique ou sous forme zéro valent. En mettant l'accent sur leur impact en tant que catalyseur ou en tant qu'agent complexant capable de modifier la réactivité des polluants. Enfin, une dernière section abordant la toxicité résiduelle associée aux produits intermédiaires et au complexe métal-polluant. L'attention est portée plus particulièrement sur *Lemna minor*, utilisée comme bioindicateur végétal modèle en écotoxicologie aquatique. Cette section permet ainsi d'étudier l'impact de ces interactions sur des paramètres clés tels que la croissance ou l'efficacité photosynthétique.

2. Chapitre 2 : Ce chapitre expose la méthodologie adoptée pour atteindre les objectifs visés et regroupe les protocoles expérimentaux et les techniques d'analyse des milieux réactionnels et de culture du bio-indicateurs ainsi que les méthodes de caractérisation des adsorbants/catalyseur utilisés tout au long de la recherche ainsi que les méthodes de préparation des échantillons, des catalyseurs argileux ainsi que leurs caractérisations. Les dispositifs des tests d'ozonation et d'adsorption ainsi que les essais écotoxicologiques sur l'organisme aquatique *Lemna minor* seront aussi présentés. Ce chapitre inclut également une description des techniques d'analyse utilisées en précisant les paramètres mesurés et les conditions opératoires.

3. Chapitre 3 : Ce chapitre fournit le contenu de la première publication dans la revue Chemosphere. Cette étude examine la dégradation oxydative de la Norfloxacin par ozonation en présence de la montmorillonite (Mt) échangée avec des cations Na^+ , Fe^{2+} et Cu^{2+} (NaMt, Fe(II)Mt et Cu(II)Mt). Les produits de dégradation ont été suivi par spectrophotométrie UV-Vis et chromatographie couplée à la spectrométrie de masse (LC-MS). Les résultats obtenus montrent que la NOF est intrinsèquement toxique pour la plante aquatique *Lemna minor*, provoquant un stress oxydatif et une réduction de croissance. L'ozonation sans catalyseur aggrave cette toxicité en générant des intermédiaires plus nocifs. L'ajout de Fe(II)Mt améliore la dégradation tout en limitant la toxicité, contrairement au Cu(II)Mt qui possède une forte activité catalytique, mais générant des dérivés plus toxiques. Ces résultats apportent des pistes sur le devenir environnemental des antibiotiques en conditions aérobies. Elles permettent de mieux comprendre

l'évolution de leurs impacts sur la biodiversité, ouvrant la voie à des stratégies de traitement de l'eau visant la minéralisation complète de l'eau.

4. Chapitre 4 : Ce chapitre contient la deuxième publication de l'article publié dans la revue International Journal of Molecular Sciences (IJMS). Cette partie traite l'influence de l'acidité générée pendant le processus d'oxydation de la Norfloxacin sur la dispersion des particules d'argiles. Ces changements physico-chimiques modifient l'efficacité catalytique ainsi que l'écotoxicité du mélange. Les changements observés concernent les interactions entre les cations et la surface argileuse avec les molécules de NOF ainsi que la diminution du pH au cours de l'ozonation. Cette diminution du pH qui influence la toxicité des sous-produits générés. De ce fait, cette étude souligne l'importance du contrôle du pH dans les procédés d'ozonation, tant que pour l'efficacité de la dégradation que pour la toxicité environnementale.

5. Chapitre 5 : Il s'agit du contenu d'un troisième article publié également dans la revue IJMS, qui porte sur l'effet de l'interaction NOF-métal, présent sous formes cationique ou zéro-valente. Les analyses par spectrophotométrie UV-Vis et la spectroscopie infrarouge à transformée de Fourier (FTIR) confirment l'implication des groupements carboxyle et carbonyle de la NOF dans la coordination avec les cations. Le rôle catalytique des métaux zéro valents supportés sur la montmorillonite a été examiné dans la dégradation du NOF. L'évaluation écotoxicologique à l'aide du bioindicateur *Lemna minor* a montré une variation de la toxicité en fonction de la spéciation chimique du métal et de la biodisponibilité du polluant. Cette étude apporte des données nouvelles sur les impacts environnementaux potentiels liés à la co-présence des fluoroquinolones et de métaux dans les milieux aquatiques.

6. Chapitre 6 : Cette partie consiste en une conclusion générale qui synthétise l'ensemble des principales conclusions tirées à chaque étape. Ces conclusions sont suivies de propositions de recherches futures dont certains concepts et phénomènes restent à élaborer et/ou élucider. Ceci concerne entre autres la dégradation réductive de polluants organiques en milieux anaérobiques.

CHAPITRE 1

REVUE BIBLIOGRAPHIQUE

1.1 Pollution de l'eau

La pollution de l'eau est aujourd'hui la principale menace environnementale, dont les impacts touchent particulièrement les milieux aquatiques ce qui affecte l'équilibre des écosystèmes et menace la santé humaine et la biodiversité. Les sources de pollution sont multiples et variées, avec des effluents :

1. domestiques contenant des polluants organiques (médicaments, détergents, surfactants, plastifiants et autres) (Pal, He et al. 2014),
2. agricoles qui libèrent des engrais azotés et phosphatés qui entraînent des phénomènes d'eutrophisation des milieux aquatiques (Smith and Schindler 2009), des pesticides et herbicides qui induisent une toxicité pour la faune et la flore aquatiques (Kumar, Dahms et al. 2014, Benghaffour, Dewez and Azzouz 2023).
3. industriels qui rejettent plusieurs types de polluants dont des métaux lourds (Nagajyoti, Lee and Sreekanth 2010), des résidus pharmaceutiques, des produits cosmétiques et diverses types de nanoparticules (Shahid, Kashif et al. 2021)

Face à cette situation, il est essentiel de développer des stratégies efficaces de traitement et de prévention. Dans cette optique, s'inspirer de la nature constitue une piste particulièrement prometteuse pour développer des procédés plus écologiques de traitement des eaux, plus particulièrement ceux utilisant des matériaux naturels. Ainsi, les matériaux argileux plus ou moins modifiés peuvent y agir comme adsorbants/catalyseurs dans des techniques d'oxydation avancée telles que l'ozonation. De ce fait, la compréhension des mécanismes de dégradation des polluants en milieux contenant ces matériaux et l'évaluation de leur toxicité sont essentielles pour envisager des voies durables de traitement des eaux avec impact minimal sur l'environnement et la biodiversité.

1.2 Pollution organique

Les rejets de concentrations élevées de composés organiques, notamment les molécules pharmaceutiques dont les antibiotiques dans les effluents aqueux inévitablement en contact avec des écosystèmes aquatiques représentent un défi majeur pour toute tentative d'assainissement de l'environnement. Leur impact négatif sur les écosystèmes se traduit non seulement par des modifications physico-chimiques sur ces milieux, mais surtout par leur toxicité sur la biodiversité.

Cette toxicité est souvent exprimée entre autres à l'aide de la concentration causant à la mortalité de la moitié d'une population biologique testée (Lethal concentration LC_{50}) et/ou la concentration efficace médiane (Half maximal effective concentration ou EC_{50}) entre la ligne de base (effet minimal) et l'effet maximum du polluant organique après une certaine durée de contact avec une espèce vivante. Comme tout paramètre de toxicité, la EC_{50} varie principalement en fonction de la structure moléculaire du polluant organique et de la durée d'exposition tel qu'illustré par quelques exemples dans le **tableau 1.1**.

Tableau 1.1. Exemples de valeurs de EC50 pour certains polluants organiques.

Polluant	Conc. max (mg/L)	Organisme testé (Bio-indicateur)	Biomarqueurs mesurés	EC50 (mg/L)	Temps de contact (Heures)	Réf.
Acetochlor Alachlor Metolachlor	100	<i>Pseudokirchneriella subcapitata</i>	Fluorescence de la chlorophylle	0.0025 0.0443 0.0066	72	(Souissi, Boucho nnet et al. 2013)
		<i>Daphnia magna</i>	Immobile Daphnia	6.29 7.50 22.3	48	
Érythromycine	20	<i>Chlorella pyrenoidosa</i>	Croissance cellulaire par densité optique	8.180	96	(Li, Liu et al. 2023)
Roxithromycine				2.128		
Sulfaméthoxazole	-	<i>Chlorella vulgaris</i>	Inhibition de croissance	0.98	48	(Boreck a, Białk-Bielińsk a et al. 2016)
Sulfapyridine				1.93		
Triméthoprim				123.22		
Oxytétracycline	10.1	<i>Pseudokirchneriella subcapitata</i>	Inhibition de croissance	1.04	72	(Kolar, Arnuš et al. 2014)
Triméthoprim	353			169		
Ampicilline	2000 200 50	<i>Pseudokirchneriella subcapitata</i>	Inhibition de croissance	>2000	72	(Magdal eno, Saenz et al. 2015)
Amoxicilline				>2000		
Céfalotine				>600		
Ciprofloxacine				11.3		
Gentamicine				19.2		
Vancomycine				724		

EC₅₀= concentration médiane efficace, la concentration d'une substance qui provoque une réponse chez 50 % du paramètre testé.

Conc. Max : concertation maximum testée; les organismes vivants étudiés ont été exposés à différentes doses en dessous de ces valeurs.

Plus de 70 % des produits pharmaceutiques et antibiotiques sont excrétés sous forme active dans les urines puis libérés dans l'environnement (Song, Liang et al. 2020). Ces substances, souvent partiellement et peu éliminées par les stations d'épuration conventionnelles, se retrouvent dans les eaux de surface, les nappes phréatiques et même dans les eaux destinées à la consommation humaine (Leite, Colombo et al. 2024). La plupart des procédés de traitement des eaux produisent également des sous-produits de dégradation issus de l'oxydation partielle de molécules-mères. Certains de ces intermédiaires ne sont pas nécessairement inoffensifs, et peuvent présenter une toxicité résiduelle pour les organismes aquatiques, qui s'ajoute à la toxicité intrinsèque de leurs

molécules-mères. La variation de la toxicité globale fait depuis longtemps l'objet d'investigations multiples et continues. Certaines études ont également recours à la modélisation QSAR/ECOSAR qui permet de prédire la toxicité des produits de dégradation à partir de leur structure chimique. Même si la modélisation ECOSAR a révélé que certains produits de transformation sont plus toxiques que l'antibiotique mère, la toxicité globale est réduite car le composé parent est éliminé et les produits formés sont en faibles quantités (Li, Liu et al. 2023).

1.3 Pollution par les antibiotiques

Les antibiotiques, largement utilisés en médecine humaine et vétérinaire et en aquaculture, sont aujourd'hui reconnus comme des polluants d'intérêt majeur. Une part importante de ces substances non métabolisée par les organismes est excrétée et rejoint les eaux usées domestiques ou les effluents agricoles. Bien que traités dans les stations d'épuration, ces composés ne sont pas totalement éliminés, ce qui entraîne leur présence résiduelle dans les sols, les eaux de surface, les nappes phréatiques et même dans l'air sous forme d'aérosols. Cette dispersion incontrôlée entraîne une contamination diffuse de l'environnement et favorise l'émergence de résistances bactériennes et une toxicité pour les organismes aquatiques avec impact direct sur la santé humaine.

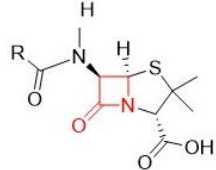
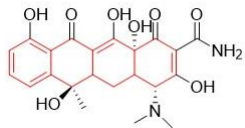
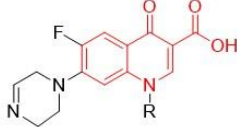
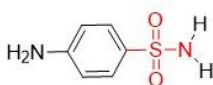
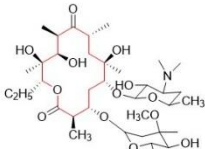
La complexité de leur dégradation nécessite des traitements spécifiques pour une élimination efficace et leurs détections requiert des techniques d'analyse avancées (Cycoń, Mrozik and Piotrowska-Seget 2019). La résistance aux antibiotiques est désormais considérée par de nombreuses institutions nationales et internationales telles que l'Union européenne et l'Organisation mondiale de la santé comme l'un des enjeux sanitaires les plus préoccupants de notre époque (Reina, Martínez-Piernas et al. 2018). Ce phénomène constitue un enjeu environnemental et sanitaire critique, d'autant plus préoccupant qu'il compromet l'efficacité future des traitements antibiotiques et la sécurité des ressources en eau.

L'un des principaux vecteurs de contamination des sols par les antibiotiques est l'utilisation agricole de fertilisants organiques tels que les fumiers, les purins et les boues d'épuration, qui peuvent contenir des résidus médicamenteux non dégradés ainsi que leurs métabolites (Akimenko 2021, Haciosmanoğlu, Mejías et al. 2022). D'après un rapport de la FDA (Food and Drug Administration), environ 14.6 millions de kilogrammes d'antimicrobiens ont été utilisés en 2014

dans les élevages d'animaux destinés à l'alimentation (Jiang, Gao et al. 2018). L'Union européenne a inscrit certains antibiotiques sur la liste de surveillance (Watch List) des polluants émergents établie dans le cadre de la Directive-cadre sur l'eau comme potentiellement dangereux pour les écosystèmes aquatiques, mais pour lesquelles des données de suivi supplémentaires sont requises afin de mieux évaluer leur niveau de risque (Hacıosmanoğlu, Mejías et al. 2022). Selon le rapport de l'Organisation mondiale de la santé, ces composés pharmaceutiques sont responsables de 25 % de la pollution de l'eau dans le monde (Zahmatkesh Anbarani, Najafpoor et al. 2023). De plus, certains d'entre eux sont susceptibles de s'accumuler dans les tissus des organismes aquatiques par bioaccumulation favorisant ainsi leur transfert le long de la chaîne alimentaire (Ciğeroğlu, El Messaoudi et al. 2024).

À l'origine, les antibiotiques désignaient des substances naturelles produites par des micro-organismes capables d'inhiber ou de tuer d'autres micro-organismes, mais la définition actuelle inclut aussi des composés antimicrobiens partiellement ou totalement synthétiques (Mora-Gamboa, Rincón-Gamboa et al. 2022). Ils peuvent être classés selon différents critères, mais les plus courantes s'appuient sur leur structure chimique, leur mécanisme d'action et leur spectre d'activité. Parmi les principales classes, les β -lactamines, les macrolides, les tétracyclines, les quinolones, les sulfonamides, les aminoglycosides et les glycopeptides (Etebu and Ariekpar 2016). Les antibiotiques exercent leur action en ciblant des structures ou fonctions essentielles des bactéries, entraînant ainsi leur inhibition ou leur destruction. Une récapitulation des principales catégories de structures moléculaires et de leurs différents mécanismes d'action sur les bactéries (Yan and Song 2014) permet de mieux comprendre les effets des antibiotiques sur la biodiversité et les écosystèmes (**Tableau 1.2**).

Tableau 1.2. Caractéristiques et propriétés physico-chimiques de certaines classes antibiotiques.

Classification	Structure chimique	Exemples	Cible d'action	Cible biologique	Réf.
Bêta-lactamines		Pénicilline, Céphalosporines Carbapénèmes Monobactames	Synthèse de la paroi bactérienne (peptidoglycane)	Gram positif Gram négatif	(Demain and Elander 1999, Worthington and Melander 2013)
Tétracyclines		Doxycycline Tétracycline Chlortétracycline Oxytétracycline	Liaison de l' aminoacyl-ARNt à la sous-unité 30S du ribosome bactérien en synthèse des protéines	Gram positif Gram négatif	(Chopra and Roberts 2001, Daghrir and Drogui 2013)
Fluoroquinolone		Norfloxacin Ciprofloxacine Ofloxacine	Enzymes de réplication de l'ADN (ADN gyrase, topoisomérase IV)	Gram positif Gram négatif	(Hooper 2000)
Sulfonamides		Sulfanilamide (sur image) Sulfaméthoxazole , Sulfadiazine	Synthèse de l'acide folique, un précurseur essentiel à la production d'ADN.	Gram positif Gram négatif	(Ovung and Bhattacharyya 2021)
Macrolides		Érythromycine Azithromycine Clarithromycine	Liaison à la sous-unité 50S du ribosome en synthèse des protéines	Gram positif	(Mazzei, Mini et al. 1993)

Les quinolones constituent une classe majeure d'antibiotiques. Leur découverte remonte aux années 1960 avec l'isolement de l'acide nalidixique, un sous-produit de la synthèse d'un antipaludique appelé chloroquine. Cet acide, premier représentant de la famille des quinolones montra une activité antibactérienne modérée principalement contre les bactéries à Gram négatif (Leshner, Froelich et al. 1962, Andriole 2005). Des modifications chimiques ont permis d'améliorer leur efficacité et leur spectre d'action. L'ajout d'un atome de fluor en position 6 du noyau quinolone et d'un groupement pipérazine ou autre en position 7 a conduit à la génération des fluoroquinolones actives sur un spectre plus large incluant les bactéries Gram positif (Serafin and Stańczak 2009). Ces molécules agissent en inhibant les enzymes clés de la réplication de l'ADN bactérien, l'ADN gyrase chez les Gram négatifs et la topoisomérase IV chez les Gram positifs entraînant un arrêt de la croissance bactérienne (Hooper 2000). Elles sont aussi efficaces contre certaines bactéries

résistantes à d'autres classes d'antibiotiques comme les tétracyclines et les β -lactamines (Serafin and Stańczak 2009). Les quinolones sont considérées comme non biodégradables (Mangla, Sharma and Ikram 2022).

Les fluoroquinolones (FQs) sont les plus utilisées dans le monde en raison de leur haute efficacité antibactérienne, de leurs faibles effets secondaires, de leurs bons effets thérapeutiques et faibles allergies (Chen, Song et al. 2024). Ils font partie des classes d'antibiotique les plus prescrites aux États-Unis et Union européenne (Cycoń, Mrozik and Piotrowska-Seget 2019). Au Canada, les FQs représentent environ 9 % des prescriptions totales, dont 13.5 % au Québec en 2019 (Crosby, von den Baumen et al. 2022). Jusqu'à présent, de nombreuses FQs ont été synthétisées, utilisées et détectées dans l'environnement. Ils ont été détectés dans les eaux de surface à une concentration de 208 ng/L (Liu, Wu et al. 2018), dans les eaux usées municipales (He, Soares et al. 2015) et dans les effluents de traitement des déchets pharmaceutiques et hospitaliers, comme par exemple la NOF (703 μ g/L), ciprofloxacin (185 μ g/L) et lomefloxacin (159 μ g/L) (Leite, Colombo et al. 2024). La NOF a aussi été détectée dans deux rivières en Inde à une concentration de 0.52 mg/L (Lin, Chang et al. 2025). Norfloxacin a même été détecté dans les organismes vivants comme dans les étangs à crevettes et les zones environnantes à des concentrations de 6.06 - 31 mg/L (Sun, Yang et al. 2022, Georgin, Franco et al. 2024).

Les fluoroquinolones ont été choisies comme cible d'étude en raison de leur usage massif, de leur forte stabilité environnementale comparée à d'autres classes d'antibiotiques comme les β -lactamines et les macrolides qui sont plus sensibles à l'hydrolyse (Timm, Borowska et al. 2019, Polianciuc, Gurzău et al. 2020). Aussi, leur implication critique dans l'émergence de résistances bactériennes, de leurs propriétés physico-chimiques favorables à l'étude des interactions environnementales ainsi que de leur difficulté à être éliminées par les procédés conventionnels. Les FQs tendent à s'accumuler dans les sédiments et à s'adsorber dans les particules argileuses (Golet, Xifra et al. 2003). Des interactions ioniques ou intercalation dans les feuillets de minéraux argileux peuvent avoir lieu dû à la présence de groupement acide et basique ainsi qu'à la structure aromatique des antibiotiques (Kümmerer 2009). Leur structure chimique leur confère donc une forte stabilité dans l'environnement, ce qui les rend difficiles à éliminer par les traitements conventionnels. Leurs interactions avec les milieux contenant des matériaux argileux peuvent être modifiées par la présence de cations métalliques polluants ou naturels. Ceci peut entraîner

inévitablement des modifications des cinétiques d'adsorption et de dégradation oxydative (en milieux aérés) ou réductive (en milieu manquant d'oxygène) et influencer grandement l'évolution dans le temps de la distribution des intermédiaires de décomposition et de celle de l'écotoxicité globale. C'est cette approche qui incite à approfondir les propriétés toxiques et catalytiques des métaux aussi bien sous forme cationique ou zéro-valente dans cette présente recherche.

1.4 Les métaux dans l'environnement

Dans un milieu aquatique ou un sol, un même métal peut exister soit sous forme dissoute (ionique) ou en suspension (nanoparticulaire). Dans ces milieux, le métal est capable d'interagir avec d'autres polluants comme les antibiotiques, il peut renforcer ou modifier leur toxicité. Un métal, selon sa nature et sa spéciation peut exercer une activité catalytique pour favoriser ou atténuer sa décomposition et même contribuer à l'écotoxicité globale ou la réduire. Ces perturbations altèrent dans un sens ou l'autre l'équilibre des écosystèmes et la biodiversité.

La pollution par les métaux lourds provient à la fois de sources anthropiques et naturelles (Ali, Abbas et al. 2020). Les sources anthropiques incluent principalement les activités humaines telles que l'exploitation minière, la gestion inadéquate des déchets municipaux et des effluents urbains et industriels, l'utilisation d'engrais et de pesticides, les émissions de gaz d'échappement, l'incinération des déchets, la production de combustibles, résidus de fonderie et autres (Nagajyoti, Lee and Sreekanth 2010). Ces activités libèrent des quantités importantes de métaux dans l'air, l'eau et les sols. Ils y sont détectés à différentes concentrations se situant autour de 4-15 $\mu\text{g.g}^{-1}$ pour le cuivre, 140 $\mu\text{g.g}^{-1}$ pour le fer et à plus faibles concentrations proches de 1 et 0.05-0.5 $\mu\text{g.g}^{-1}$ pour le nickel et le cobalt, respectivement (Nagajyoti, Lee and Sreekanth 2010). Au Canada, des concentrations en cobalt mesurées dans les eaux de surface à proximité d'activités minières peuvent atteindre 2 ppm (Sree, Keresztes et al. 2015).

Paradoxalement, certains métaux potentiellement toxiques en doses suffisantes, comme le cuivre et le zinc sont utilisés comme additifs alimentaires du bétail pour favoriser la croissance et prévenir les maladies (Zhai, Fu et al. 2023). En effet, certains métaux même très toxiques comme l'arsenic sont en réalité des oligo-éléments essentiels pour les organismes vivants car ils participent à des processus métaboliques vitaux. Le cuivre (Cu) joue un rôle clé dans la photosynthèse en tant que

composant essentiel de la plastocyanine et cofacteur d'enzymes antioxydantes (Yruela 2005). Le nickel (Ni) est indispensable à l'uréase, enzyme essentielle qui hydrolyse l'urée en ammoniac et CO₂, de plus l'uréase jouerait aussi un rôle de défense contre les pathogènes (Hänsch and Mendel 2009). Le cobalt (Co) est intégré dans la vitamine B₁₂, indispensable à la fixation de l'azote (Genchi, Lauria et al. 2023). Toutefois, le rôle métabolique du cobalt chez les plantes et ses interactions avec d'autres métaux reste à élucider (Hu, Wei et al. 2021). Enfin, le fer (Fe), est présent au sein des centres fer-soufre Fe-S des photosystèmes I et II et dans les cytochromes, où il intervient activement dans le transport d'électrons au cours de la photosynthèse (Hänsch and Mendel 2009).

Bien qu'indispensables à faibles concentrations, ces métaux peuvent devenir toxiques en excès. Ils peuvent perturber divers processus physiologiques, notamment les échanges gazeux, la fixation du CO₂, la respiration, l'absorption des nutriments, comme ils peuvent altérer directement le fonctionnement des chloroplastes en se liant aux groupes thiols (-SH) des enzymes (Hou, Chen et al. 2007, Nagajyoti, Lee and Sreekanth 2010). Chez les poissons, les cations Cu²⁺ ont été identifiés comme inhibiteur de l'enzyme Na⁺/K⁺-ATPase entraînant une réduction de l'absorption intracellulaire du sodium conduisant à une défaillance respiratoire (Liao, Zhu et al. 2023). Chez la plante aquatique *Lemna minor* les métaux induisent des dommages oxydatifs, l'exposition au Co²⁺ entraînent une désactivation du superoxyde dismutase et au Cr⁶⁺ a une diminution des teneurs en sucre, chlorophylles et caroténoïdes (Begović, Mlinarić et al. 2016, Aslanzadeh, Saboori and Moradlou 2024). La présence de Zn²⁺ peut conduire à la substitution de l'atome centrale Mg²⁺ de la chlorophylle, ce qui altère le fonctionnement du système photosynthétique (Tarrahi, Khataee et al. 2018). De même, le Cu²⁺ ou le Cd²⁺ peuvent inhiber la croissance, réduire la production de chlorophylle et altérer la structure des frondes (Hou, Chen et al. 2007). De plus, les métaux peuvent modifier la composition des populations bactériennes réduisant la diversité fonctionnelle des sols et des sédiments (Sazykin, Khmelevtsova et al. 2023). Le tableau ci-dessous synthétise quelques effets toxiques des métaux sur les espèces aquatiques (**Tableau 1.3**).

Tableau 1.3. Quelques effets toxiques des métaux.

Métal	Espèce	Concentration (mg/L)	Biomarqueurs	Durée (jours)	Résultats/ observations	Réf.
Pd ²⁺	<i>Lemna minor</i>	0.2, 0.4, 0.8, 1.3, 2.6 et 5.3	Inhibition de la croissance Viabilité cellulaire Production d'espèces réactives de l'oxygène (ROS) Fluorescence rapide de la chlorophylle <i>a</i> Teneur en pigments photosynthétiques Protéines totales Activités enzymatiques Teneur en thiols totaux Proline, lignine, sucres réducteurs, polysaccharides Composés phénoliques totaux et flavonoïdes	7	Les biomarqueurs les plus sensibles sont la taille des racines et l'inhibition de croissance Stress oxydatif	(Jmii and Dewez 2021)
Cu ²⁺ Cd ²⁺		0, 0.05, 0.5, 5, 10 et 20		4	Cd plus perturbateur que Cu	(Hou, Chen et al. 2007)
Zn ²⁺		0.5, 5, 10, 15, 20		4	Inhibition enzymatique par complexation métal-enzyme	(Jayasri and Suthindhira n 2017)
Pb ²⁺		1, 2, 4, 6, 8		1, 2 et 3	Augmentation de la peroxydation lipidique	(Begović, Mlinarić et al. 2016)
Co ²⁺		0.6 et 59		0.5, 1, 2, 3, 4, 6 et 7	Stress oxydatif	(Aslanzadeh, Saboor and Moradlou 2024)
Cr ⁶⁺		1, 10, 50 et 100		2	Perturbation du transport d'électrons Accumulation de cuivre dans les tissus	(Perreault, Samadani and Dewez 2014)
Cu ²⁺	<i>Lemna gibba</i>	4, 8, 16 et 32				

La plupart des métaux induisent un stress oxydatif caractérisé par une augmentation des ROS, une perturbation du système antioxydant enzymatique et une accumulation de malondialdéhyde (MDA), indicateur de peroxydation lipidique (Begović, Mlinarić et al. 2016, Aslanzadeh, Saboor and Moradlou 2024). Les cations de métaux de transition tels que Cu²⁺, Cd²⁺, Zn²⁺ et Pb²⁺ provoquent également des altérations des systèmes photosynthétiques, observées à travers la variation de la distribution des pigments photosynthétiques surtout par la fluorescence de la chlorophylle *a*. Les réponses enzymatiques de la peroxydase (POD), la catalase (CAT) ou le superoxyde dismutase (SOD), ainsi que les variations de composés antioxydants et osmoprotecteurs (thiols, proline, composés phénoliques), illustrent les mécanismes de défense de la plante face à la toxicité métallique (Hou, Chen et al. 2007, Perreault, Samadani and Dewez 2014, Begović, Mlinarić et al. 2016, Jayasri and Suthindhira n 2017). La combinaison des paramètres

morphologiques et biochimiques permet une évaluation plus complète de la toxicité (Jmii and Dewez 2021).

En parallèle, des évènements naturels tels les incendies spontanés ou causés par la foudre, l'érosion et les éruptions volcaniques, en autres, contribuent également à la libération de métaux lourds et autres polluants dans l'environnement (Garrett 2000). Selon les visions écologiques en vigueur, cette pollution naturelle par les métaux peut être prise en charge par la nature. Celle-ci rétablira ses propres équilibres, qui ne tiennent pas obligatoirement compte de l'existence de communautés humaines avoisinantes (Villes, villages,...).

1.5 Interactions [Métal:Substrat organique]

Quelles que soient leur nature et leur spéciation, les métaux interagissent inévitablement comme acides de Lewis avec plusieurs types de molécules portant des atomes pourvus de paires d'électrons libres. Ces interactions entraînent un processus de complexation ou chélation de type [Métal:Substrat organique] de diverses stœchiométries. Dans les milieux naturels, la matière organique est souvent présente sous forme dispersée étant dissoute, en suspension ou en émulsion, tous ses états étant improprement assimilés à une dissolution (Wei, He et al. 2024). La chélation, dans ce cas, peut modifier son affinité pour les surfaces solides influençant ainsi son comportement d'adsorption, sa mobilité et sa dispersion dans l'environnement.

La coexistence de plusieurs polluants dans ces milieux favorise la formation de produits secondaires ainsi que des espèces réactives de l'oxygène (ROS), ce qui accentue la formation de complexes entre métaux et substrats organiques. Ces complexes "secondaires" présentent des propriétés telles que la solubilité, l'aptitude à la photolyse, la réactivité aux agents d'oxydation et la biodisponibilité différentes de celles des molécules d'origine (Cuprys, Pulicharla et al. 2018, Baral, Bhattarai and Chaudhary 2024). À titre d'exemple, les antibiotiques comme les fluoroquinolones possèdent des groupements fonctionnels capables de fournir des paires d'électrons non-liantes pour se lier à des métaux. Leurs groupements carboxyle, cétone, hydroxyle et/ou pipérazine sont capables de former des complexes (Zhang, Cai et al. 2012).

L'étude des interactions menant à la formation de tels complexe est donc essentielle pour comprendre l'évolution de la dégradation et de la toxicité d'un polluant organique et de celles des différentes espèces polluantes qui peuvent l'accompagner dans un même milieu. Plus précisément, ceci permettra d'identifier et de quantifier les risques associés à la coexistence de métaux et d'antibiotiques dans l'environnement par l'utilisation de différentes techniques d'analyse dont la spectroscopie et la modélisation moléculaire (Baral, Bhattarai and Chaudhary 2024). Ce type d'études permettra de comprendre les voies d'autorégénération des milieux naturels et élaborer des voies de traitement qui s'en inspirent.

1.6 Tentatives d'élimination

À ce jour, plusieurs techniques ont été développées pour tenter de réaliser une élimination la plus efficace possible de ces polluants organiques (**Tableau 1.4**). Parmi les méthodes couramment employées figurent les procédés d'adsorption, de traitement biologique, de filtration membranaire, d'oxydation, d'osmose inverse, ainsi que de dégradation par les nanoparticules de métaux (Sousa, Oliveira et al. 2019).

Lorsque la problématique de la pollution par les antibiotiques et/ou les métaux, l'approche spontanée et quasi-générale est sans conteste leur rétention réversible sur des solides abordables et non polluants, mais en aucun cas leur extraction liquide-liquide qui se justifie rarement à cause des faibles concentrations. Ainsi, l'adsorption est la tentative première pour capturer et éliminer ce genre de polluants, surtout en raison de sa facilité de combinaison avec d'autres procédés pour compléter l'efficacité des stations de traitement (Mangla, Sharma and Ikram 2022).

Tableau 1.4. Méthodes d'élimination de quelques antibiotiques.

Antibiotiques	Procédé	[Polluant] mg/L	Conditions opératoires	Élim. (%)	Réf.
Difloxacin Sarafloxacin	Photolyse	10	35 °C ; Radiation: 500 W/m ² , 290-800 nm ; 24 h	-	(Prabhakaran, Sukul et al. 2009)
Norfloxacin	Adsorption et dégradation sur oxyde de graphène (OG) dans une matrice aqueuse	30	303 K ; [OG] = 200 mg/L ; pH = 7 ; 4 h	94.8	(Moreira, Lebron et al. 2020)
Amoxicilline	Symbiose entre micro-algue et bactérie	5-50	35 °C ; pH = 7 ; Agitation = 180 rpm ; 3 jours	>90	(Wang, Liu et al. 2023)
Tétracycline	Photocatalytique en présence de TiO ₂ dopé en azote	10	25 °C ; Radiation: 20 mW/cm ² , 350-850 nm, [TiO ₂] = 20 mg ; 120 min	94.8	(Wu, Hu et al. 2020)
Ciprofloxacin	Photocatalytique ozonation en présence Fe-zeolite 5A (Fe-Z5A)	50	Radiation : 20 W, 254 nm ; [Fe-Z5A] = 25 g ; pH = 6.5 ; Débit d'ozone = 0.9 mg/L ; 60 min	75	(Ikhlaq, Javaid et al. 2022)
Sulfamethoxazole	Ozonation catalytique avec Fe ²⁺ - montmorillonite (Fe(II)Mt)	76	Débit d'ozone = 6 mg/min ; [Fe(II)Mt] = 1.91 g/L ; pH = 2.88 ; 20 min	99	(Shahidi, Moheb et al. 2015)
Rifampicin	Nanoparticule d'oxyde de cuivre biosynthétisé (CuO)	10	65 °C ; [CuO] = 50 mg ; pH = 2 ; 8 min	98.43	(Nzilu, Madivoli et al. 2023)
Métronidazole	Photolyse par UV et UV/H ₂ O ₂	1.7	Radiation : $I_0 = 7.50 \times 10^{-6}$ Einstein L ⁻¹ s ⁻¹ , 254 nm ; [H ₂ O ₂] = 100 µM ; pH 3.00 ; 60 min	67.5 >99	(Su, Dai et al. 2022)
Sulfamethoxazole Trimethoprim Norfloxacin Roxithromycin	Osmose directe suivie d'oxydation électrochimique	-	Densité de courant J = 1 mA/cm ²	>99	(Liu, Zhang et al. 2015)
Amoxicilline	Photocatalytique ozonation	30	Débit d'ozone = 7.5 mg/min ; [TiO ₂] = 0.5 g/L ; 30 min	100	(Moreira, Orge et al. 2015)
Enrofloxacin	Photolyse suivie d'une filtration membranaire	10	25 °C ; Radiation : 500 W/cm ² , 300-800 nm ; pH = 8 ; 2 h Pression 10 bar ; Débit = 750 mL/min	99	(Dolar, Košutić et al. 2013)

Élim. (%) : taux d'élimination de l'antibiotique par le procédé correspondant (%).

Parmi les adsorbants qui ont déjà prouvé leurs efficacités et qui ressemblent le plus ceux contenus dans des milieux naturels figurent les aluminosilicates et plus particulièrement les minéraux argileux. Des matériaux de type argile pontée à la silice (Si-PILC, silica-pillared clay) aurait présenté une capacité d'adsorption élevée pour l'antibiotique ciprofloxacine (Roca Jalil, Toschi et al. 2018). Sa faible taille moléculaire semble favoriser sa diffusion dans les pores du matériau et l'accès aux sites d'adsorption des piliers. Il existe actuellement une ample littérature dédiée à des tentatives d'élimination d'antibiotiques par adsorption sur une gamme variée de matériaux plus ou moins poreux, certains travaux étant cités ultérieurement dans cette présente recherche.

De nombreuses molécules d'agents antibiotiques portent des groupements -OH, -NO₂ et/ou -Cl avec une densité électronique élevée qui induit des interactions sur des espèces chimiques environnantes. Cela influence la réactivité du composé organique vis-à-vis de l'ozone, un agent électrophile, ou de tout autre agent oxydant en facilitant ou en inhibant l'attaque sur certaines positions du cycle (Wang and Chen 2020). À titre d'exemple, les premières étapes de la photolyse tendent à affecter principalement les groupes fonctionnels périphériques ou substituants moins stables de la molécule.

1.7 Action de l'ozone sur les espèces organique

Contrairement à l'utilisation du chlore et d'autres techniques de traitement de l'eau, le procédé d'ozonation n'est pas si couramment utilisé pour assurer une qualité microbiologique et chimique conforme aux normes (Salimi, Esrafil et al. 2017, Ghuge and Saroha 2018) même s'il présente de nombreux avantages tels que l'absence de résidus chlorés potentiellement cancérigènes. L'ozone est un constituant naturel de l'atmosphère présent aussi bien dans la troposphère où il forme une couche protectrice ou dans la stratosphère. Ce dernier est produit par l'action des rayons UV sur l'oxygène (Rowland 2006). Dans d'autres couches de l'atmosphère, l'ozone agit comme polluant nocif et il résulte de réactions photochimiques impliquant des précurseurs naturels ou anthropiques (Loya, Pregitzer et al. 2003, Monks, Granier et al. 2009).

A basses altitudes à la surface des milieux aquatiques et des sols, l'ozone se forme de très faibles quantités à cause de la présence de faibles flux de photons UV ayant réuni à traverser l'atmosphère. Cependant, ces basses concentrations d'ozone contribuent quand même et d'une manière non

négligeable à l'oxydation naturelle de toutes espèces organiques à côté d'autres agents oxydants et bactéries diverses. À ce niveau-ci, l'ozone agit comme un oxydant puissant capable de dégrader ou éliminer différents types de polluants organiques tels les antibiotiques, les pesticides, les colorants organiques et autres (Wang and Chen 2020).

L'ozone présente un pouvoir oxydant de 2.07 V, qui est supérieur à celui du chlore et d'autres oxydants classiques capables d'attaquer une grande variété de composés organiques (Wang and Chen 2020). D'une part, il peut agir par attaque directe, en interagissant sous sa forme moléculaire en milieu acide (**Schéma 1.1**). D'autre part, à des pH élevés, l'ozone peut subir une décomposition radicalaire et convertir les anions hydroxyle en espèces réactives dont le radical hydroxyle (Shahidi, Roy and Azzouz 2015). Ce dernier est un oxydant extrêmement puissant et non sélectif capable d'attaquer un large éventail de polluants organiques (El Hassani, Kalnina et al. 2019). Sa formation reste très limitée en milieu neutre ou acide (Wang and Chen 2020).

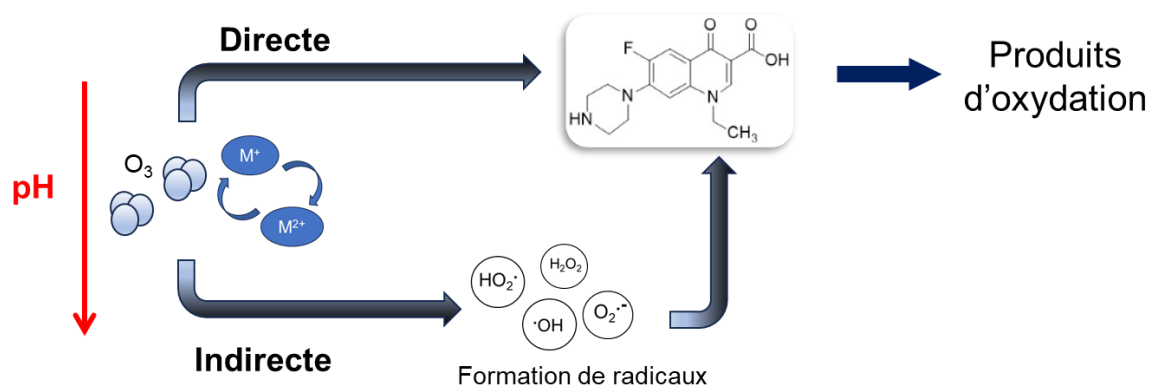


Schéma 1.1. Illustration schématique de l'action directe et indirecte de l'ozone sur un polluant organique.

L'ozone agit comme un dipôle réactif, capable de se comporter à la fois comme un électrophile et comme un nucléophile selon les formes de résonance qu'il adopte (**schéma 1.2**). Cette particularité induit la coexistence de centres électrophiles et nucléophiles expliquant ainsi la grande diversité des réactions chimiques possibles. L'ozone peut ainsi réagir par cycloaddition sur les doubles liaisons C=C, mais également par attaques électrophiles sur des sites riches en électrons, tels que les noyaux aromatiques activés ou certains groupements fonctionnels. À l'inverse, des attaques

nucléophiles peuvent également se produire sur les centres électrophiles de l’ozone, notamment en présence de composés fortement polarisés ou dans des conditions favorisant sa décomposition.

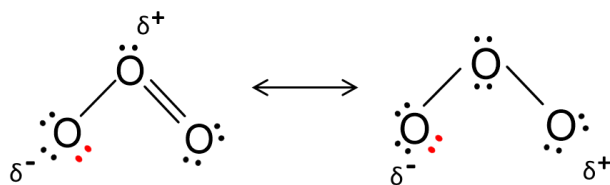
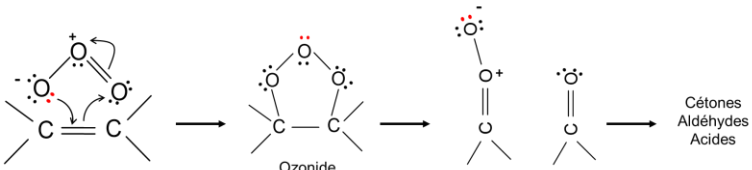
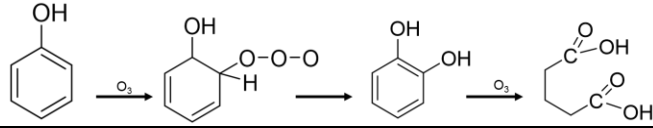


Schéma 1.2. Structure de résonance de l’ozone.

Ainsi, en fonction du composé chimique et du milieu réactionnel, l’ozone peut être impliqué dans différents types de réactions. Les catégories de réactions directe et indirecte de l’ozone vis-à-vis des polluants sont résumées dans le **Tableau 1.5**. La première réaction (**équation 1.1**) montre le rôle central de l’ozone en tant qu’oxydant fort dans les réactions d’oxydo-réduction impliquant des polluants organiques ou des espèces métalliques, lesquelles fournissent les électrons nécessaires à la réduction de l’ozone (Shahidi, Roy and Azzouz 2015). En milieu aqueux, l’ozonation des composés insaturés conduit, après formation d’un ozonide, à la formation de composés carbonylés tels que des aldéhydes et des cétones par cycloaddition (mécanisme de Criegee). Les aldéhydes peuvent ensuite être oxydés en acides carboxyliques, notamment en présence du peroxyde d’hydrogène généré in situ. Dans certains cas, l’ozone peut entraîner une réaction de type substitution électrophile sur l’aromatique. L’aromatique activé réagit d’abord par cycloaddition dipolaire avec l’ozone, conduisant à la formation d’un melozonide aromatique en position para. Cet intermédiaire, instable, se réarrange rapidement et entraîne la substitution d’un atome d’hydrogène du cycle par un fragment issu de l’ozone. Cette transformation constitue donc une réaction de substitution aromatique passant par un mécanisme d’addition-réarrangement. L’ozone grâce à la charge négative partielle sur l’un de ses atomes d’oxygène terminaux peut se comporter comme un nucléophile. Dans des systèmes non aqueux, l’ozone peut présenter un caractère nucléophile et réagir avec des sites électrophiles de certaines molécules tels que le carbone des groupements nitrile ($C\equiv N$) ou imine ($C=N$), mécanisme qui reste toutefois limité en milieu aqueux (Wang and Chen 2020). À mesure que le pH augmente, la réaction indirecte de l’ozone devient

prépondérante et correspond à une voie d'oxydation radicalaire initiée par la décomposition de l'ozone sous l'effet d'espèces nucléophiles, principalement les ions hydroxyle (OH^-). Cela conduit à la formation d'espèces réactives de l'oxygène, en particulier les radicaux hydroxyles ($\bullet\text{OH}$), caractérisés par un potentiel oxydant très élevé et une faible sélectivité. La génération de ces espèces radicalaires peut être décrite par les réactions 1.2-1.4 présentées dans le **Tableau 1.5**.

Tableau 1.5. Mécanismes de réaction de l'ozone (Wang and Chen 2020).

Action prépondérante de l'ozone	Type de réaction	Équation/Schéma
Directe	Oxydation-réduction	$\text{O}_3 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{O}_2 + \text{H}_2\text{O}$ (1.1)
	Réaction de cycloaddition	
	Substitution électrophile	
Indirecte	Radicalaire	$\text{O}_3 + \text{OH}^- + \text{H}^+ \rightarrow 2\text{OH}^\bullet + 4\text{O}_2$ (1.2)
		$\text{O}_3 + \text{OH}^\bullet \rightarrow \text{O}_2 + \text{HO}_2^\bullet$ (1.3)
		$\text{O}_3 + \text{OH}^- \rightarrow \text{O}_2^{\bullet-} + \text{HO}_2^-$ (1.4)
		$2\text{HO}_2^\bullet \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$ (1.5)

Malgré sa forte réactivité envers les composés organique et sa production relativement simple par arc électrique dans des dispositifs peu volumineux et mobiles, son utilisation seul dans l'élimination de polluants organiques reste souvent désavantageuse. Ceci est principalement due à sa faible solubilité qui impose une consommation élevée d'énergie électrique pour sa production. Afin de dépasser cet obstacle, de nombreux travaux ont été menés pour accélérer le processus d'ozonation par l'utilisation de catalyseurs dispersés ou supportés. D'autres recherches ont permis de démontrer le rôle incontournable de matériaux catalytiques dotés de capacités d'adsorption. Parmi ces matériaux, une place privilégiée revient aux aluminosilicates dont les argiles de type smectite (Shahidi, Roy and Azzouz 2014, Shahidi, Moheb et al. 2015, Boudissa, Mirilà et al. 2019,

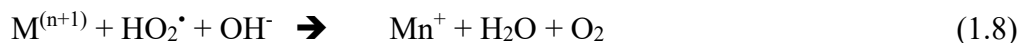
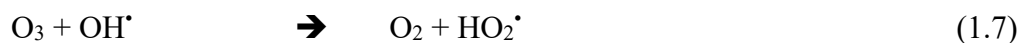
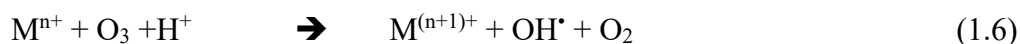
Boudissa, Zekkari et al. 2020, Mirilă, Boudissa et al. 2020, Benghaffour, Foka-Wembe et al. 2022, Zekkari, Ouargli-Saker et al. 2022, Benghaffour, Azzouz and Dewez 2023, Benghaffour, Dewez and Azzouz 2023, Boudissa, Arus et al. 2023).

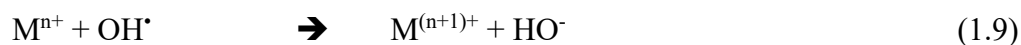
1.8 Ozonation catalytique

L'ozonation catalytique se décline en deux approches principales, dont l'une est abusivement dénommée "homogène", car même l'utilisation de cations métalliques dissous comme catalyseurs n'enlève pas l'hétérogénéité du processus global d'un gaz barbotant dans un milieu aqueux. De plus, l'oxydation de molécules organique produit inévitablement du dioxyde de carbone, qui est gazeux ou devient gazeux dans un milieu aqueux créant une interface gaz-liquide.

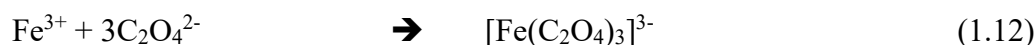
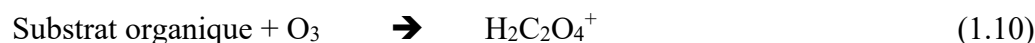
La dissolution et la décomposition d'une partie de l'ozone dissout ne sont que des étapes de ce processus global. Ce dernier inclue aussi l'action directe de l'ozone gazeux sur les molécules organiques et sur les cations métalliques à l'interface même gaz-liquide (surface du liquide et bulles gazeuses). Les valences multiples permettent aux cations de métaux de transitions d'interagir souvent simultanément avec l'ozone et le polluant organique selon un mécanisme complexe qui générant diverses espèces oxydantes réactives (Azzouz, Kotbi et al. 2010, Azzouz 2012, Azzouz 2012 2nd quarter, Shahidi, Roy and Azzouz 2014, Shahidi, Moheb et al. 2015, Shahidi, Roy and Azzouz 2015, Azzouz A., Mirilă D. et al. 2019, Wang and Chen 2020, Azzouz, Dewez et al. 2024).

La présence de cations, s'ils ne sont pas chélatés, facilitent la décomposition de l'ozone et la génération d'espèces radicalaires, ouvrant la voie à une oxydation indirecte même à faible pH. La décomposition se fait selon les réactions **1.6-1.9** par régénération du métal par les espèces radicalaires issues de cette décomposition (Shahidi, Roy and Azzouz 2015, Wang and Chen 2020).





Une autre voie possible, repose sur la formation de complexes métal-organique, dans lesquels ces ligands organiques causent l'oxydation initiale d'une fraction des polluants et de la génération de traces d'oxalates ou d'anions alkylates capables de complexer les ions métalliques (2006, Inchaurren and Font 2022). Comme l'illustre l'exemple suivant (équation **1.10-1-12**), montrant la dégradation partielle d'une molécule organique par l'ozone qui conduit à la formation d'acide oxalique. Celui-ci se dissocie ensuite en solution aqueuse, libérant des protons et générant l'anion oxalate, qui peut agir comme ligand pour complexer le fer.



Ainsi, la présence d'un cation métallique surtout de transition permet de transférer le pouvoir oxydant de l'ozone seul à un complexe $[Fe^{2+}:\text{Alkylate}]$ par oxydation du cation métallique en $[Fe^{3+}:\text{Alkylate}]$, qui revient à son état d'oxydation de départ après acte catalytique et oxydation du substrat organique. Ceci entraîne une prolongation dans le temps de l'action de l'ozone, souvent limité par sa faible solubilité dans les milieux aqueux, et compense ainsi une consommation élevée de gaz. Cependant, cette approche présente certains inconvénients dont la difficulté de récupération des catalyseurs métalliques dissous et le risque de contamination secondaire lié à la présence de métaux résiduels dans l'eau traitée.

À l'inverse, l'ozonation par catalyse hétérogène (OCH) permet d'éviter ces inconvénients avec un avantage supplémentaire de la contribution additionnelle de l'étape d'adsorption sur des catalyseurs solides. À cet effet, une grande gamme de matériaux ont été testés dont des oxydes métalliques, des matériaux à base d'argiles et de zéolites, des charbons actifs et autres (Shahidi, Roy and Azzouz 2015, Wang and Chen 2020). À ce jour, plusieurs travaux ont été réalisés dans notre laboratoire et ailleurs dans cette direction (**Tableau 1.6**). Les procédés OCH se sont révélés

particulièrement efficaces pour le traitement des eaux offrant plusieurs avantages par rapport à l’ozonation en l’absence de catalyseurs.

Tableau 1.6. Composé organique ozoné en présence de matériaux argileux.

Composé organique	Concentration du polluant Mg/L	Catalyseur		Ozone dosage (mg/h)	Efficacité d'élimination (%)	Temps (min)	pH	Réf.
		Nature	Conc. g/L					
Norfloxacin	1	Fe(II)Mt Cu(II)Mt	2	600	99	0.5 6	5.28	(Djidja, Dewez and Azzouz 2025)
Diclofenac	100	Fe(II)Mt	2	600	90	30	5.8	(Benghaffour, Foka-Wembe et al. 2022)
		NaMt				10		
Diazinon	10	NaMt Fe(II)Mt	2	600	98	20	-	(Benghaffour, Azzouz and Dewez 2023)
					98	30		
Sulfamethoxazole	75	Fe ²⁺ -montmorillonite (Fe(II)Mt)	1.91	360	100	10	2.88	(Shahidi, Moheb et al. 2015)
3-hydroxybenzaldehyde	35							
Phénol	30							
Orange G	45	SBA-16	2	360	98	5	7.37	(Ouargli, Larouk et al. 2016)
Phénol	95				84		8.16	
Acide oxalique	90				8.3		2.79	
Bleu de méthylène Vert de méthyle	30	Bentonite activée par l'acide sulfurique (HMT)	2	600	100	5	-	(Boudissa, Mirilä et al. 2019)
17 α -ethinylestradiol	3	HMT NaMt Fe(II)Mt	2	600	99.99	15	3	(Boudissa, Zekkari et al. 2020)
Bleu de méthylène	15	Montmorillonite K10	0.25	-	99	-	-	(Mirilä, Boudissa et al. 2020)
Vert malachite					95			
Acide oxalique Norfloxacin	10	Bismuth ferrite (BiFeO ₃) magnetic nanoparticles	-	75	83.3 63.0	60 120		(Yin, Liao et al. 2016)
Orange 4	100	Cu/ SBA-15	-	-	86	60	9	(Ghughe and Saroha 2018)
Norfloxacin	10	MnOx/SBA-15	0.1	100	90	15	5	(Chen, Li et al. 2017)
Ibuprofen	10	Fe ₂ O ₃ /Al ₂ O ₃ @SBA-15	-	-	90	60	7	(Bing, Hu et al. 2015)
Orange de méthylène	500	Ni-LDHs	1	109	96	60	9	(El Hassani, Kalnina et al. 2019)
N,N-diméthylacetamide	200	CuFe ₂ O ₄	30	-	95.4	120	6.7	(Zhang, Ji et al. 2018)
Ceftazide	10	TiO ₂	1	220	75.5	15	11	(Asgari, Farzadkia et al. 2020)

Conc. : Concentration du catalyseur.

Un processus OCH est un système d'oxydation complexe reposant principalement sur sept étapes successives, à savoir: 1. La diffusion des réactifs vers la surface du catalyseur solide; 2. La diffusion des réactifs dans les pores du catalyseur; 3. L'adsorption de l'ozone et du polluant organique sur la surface du catalyseur suivie de la formation d'espèces réactives capables de déclencher l'acte catalytique; 4. Réaction d'oxydation sur les sites actifs; 6. La diffusion des produits vers la sortie des pores; 7. La diffusion des produits d'oxydation loin de la surface du catalyseur (Zhang, Ji et al. 2018). La performance d'un catalyseur est déterminée par plusieurs paramètres dont une capacité d'adsorption et une affinité suffisante pour la rétention de l'ozone et du substrat organique ainsi que son activité catalytique dans la réaction ciblée. Ces paramètres imposent un nombre suffisant de sites d'adsorption et catalytiques sur une surface de contact élevée ainsi qu'une structure poreuse permettant l'accessibilité des molécules (Singh, Martínez-Ortigosa et al. 2024).

1.9 Traitements des eaux par les argiles

Naturellement abondants, peu coûteux et respectueux de l'environnement, les matériaux argileux représentent une solution technologique prometteuse et durable pour le traitement des eaux contaminées. Ce sont des matériaux présentant une bi-fonctionnalité comme adsorbant et catalyseurs et des propriétés physico-chimiques intéressantes aussi bien pour l'adsorption que pour la catalyse. Ces propriétés consistent essentiellement en leur structure en feuillets superposés et mobiles en fonction de la teneur en humidité ou en eau du milieu, leur grande surface spécifique, leur porosité appréciable ainsi que leur capacité d'échange cationique (CEC) élevée qui permettent la diffusion et l'adsorption efficace d'une grande variété de particules et d'espèces chimiques (Bananezhad, Islami et al. 2019). Les groupements fonctionnels présents sur la surface peuvent interagir avec des molécules organiques par des liaisons électrostatiques, des ponts hydrogène, des interactions hydrophobes et/ou acide-base de Lewis (Sadiku, Selimi et al. 2022).

Les argiles seules, modifiées ou combinées à d'autres matériaux sont largement utilisées comme adsorbants, agents de décoloration, échangeurs d'ions, tamis moléculaires et/ou catalyseurs (El Hassani, Kalnina et al. 2019, Joseph, Vellayan et al. 2019). Par exemple, l'intégration de charbon actif et d'oxydes de fer à une matrice argileuse a permis d'obtenir un matériau efficace pour l'élimination du plomb, du cadmium et de l'arsenic dans les eaux usées (Pawar, Lalhmunsiam et al. 2018). Plusieurs études ont démontré l'efficacité des smectites comme supports catalytiques ou

adsorbants pour l'élimination d'antibiotiques et de colorants organiques (Ding and Bian 2015, Mahmoud, El-Ghanam et al. 2020).

L'adsorption des antibiotiques sur les minéraux argileux tels que la montmorillonite dépend de la nature et de la distribution de l'affinité, et donc de la force de rétention du substrat organique sur les sites de fixation la surface solide (Wang, Yang et al. 2023). Les argiles naturelles peuvent être modifiées chimiquement par exemple par échange avec des cations métalliques de transition (Fe^{2+} , Cu^{2+} , Co^{2+}) (Hong, Zhang et al. 2019) ou des agents tensioactifs (Rahardjo, Susanto et al. 2011, Sun, Shi et al. 2017) dans le but d'améliorer leur affinité pour des polluants spécifiques ou d'y conférer des propriétés catalytiques. Une bentonite modifiée par un tensioactif cationique (CTAB) a montré une capacité d'adsorption élevée grâce à des interactions électrostatiques entre les groupes ammoniums positifs (CTA^+) et les groupements carboxylates de l'antibiotique ampicilline (COO^-). Par contre en eaux réelles, l'adsorption est moins efficace à cause de la compétition avec d'autres composés et des forces de répulsions qui se produisent entre les groupements silanol déprotonés (Si-O^-) de la bentonite et les anions (COO^-) de l'ampicilline (Rahardjo, Susanto et al. 2011). Ces argiles modifiées sont alors capables d'activer des oxydants comme l'ozone, rendant possible la dégradation avancée des polluants organiques.

1.10 Structure des adsorbants/catalyseurs à base de montmorillonite

Le terme argile désigne de manière générale un matériau naturel, comme la bentonite, pouvant contenir un mélange de minéraux argileux (montmorillonite, illite,...) et d'autres composants minéraux (divers types de silices cristallines et amorphes, sels, oxydes métalliques) ou non minéraux (molécules organiques, débris animaux et végétaux) (Inchaurredo and Font 2022). La bentonite est une argile naturelle majoritairement composée de smectites, une famille de minéraux argileux regroupant notamment la montmorillonite, la saponite, la vermiculite, la nontronite, l'hectorite, la sauconite et la beidellite (Shahidi, Roy and Azzouz 2015).

La montmorillonite figure parmi les minéraux argileux les plus utilisés. C'est un minéral argileux de type 2:1 constitué de lamelles d'aluminosilicate pouvant interagir avec diverses espèces présentes dans un milieu aqueux selon le pH du système. Chaque lamelle est formée de deux couches tétraédriques de silice encadrant une couche octaédrique centrale dans laquelle certains

ions Al^{3+} sont remplacés isomorphiquement par des cations bivalents Mg^{2+} , Fe^{2+} et/ou autres (**Schéma 1.3**). Cette substitution isomorphique consiste à imposer une structure octaédrique et donc une troisième liaison avec un troisième atome d'oxygène aux cations bivalents qui ne peuvent en avoir que deux en situation normale. Ceci génère des charges négatives permanentes qui sont compensées par la présence de cations échangeables situés dans l'espace interlamellaire (Lin, Jiang and Zhan 2018, Mishra, Mehta and Basu 2018).

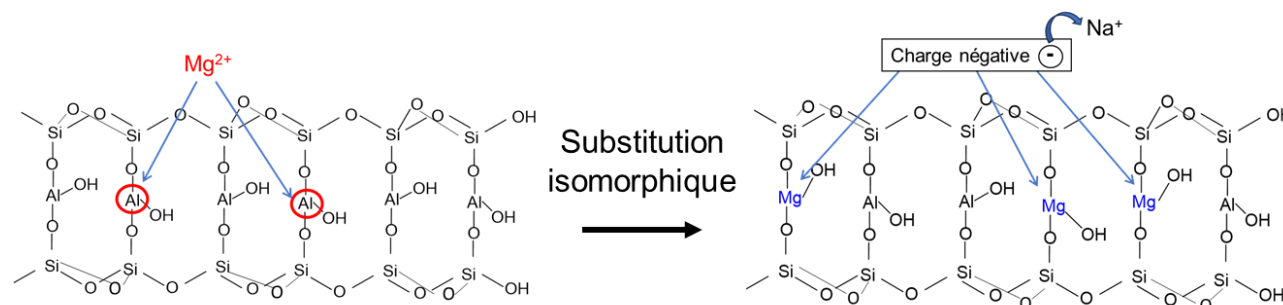


Schéma 1.3. Structure de la montmorillonite.

La montmorillonite se démarque des autres argiles étudiées par sa très grande surface spécifique allant de 600 à 800 m^2/g et sa capacité d'échange cationique élevée variant de 80 à 120 $\text{mEq}/100 \text{ g}$ (Haciosmanoğlu, Mejías et al. 2022). Elle présente aussi une aptitude au gonflement contrairement aux argiles peu expansibles telles que la kaolinite ou l'illite (Nagendrappa and Chowreddy 2021). Ces propriétés la rendent particulièrement efficace pour l'élimination des polluants dans les traitements de l'eau notamment par ozonation catalytique et favorisent l'adsorption. De plus, les cations échangeables situés entre les feuillets d'argile peuvent être remplacés par d'autres ions métalliques. La combinaison synergique de l'adsorption et autre méthode de dégradation constitue une stratégie prometteuse pour l'élimination des polluants organiques dans l'eau. Par exemple, le nanocomposite phosphate d'argent couplé aux nanotubes d'halloysite ($\text{Ag}_3\text{PO}_3/\text{HNTs}$) a été testé dans la dégradation de la rhodamine B, le bleu de méthylène, l'ibuprofène, le diclofenac, le naproxène, le flurbiprofène et la carbamazépine (Nyankson and Kumar 2019). Dans ce système hybride, les HNTs concentrent les contaminants à proximité des sites photocatalytiques favorisant ainsi leur dégradation rapide par les radicaux oxydants générés sous irradiation.

De nombreux matériaux argileux sont compatibles avec différents procédés de traitement (ozonation, filtration, coagulation, etc.) et peuvent être régénérés et réutilisés. La montmorillonite a fait l'objet de peu d'études concernant les quinolones. Toutefois, certaines limites subsistent, comme la saturation rapide du matériau en cas de forte charge polluante, la compétition entre les ions en solution ou encore la nécessité de stabiliser le matériau pour éviter un relargage potentiel de polluants.

1.11 Cations métalliques dans la dégradation des antibiotiques

Les métaux, plus particulièrement ceux de transition, présentent des propriétés chimiques qui les rendent efficaces dans la dégradation de molécules organiques dont les antibiotiques. À ce jour, une ample littérature a été consacrée à l'utilisation des cations métalliques, des oxydes métalliques ou des métaux à l'état zéro valent dans la dégradation des antibiotiques. Leur capacité à agir en tant qu'accepteurs ou donneurs d'électrons dépend de leur état d'oxydation, leur potentiel redox et leur spéciation chimique. Cette dernière est définie comme étant la forme sous laquelle le métal existe en fonction du pH (Girma 2023), du potentiel redox, de la force ionique (Ben-Moshe, Frenk et al. 2013) et de la température.

En solution ou supportées sur des matériaux solides comme les argiles ou les oxydes, les espèces métalliques comme celles issues de métaux de transition comme Fe, Cu, Co ou Mn interviennent dans plusieurs procédés de dégradation des antibiotiques. Ces espèces peuvent également interagir directement avec les antibiotiques par des mécanismes de complexation ou de chélation, modifiant leur réactivité et facilitant leur décomposition. Lorsqu'elles sont intégrées dans des matériaux hybrides (argiles modifiées, nanoparticules, biochars), les espèces métalliques permettent d'optimiser la dispersion des sites actifs et d'améliorer la stabilité du système catalytique. À l'échelle nanométrique, les nanoparticules métalliques offrent une grande surface réactive et peuvent être supportées sur des matériaux tels que la montmorillonite pour améliorer leur stabilité et leur dispersion.

L'un des systèmes les plus étudiés dans les procédés d'oxydation de substrats organiques est le système Fenton ($\text{Fe}^{2+}/\text{H}_2\text{O}_2$). L'origine de la chimie de Fenton remonte à 1894, lorsque Henry J. Fenton a démontré que le H_2O_2 pouvait être activé par des sels de fer(II) pour oxyder l'acide

tartrique (Pignatello, Oliveros and MacKay 2006). Des études ultérieures ont élargi le champ d'application à d'autres métaux comme Cu^{2+} ou Co^{2+} qui peuvent également catalyser la génération de radicaux dans des conditions acides, neutres ou basiques. La réactivité des métaux dépend fortement de plusieurs paramètres qui déterminent également d'autres propriétés physico-chimiques dont le pouvoir polarisant, l'acidité de Lewis et leur potentiel Radox (**Tableau 1.7**). Ces propriétés gouvernent, à leur tour, leurs comportements dans différents milieux notamment, dans les suspensions aqueuses de matériaux argileux qui font l'objet de la présente recherche.

Tableau 1.7. Quelques propriétés chimiques des métaux étudiés.

Élément	Masse atomique g/mol	Pouvoir polarisant ¹	Acidité de Lewis ¹	Potentiel redox standard (E°) ²
Sodium (Na^+)	22.99	1.0	0.16	$\text{Na}^+/\text{Na} : -2.71$
Fer (Fe^{2+})	55.85	3.3	0.34	$\text{Fe}^{2+}/\text{Fe} : -0.44$
Cuivre (Cu^{2+})	63.55	3.8	0.50	$\text{Cu}^{2+}/\text{Cu} : +0.34$
Nickel (Ni^{2+})	58.69	4.2	0.45	$\text{Ni}^{2+}/\text{Ni} : -0.26$
Cobalt (Co^{2+})	58.93	3.6	0.40	$\text{Co}^{2+}/\text{Co} : -0.28$

¹Pouvoir polarisant, acidité de Lewis (Djidja, Dewez and Azzouz 2025).

²Le potentiel rédox standard (E°) correspond au potentiel électrique d'un couple oxydant/réducteur mesuré dans des conditions standards (25 °C, pression de 1 bar) par rapport à l'électrode standard à hydrogène (Kinraide, Poschenrieder and Kopittke 2011).

Les métaux sous forme cationique peuvent aussi former des complexes par chélation avec les antibiotiques. Certains de ces complexes ont d'abord été synthétisés en laboratoire à des fins pharmaceutiques pour le traitement de maladies graves telles que le cancer et les troubles neurodégénératifs (Baral, Bhattarai and Chaudhary 2024). Ils peuvent également se former spontanément dans l'environnement, en particulier dans les sols et milieux aquatiques riches en métaux, antibiotiques et matière organique. Ainsi, la pollution par les métaux produit inévitablement de tels complexes, qui peut modifier les propriétés physiques essentielles d'un sol comme la plasticité, à titre d'exemple (Muththalib and Baudet 2019). La complexation de certains cations métalliques peut aussi modifier la capacité d'un sol pollué par des molécules organiques à s'autorégénérer, en accélérant ou inhibant des réactions de dégradation. Par exemple, le cation Ni^{2+} a un effet catalytique positif en présence de fer zéro valence (Fe^0 ou ZVFe) pour la déchloration de certains polychlorinated biphenyls (PCB153) (Lou, Cai et al. 2019).

Le pouvoir polarisant de certains cations métalliques induit des interactions électrostatiques entre les charges positives des cations et les charges négatives partielles de molécules d'eau environnantes. Ceci entraîne leur dissociation et l'apparition d'une acidité Bronsted dans le milieu liquide (**Schéma 1.4.**).

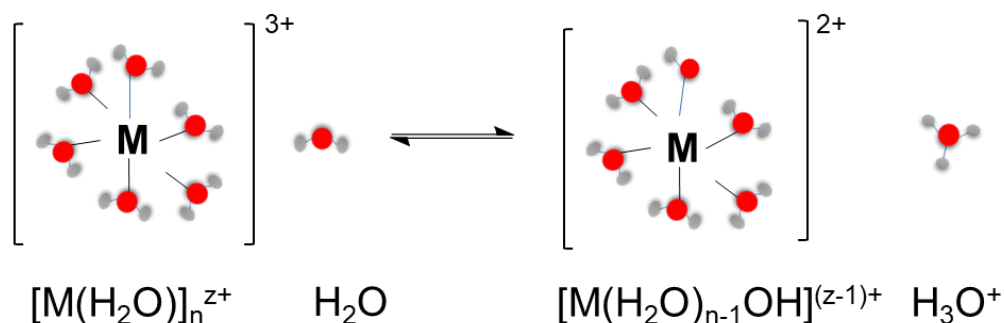


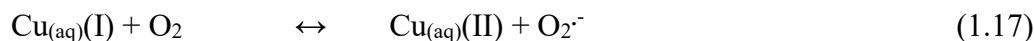
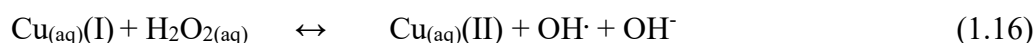
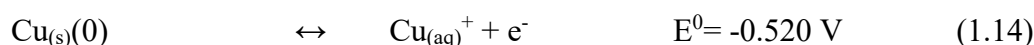
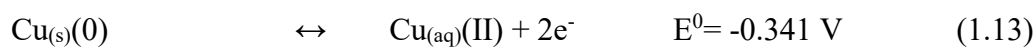
Schéma 1.4. La genèse de l'acidité de Bronsted par les complexes hydratés des cations métalliques dotés d'un pouvoir polarisant favorisé par une petite taille et une charge électrique élevée.

Des mécanismes supplémentaires comme la précipitation, la complexation directe aux groupements fonctionnels d'une surface argileuse ($\equiv Si-OH$, $\equiv Al-OH$), la co-complexation ou encore des réactions redox peuvent intervenir (Wang, Shen et al. 2024). Les métaux sur la surface des adsorbants peuvent créer de nouveaux sites actifs capables de se complexer avec les molécules organiques formant ainsi des complexes ternaires (adsorbant-métal-polluant organique) ou quaternaires (Zhai, Fu et al. 2023).

1.12 Métaux zéro valents

Les métaux à zéro valence peuvent éliminer une large gamme de polluants organiques par deux mécanismes principaux, réduction et oxydation (Sousa, Oliveira et al. 2019). Dans le processus réducteur, le métal est donneur d'électrons. Les molécules du polluant sont d'abord adsorbées à la surface du matériau ce qui facilite le transfert d'électrons et permet la réduction chimique de ces substances comme le cas pour le 4-nitrophénol (Bouazizi, Campagne et al. 2018). En parallèle, ces métaux peuvent aussi favoriser un processus oxydatif. Dans ce mécanisme, le métal réagit avec l'oxygène dissous dans l'eau pour générer des espèces réactives de l'oxygène (ROS), qui vont

ensuite oxyder les polluants. Certains colorants azoïques peuvent subir une rupture de liaison (-N=N-) en présence de cuivre zéro-valence (Dong, Ai and Zhang 2014). Dans un premier temps, Cu^0 s'oxyde pour former Cu^+ . Par ailleurs, le cuivre catalyse la génération d'espèces réactives de l'oxygène, selon les équations d'oxydoréduction **1.13-1.17** pour le cuivre zéro-valence (de Sousa, de Oliveira et al. 2019, Sousa, Oliveira et al. 2019).



Le fer zéro-valent (Fe^0) est largement utilisé dans les procédés de traitement avancé de l'eau comme donneur d'électrons pour éliminer les métaux, tandis qu'avec les polluants organiques il sert à activer des oxydants comme le persulfate (Liu, Diao et al. 2018). De même pour d'autres métaux de transition, l'ajout de zinc zéro-valent (ZVZ) semble améliorer le procédé d'ozonation du di-n-butylphthalate grâce à l'oxydation du zinc. Ce processus conduit à la formation de radicaux superoxyde ($\text{O}_2^{\cdot-}$) par réduction de l'oxygène dissous (Wen, Wang et al. 2014). Le tableau suivant montre quelques exemples d'élimination de certains polluants en présence métaux à valence zéro (**Tableau 1.8**).

Tableau 1.8. Catalyseurs à base de nanoparticules métalliques en dégradation des polluants organiques.

Polluants	Concentration (mg/L)	Catalyseur	Quantité de catalyseur (g/l)	Élimination (%)	pH	Temps (min)	Réf.
Trichloréthylène	13	Bentonite-Fe/Ni	0.5	100	8.2	1860	(Wang, Han et al. 2016)
Phosphate	10	nZVI	-	99	3	5	(Wen, Zhang and Dai 2014)
Cu ²⁺ / Bisphénol A	5 et 10	nZVI	1	98 96	3	60	(Liu, Diao et al. 2018)
Orange II	90	NiO NPs NiO/Nc	2	93 96	2	20	(Khan, Saeed et al. 2022)
2,4-dichlorophenol	20	Pd-Fe nanoparticules	10	100	7	180	(Wang, Zhu et al. 2013)
Méthyl orange	10	NiO NPs	2	96	3	0.5	(Barzinjy, Hamad et al. 2020)
Orange II	100	Diatomite-Fe/Ni nanoparticules	4	99	6.84	360	(Ezzatahmadi, Bao et al. 2018)
Bleu de méthylène Rouge congo	5 20	Bentonite/Cu NPs	0.4	-	-	0.67 5	(Issaabadi, Nasrollahzadeh and Sajadi 2017)
Ciprofloxacine	20	Cuivre zéro valence nZVC	0.5	100	3.5	120	(Sousa, Oliveira et al. 2019)
Méthyl orange Rouge congo	20	Cuivre zéro valence nZVC	0.3	100	6.6 7.2	240	(Dong, Ai and Zhang 2014)
Aniline	10	Cu(0)	2	98	4	24	(Zhang, Guo et al. 2017)
sulfamethoxazole	1.3	zero-valent cobalt ZVCo/PAA	0.1	99.4	7	10	(Zhou, Zhou et al. 2022)

Un rendement élevé de l’ozonation de l’aniline atteignant 90 % en 40 minutes avec du cobalt métallique Co⁰ est expliqué par deux mécanismes : i. Les ions Co²⁺ et Co³⁺ issus de l’oxydation partielle du Co⁰ induisent efficacement la décomposition de l’ozone en OH[•] ; ii. Le cobalt possède une capacité de complexation plus marquée avec les groupes amines de l’aniline rapprochant ainsi

la molécule du site catalytique et facilitant son oxydation (Zhang, Guo et al. 2017). Une étude a montré que Fe^0 supporté sur une surface argileuse comme la bentonite améliore sa dispersion et empêche son inactivation par agglomération ou oxydation (Baldermann, Kaufhold et al. 2021).

En raison de la toxicité secondaire que peuvent engendrer les métaux libérés dans les milieux aqueux, leur récupération après usage est essentielle pour limiter leur impact environnemental. Plusieurs techniques physico-chimiques peuvent être mises en œuvre à cet effet, notamment la précipitation (Wei, Viadero Jr and Buzby 2005), l'adsorption (Wang, Shen et al. 2024), la nanofiltration (Wanjiya, Zhang et al. 2024) ou même le traitement électrochimique (Du, Waite et al. 2023). Cependant, ces métaux peuvent être entravés par leur capacité à former des complexes stables avec des polluants organiques présents dans le milieu. Ces complexes métal-polluant peuvent présenter une biodisponibilité accrue et une toxicité renforcée envers les microorganismes aquatiques (Djidja, Dewez and Azzouz 2026). Ainsi, une attention particulière doit être portée à la présence simultanée de métaux et de polluants organiques, en tenant compte de leurs interactions et de leurs effets synergiques sur l'environnement.

1.13 Biomarqueurs et paramètres de toxicité

Un biomarqueur c'est la réponse physiologique ou biochimique mesurable traduisant une exposition à des polluants (Radić, Stipaničev et al. 2011). Chez la plante aquatique *Lemna minor*, les biomarqueurs utilisés peuvent être regroupés en deux grandes catégories complémentaires (**Schéma 1.5**). La première catégorie correspond aux biomarqueurs de croissance. Ils incluent le nombre total de frondes, la surface foliaire, le poids frais ou sec ainsi que le taux de croissance relatif calculé à partir de ces paramètres (Ziegler, Sree and Appenroth 2019). La seconde catégorie regroupe les biomarqueurs physiologiques et biochimiques. Cela inclut la mesure de la teneur en pigments photosynthétiques comme la chlorophylle *a* et *b*, les activités enzymatiques, en particulier celles des enzymes antioxydantes comme la peroxydase, la catalase ou le superoxyde dismutase, les espèces réactives de l'oxygène (ROS) et la cinétique de fluorescence de la chlorophylle *a* (Brain and Cedergreen 2008). Les tests standardisés donnent une évaluation globale de l'effet des contaminants sur les plantes aquatiques, tandis que l'analyse des biomarqueurs apporte des informations sur les mécanismes de toxicité c'est-à-dire comment les contaminants perturbent les

processus biologiques ou physiologiques tels que la photosynthèse, le métabolisme ou la croissance (Ziegler, Sree and Appenroth 2019).

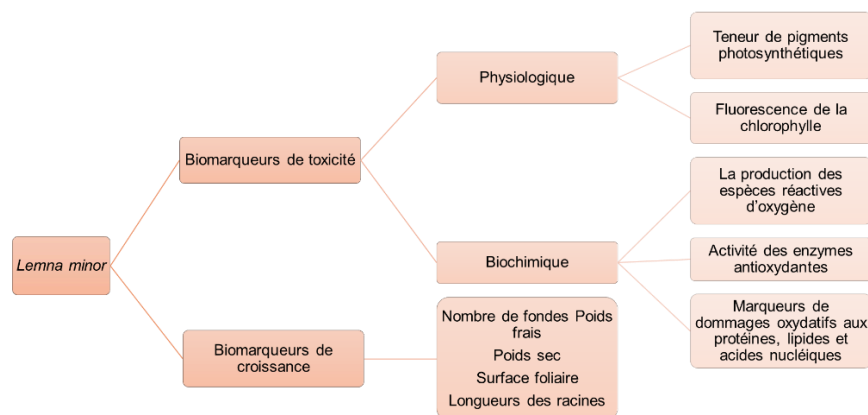


Schéma 1.5. Biomarqueurs et paramètres utilisés pour l'évaluation de la toxicité sur *L. minor*.

Certains biomarqueurs se modifient très tôt et reflètent une réponse rapide de l'organisme, tandis que d'autres ne sont pas affectés qu'à des concentrations plus élevées. L'utilisation de LOED (Lowest Observed effect dose) a permis de hiérarchiser la sensibilité des biomarqueurs et de déterminer lequel présentait la réponse la plus précoce face au contaminant (Jmii and Dewez 2021). Plusieurs études ont évalué la toxicité de polluants organiques sur *L. minor* en s'appuyant sur une combinaison de biomarqueurs. L'exposition de *L. minor* au paracétamol, au diclofénac et à la chlorpromazine pendant une durée de quatre jours a notamment été associée à des variations de la teneur en pigments photosynthétiques, de la fluorescence rapide de la chlorophylle *a*, des activités enzymatiques antioxydantes, ainsi qu'à une inhibition de la croissance (Alkimi, Daniel et al. 2019). Des réponses similaires ont été observées après une exposition à des herbicides tels que l'isoproturon et le glyphosate (Dosnon-Olette, Couderchet et al. 2011). D'autres paramètres ont également été évalués, notamment la teneur en protéines totales, le nombre de frondes, la surface foliaire, la longueur des racines et le rendement quantique du photosystème II lors de d'expositions au 17 α -ethynylestradiol, Methotrexate et Fluoxetine (Amy-Sagers, Reinhardt and Larson 2017, Markovic, Neale et al. 2021). La teneur en chlorophylle *a* été démontrée comme un indicateur sensible pour évaluer l'effet de différents polluants comme le bisphénol A, 3,4-dichlorophenol, acetochlor, atrazine et diuron (Fekete-Kertész, Kungléné-Nagy et al. 2015) ou pour les mélanges

binaires de contaminants (Markovic, Neale et al. 2021). L'utilisation combinée de ces indicateurs permet d'obtenir une vision plus complète des mécanismes de toxicité et de relier les réponses précoces au stress aux effets biologiques observés à des niveaux d'organisation supérieurs. Dans ce contexte, *Lemna minor* a été retenue comme organisme modèle pour l'évaluation de la toxicité dans cette présente recherche.

1.14 *Lemna minor* comme organisme modèle en écotoxicologie

La lentille d'eau *lemna minor* de la famille des lemnacées, mesurant 2 à 4 mm. C'est un monocotylédone macrophyte flottant qui forme un tapis dense à la surface des eaux de lacs et des étangs. Elle se disperse dans les milieux aquatiques grâce à sa racine adhésive capable de s'accrocher aux oiseaux d'eau qui assurent ainsi son transport vers d'autres plans d'eau, d'où le nom anglais duckweed (Aslanzadeh, Saboor and Moradlou 2024). Elle est constituée d'un ou de quelques thalles appelés frondes et d'une seule racine. Sa reproduction est principalement végétative par division avec un temps de doublement des frondes d'environ 1.4 jour. *L. minor* tolère un pH allant de 5 à 9 et une température entre 6 et 33 °C (Leng, Stambolie and Bell 1995, Van Hoeck, Horemans et al. 2015, Ekperusi, Sikoki and Nwachukwu 2019, Maldonado, Terrazas and Vilca 2022). En conditions de laboratoire, avec un apport continu en nutriments, lumière et eau, elle peut croître indéfiniment, produisant de nombreuses frondes filles au cours de sa vie. Toutefois, la fronde mère meurt généralement après avoir donné six générations (Ekperusi, Sikoki and Nwachukwu 2019).

L. minor est largement utilisée comme organisme modèle en écotoxicologie pour évaluer la toxicité de divers contaminants de l'eau en raison de sa sensibilité aux contaminants, culture facile, petite taille et de sa structure simple (Clevers 2003, Fekete-Kertész, Kungl-Nagy et al. 2015, Markovic, Neale et al. 2021). Ces évaluations sont essentielles pour orienter les stratégies de remédiation visant à restaurer la qualité de l'eau et à préserver la vie aquatique. *L. minor* fait l'objet de plusieurs protocoles normalisés, notamment ceux établis par l'ASTM (*American Society for Testing and Materials*), l'ISO-20079 (*International Organization for Standardization*), l'OCDE (*Organisation de coopération et de développement économiques*), Environnement Canada et l'EPA (*United States Environmental Protection Agency*) (Van Hoeck, Horemans et al. 2015). Elle contient une variété de nutriment comme les protéines, les carbohydrates et vitamines formant une

base nutritive pour de nombreux organismes, contribuant ainsi à la production primaire (Jaimes Prada, Lora Díaz and Tache Rocha 2024). Elle est aussi utilisée en phytoremédiation car elle possède la capacité d'accumuler ou d'éliminer les contaminants dans ses tissus (Ekperusi, Sikoki and Nwachukwu 2019), tels que les métaux lourds (Hou, Chen et al. 2007, Ali, Abbas et al. 2020), pesticides ou résidus pharmaceutiques jouant ainsi un rôle dans l'épuration naturelle des eaux (Maldonado, Terrazas and Vilca 2022). De plus, elle contribue aussi à la phytoremédiation des microplastiques de polyéthylènes (Rozman, Kokalj et al. 2022). Comme elle participe à l'élimination de l'azote et du phosphore contribuant ainsi à la réduction des phénomènes d'eutrophisation (Chen, Fang et al. 2018).

CHAPITRE 2

APPROCHES MÉTHODOLOGIQUES ET PRINCIPES DES MÉTHODES D'ANALYSES

Ce travail repose sur une approche combinée de dégradation catalytique de la Norfloxacin et d'évaluation écotoxicologique sur un bioindicateur végétal pour représenter une espèce de la biodiversité. Dans un premier temps, des tests d'ozonation et d'adsorption ont été réalisés en présence de montmorillonite échangés par différentes espèces métalliques. Par la suite, le suivi du pH, potentiel zêta et la taille de particules ont été effectués tout au long des expériences. Les cinétiques de dégradation ainsi que les rendements d'élimination ont été déterminés à l'aide de la spectrophotométrie UV-Visible et de la chromatographie liquide à haute performance (HPLC). Les produits intermédiaires formés ont été identifiés par spectrométrie de masse (LC-MS). Dans un second temps, les solutions traitées ont été soumises à une évaluation écotoxicologique à l'aide du bioindicateur *Lemna minor*. Différents biomarqueurs ont été mesurés.

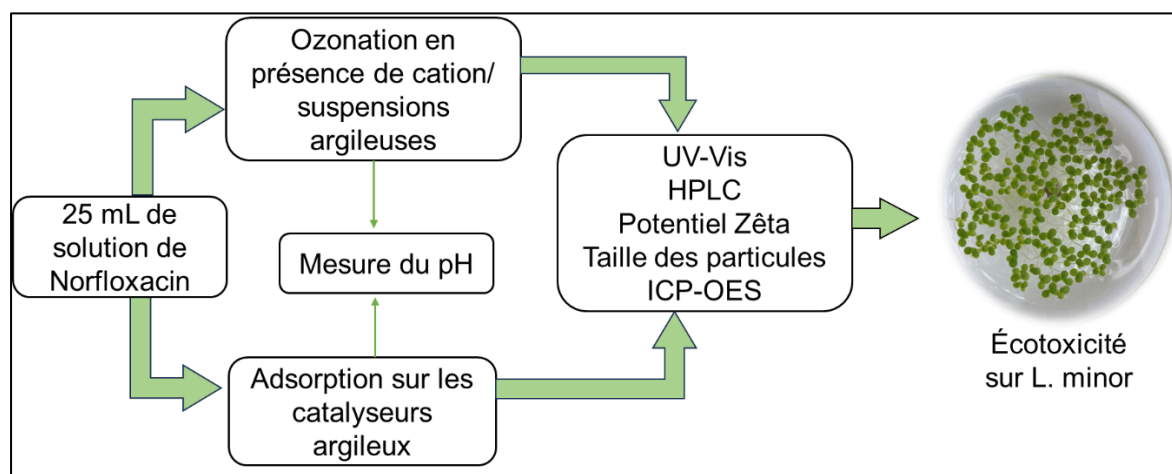


Schéma 2.1. Illustration schématique et concise de la méthodologie adoptée dans cette recherche.

2.1 Préparation des matériaux argileux

Les différents matériaux argileux utilisés dans cette recherche ont été obtenus à partir d'une bentonite commerciale (Sigma-Aldrich). La bentonite brute est composée de diverses substances minérales, telles que le quartz, illite, plagioclase, orthoclase et plusieurs sortes de minéraux.

argileux. La purification de la bentonite en Na^+ -montmorillonite (NaMt) est réalisé par échange ionique entre la bentonite et le chlorure de sodium (NaCl). Pour ce faire, 400 g de bentonite et 50 g de NaCl ont été introduits dans 2 L d'eau distillée puis chauffés à 80 °C sous forte agitation pendant 7 heures. Après avoir laissé reposer pendant 24 h, le mélange a été centrifugé et lavé à l'eau distillée jusqu'à ce qu'aucun chlorure ne soit détecté par le test aqueux AgNO_3 . Le matériau riche en montmorillonite (NaMt) échangé au Na^+ a été récupéré par centrifugation et séché à l'air à 40 °C pendant toute la nuit (Benghaffour, Foka-Wembe et al. 2022).

Diverses montmorillonites échangées en cations tels que Fe(II)Mt , Ni(II)Mt , Co(II)Mt et Cu(II)Mt ont été préparés en imprégnant 9 g de NaMt sec dans 6 g/L de la solution de sel de cation bivalent respectif $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 7\text{H}_2\text{O}$ fournie par Fisher Scientific (pureté de 99 %) et $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (Sigma-Aldrich, pureté de 99.995 %). La suspension a été maintenue à 70-80 °C sous agitation vigoureuse pendant 5 h. Les suspensions résultantes des différents catalyseurs argileux ont ensuite été dialysées à plusieurs reprises dans de l'eau fraîche désionisée à température ambiante, puis centrifugées et séchées à l'air pendant une nuit à 60°C (Azzouz, Kotbi et al. 2010, Shahidi, Moheb et al. 2015).

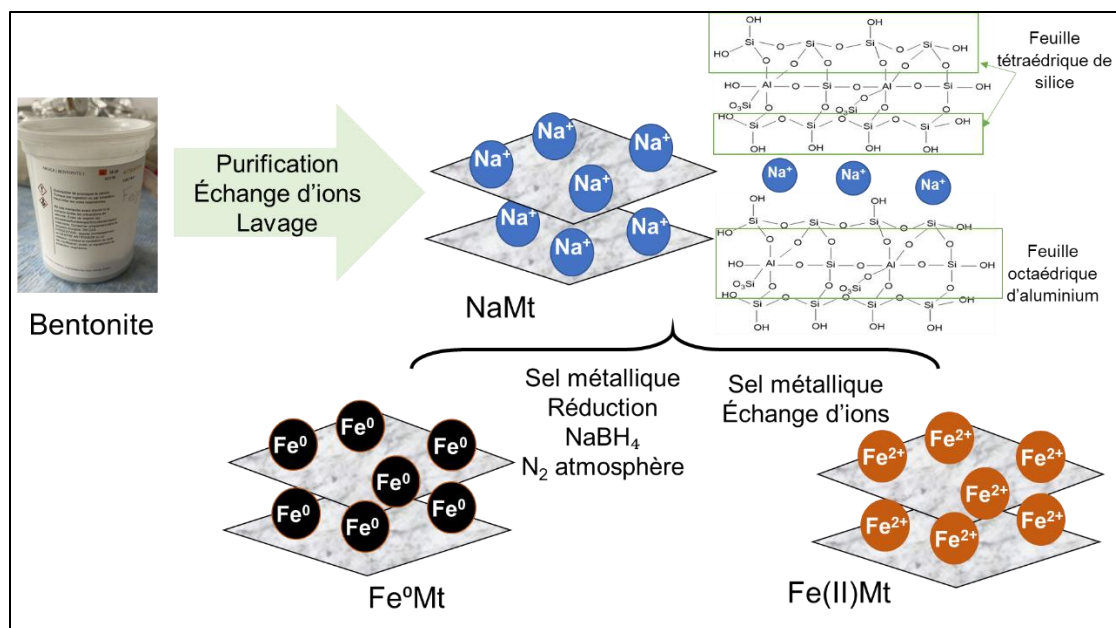


Schéma 2.2. Préparation des matériaux argileux

Les montmorillonites modifiées par des métaux à valence zéro tels que Fe⁰Mt, Cu⁰Mt, Ni⁰Mt et Co⁰Mt ont été préparées en dispersant 0.2 g de montmorillonite sodique (NaMt) dans 15 mL de solutions aqueuses 0.12 M des sels métalliques correspondants, sous agitation vigoureuse pendant 2 h. Ensuite, 10 mL d'une solution de NaBH₄ (0.2 M) ont été ajoutés goutte à goutte en tant qu'agent réducteur, sous atmosphère d'azote et le mélange a été agité pendant 15 min supplémentaires. Les matériaux obtenus (M⁰/Mt) ont ensuite été filtrés, lavés et séchés à 80 °C pendant 3 h sous atmosphère d'azote, puis conservés dans une enceinte hermétique sous azote sec exempt d'oxygène (Bouazizi, Barrimo et al. 2017, Noori, Neree et al. 2021).

La caractérisation des différents matériaux argileux a été réalisée par diffraction des rayons X et par spectroscopie infrarouge à transformée de Fourier.

2.2 Caractérisation des matériaux argileux

2.2.1 DRX diffraction des rayons X

Diffraction des rayons X (DRX) est une méthode rapide, non destructive utilisée pour identifier les phases cristallines présentes dans un matériau en les comparant à des références dans une base de données (Khan, Yerramilli et al. 2020). Ce dernier est composé d'un tube à rayons X monochromatique, un porte-échantillon, un détecteur d'intensité des faisceaux diffractés et au centre un goniomètre qui permet d'orienter le cristal dans l'espace pour satisfaire la loi de Bragg. L'émission des rayons x se fait grâce à un filament en tungstène dans la cathode, qui sous l'effet de la chaleur va émettre des électrons. En appliquant une tension les électrons sont accélérés vers l'anode en cuivre (CuK alpha = 1.54 Å) qui va produire des rayons X (Bunaciu, Udriștioiu and Aboul-Enein 2015). Le faisceau de rayons X ainsi produit interagit avec le matériau cristallin, et le détecteur enregistre l'intensité des faisceaux diffractés en fonction de l'angle 2θ. La direction et la condition de diffraction du faisceau sont régies par la loi de Bragg, décrite par l'équation suivante :

$$2d \sin \theta = n\lambda \quad (2.1)$$

Avec d : distance interarticulaire du réseau cristallin (entre deux plans cristallographiques).

λ : longueur d'onde des rayons X du faisceau incident ($\lambda = 1,540 \text{ \AA}$).

n : l'ordre de la diffraction

θ : demi-angle de déviation (l'angle d'incidence des rayons X par rapport à la surface de l'échantillon)

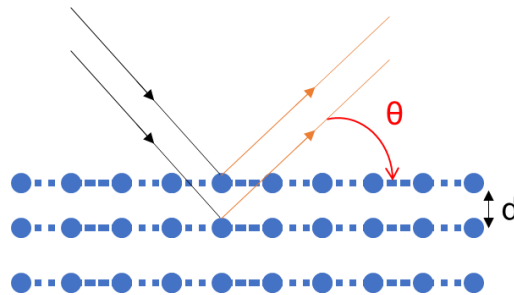


Schéma 2.3. Principe de diffraction des rayons

Dans cette étude, les analyses de DRX pour les matériaux argileux ont été déterminées par DRX (D8 Advance Bruker) avec rayonnement $\text{Cu K}\alpha$ ($\lambda = 1.5406 \text{ \AA}$), une tension de 40 kV et angles de balayage de 2° à 70° .

L'analyse DRX de la bentonite (**Figure 2.1**) a montré un diagramme de diffraction similaire à ceux rapportés dans la littérature. La bentonite est composée de plusieurs phases minérales, incluant la smectite, l'illite, le plagioclase, l'orthose (orthoclase) et le quartz (Cuevas, Cabrera et al.). Un pic intense à 7.5° en 2θ , attribué au plan (001), ainsi que des pics à 35° correspondant aux plans (040) et (220) de la montmorillonite confirment sa structure lamellaire (Cuevas, Cabrera et al. , Ahmed, Chaker et al. 2018). La présence de quartz est mise en évidence par un pic caractéristique à 26.6° en 2θ correspondant au plan (101). Des pics situés entre 21.0 et 31.0° en 2θ sont attribués à la présence de plagioclase (Cuevas, Cabrera et al.). La présence d'illite est indiquée par des réflexions caractéristiques à 20.9° , et une contribution de la kaolinite est détectée autour de 62° (Lamrani, Aabi et al. 2021).

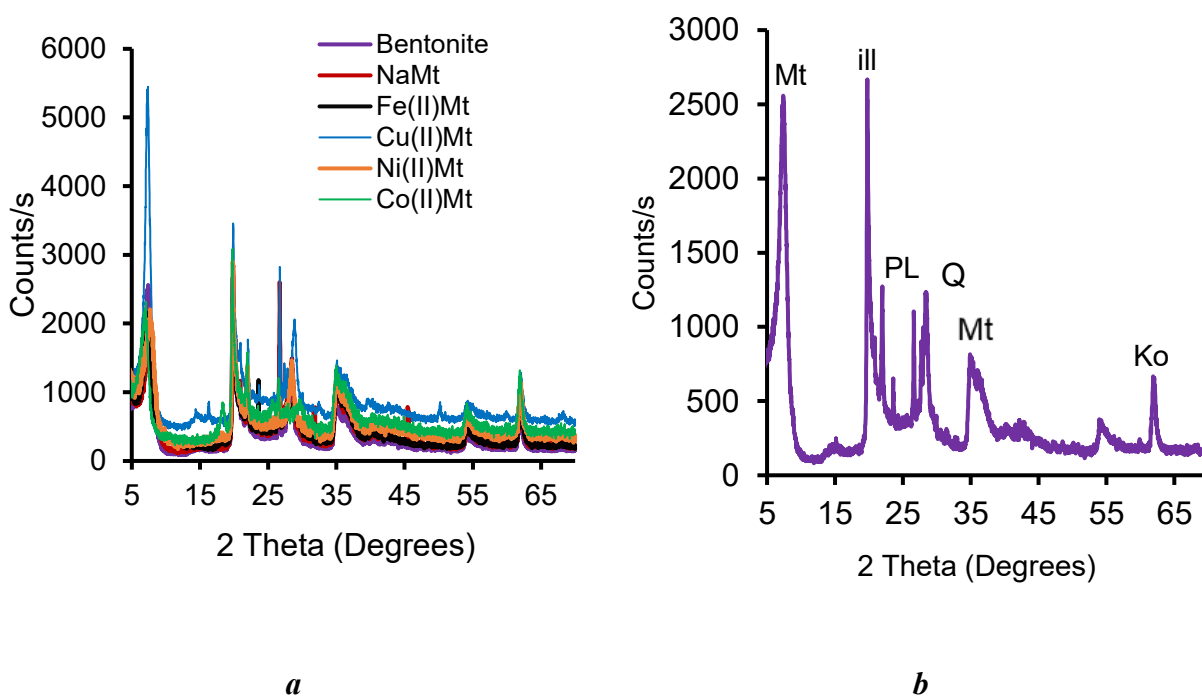


Figure 2.1. Diffractogramme de rayons X des matériaux argileux étudiés (a), identification des phases minérales associées à la bentonite (b).

La caractérisation de tous les matériaux argileux a montré une amélioration de la cristallinité lors de la purification de la bentonite en NaMt comme le montre le diagramme de diffraction (**Figure 2.1**). Les montmorillonites échangées par cations ont également montré une cristallinité élevée grâce au pic (001) net et intense. La diminution de l'angle theta par rapport à NaMt montre que l'espace interfoliaire entre les feuillets d'argile est plus grand à cause de la densité plus grande des cations Fe^{2+} et Cu^{2+} incorporés par rapport à Na^+ .

2.2.2 Potentiel zêta

Une particule en solution entraîne la formation d'une charge électrique à la surface des particules. Potentiel zêta (PZ) est une propriété interfaciale mesurée en millivolts (mV) qui représente la différence de potentiel électrique entre le milieu liquide mobile et le milieu liquide stagnant

entourant la particule au niveau du plan de glissement (cisaillement), ce dernier est le point où la couche Stern rencontre la couche diffuse (Lunardi, Gomes et al. 2021). La technique la plus utilisée pour le mesurer est la diffusion électrophorétique de la lumière (Serrano-Lotina, Portela et al. 2023). Lors de l'électrophorèse, les particules se déplacent sous l'effet d'un champ électrique, elles diffusent la lumière à une fréquence différente de celle du laser d'origine. Ce décalage de fréquence est dû à l'effet Doppler, qui est proportionnel à la vitesse des particules, de la longueur d'onde de la lumière dans le milieu et l'angle de diffusion et qui permet de déterminer PZ avec équation ci-dessous (Bhattacharjee 2016, Karmakar 2019). Le PZ a été mesuré à l'aide d'un appareil Zetasizer model Malvern, Zetasizer Ultra Red Label (Malvern Panalytical, UK). Dans les cellules capillaires, un faisceau laser traverse l'échantillon, charge la particule et celle-ci se déplace à une fréquence f , en fonction de la longueur d'onde du laser, λ , et de l'angle de diffusion θ .

$$\Delta f = (2 v \sin(\theta/2)) / \lambda \text{ where } v \text{ is the particle velocity} \quad (2.2)$$

Le potentiel zêta est un paramètre clé pour la stabilité colloïdale et est influencé par différents facteurs tels que pH, la concentration, la taille des particules en suspensions, la température ainsi que de la viscosité du milieu de dispersion (Habib, Kumara et al. 2018). Il est mesuré dans de nombreux secteurs industriels comme les industries biopharmaceutiques, cosmétiques, agroalimentaires, peintures, revêtements et traitement des eaux. Un PZ élevé positif ou négatif indique généralement des forces de répulsion plus fortes entre les particules, ce qui aide à maintenir la dispersion et empêche l'aggrégation. En revanche, un PZ faible peut entraîner une instabilité et une floculation, où les particules s'agglutinent en raison d'une répulsion électrostatique insuffisante.

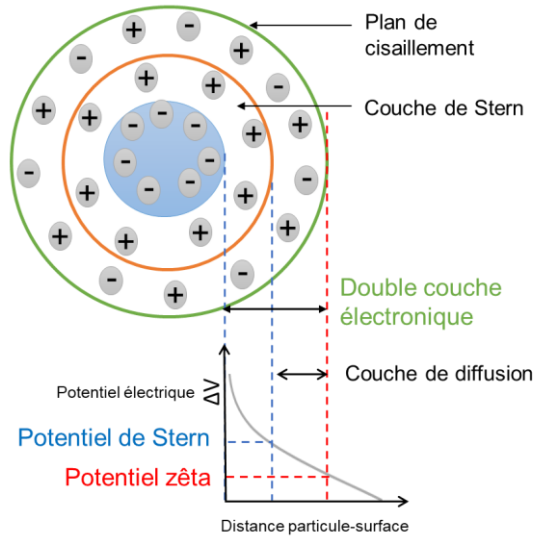


Schéma 2.4. Double couche électrique autour d'une particule colloïdale (Muththalib and Baudet 2019).

Dans le cadre de ce projet, le PZ a été mesuré afin de déterminer le comportement des suspensions argileuses en solution dans l'eau et la NOF. Pour ce fait, un appareil Zetasizer Ultra Red Label (Malvern Panalytical, UK) a été utilisé équipé d'un laser He-Ne ($\lambda = 632.8 \text{ nm}$) et d'un détecteur en rétrodiffusion (angle de 173°) en utilisant une cuve en plastique avec électrodes intégrées (DTS 1070) capables à la fois de mesurer le PZ et la taille de particule (par DLS). Tandis que l'acquisition et le traitement des données ont été effectués à l'aide du logiciel Zeta potential analyzer, Version 5.68.

2.2.3 Tailles des particules

La technique de diffusion dynamique de la lumière (DLS) est une technique optique qui mesure les fluctuations de l'intensité de la lumière diffusée par des particules en suspension due à leur mouvement brownien. Le coefficient de diffusion est obtenu, par la suite le rayon hydrodynamique est déduit par l'équation de Stokes-Einstein (Stetefeld, McKenna and Patel 2016).

$$D = \frac{K_B T}{6\pi\eta R_h} \quad (2.3)$$

Où D = coefficient de diffusion translationnelle, k_B = constante de Boltzmann (1.380×10^{-23} kg.m².s⁻².K⁻¹), R_h = rayon hydrodynamique, T = température absolue, η = viscosité du milieu.

Un faisceau laser est envoyé sur un échantillon contenant des particules en suspension. La lumière diffusée par ces particules en mouvement est ensuite captée par un détecteur, positionné généralement à un angle fixe (ex. 90° ou 173°). Dans cette étude, la taille des particules des suspensions argileuse a été analysée à l'aide d'un instrument Zetasizer Ultra Red Label (Malvern Panalytical, UK).

2.2.4 La spectroscopie infrarouge à transformée de Fourier (FTIR)

La spectroscopie infrarouge à transformée de Fourier (FTIR) est une basée sur la détermination de l'interaction entre un rayonnement IR et un échantillon qui peut être solide, liquide ou gazeux. Utilisée pour l'identification des bandes d'absorption infrarouge, qui est généralement entre 1000 et 4000 cm⁻¹ qui correspondent aux vibrations asymétriques d'élongation ou de déformation des groupes fonctionnels d'un composé (Berthomieu and Hienerwadel 2009). Le FTIR est constitué d'une source, un compartiment à échantillons, un interféromètre, un détecteur et un ordinateur (Ojeda and Dittrich 2012).

En bref, la source produit un rayonnement IR, le champ électrique induit par l'onde électromagnétique peut interagir avec un moment dipolaire d'un composé présent dans l'échantillon. Lorsque la fréquence du champ incident coïncide avec la fréquence de vibration caractéristique d'une liaison chimique dans la molécule, il se crée une interaction qui engendre la vibration de cette liaison et l'absorption de l'énergie du rayonnement. La fréquence d'absorption dépend de la nature des liaisons chimiques, de la masse des atomes impliqués et de l'environnement du groupement fonctionnel. Lorsque l'échantillon est traversé par le rayonnement IR. L'échantillon absorbe une partie du rayonnement infrarouge, tandis qu'une autre partie est transmise. L'absorption et la transmission sont enregistrés sous forme d'un signal interférométrique avant d'atteindre le détecteur. Le signal recueilli est ensuite amplifié et converti en un signal lisible, puis envoyé à un ordinateur où est effectuée la transformation de Fourier permettant d'obtenir le spectre infrarouge correspondant (Saji, Ramani et al. 2024).

Dans le cadre de ce travail, la spectroscopie FTIR a été utilisée pour caractériser et observer les modifications structurales de la montmorillonite modifiées. L'étude a révélé des bandes de vibration caractéristiques à 3626 cm^{-1} (étirement Al-OH), 985 cm^{-1} (étirement Si-O), 908 cm^{-1} (déformation Al-OH) et 796 cm^{-1} (étirement Si-O), en accord avec les valeurs déjà rapportées dans la littérature (Benghaffour, Dewez and Azzouz 2023, Djidja, Dewez and Azzouz 2025).

La FTIR a également permis de suivre les légères modifications de la molécule de NOF induites par la complexation avec les métaux. L'addition de cations à la norfloxacin a induit une diminution de l'intensité ainsi qu'un léger déplacement de la large bande située autour de 3260 cm^{-1} vers des nombres d'onde plus élevés, atteignant environ 3270 cm^{-1} pour le complexe $[\text{NOF}:\text{Na}^+]$, 3370 cm^{-1} pour $[\text{NOF}:\text{Fe}^{2+}]$, 3380 cm^{-1} pour $[\text{NOF}:\text{Cu}^{2+}]$, 3280 cm^{-1} pour $[\text{NOF}:\text{Ni}^{2+}]$ et 3320 cm^{-1} pour $[\text{NOF}:\text{Co}^{2+}]$. Ces déplacements suggèrent l'existence d'interactions entre les cations métalliques et le groupement N-H du groupement pipérazinyle et/ou le groupement O-H de la fonction carboxylique, en accord avec les observations précédentes. Par ailleurs, de nouveaux signaux observés dans la région $1620\text{-}1670\text{ cm}^{-1}$ ont été attribués aux vibrations de déformation N-H du groupe quinolone (Sahoo, Chakraborti et al. 2012, Ahmad, Ziaullah et al. 2024, Cao, Zhang et al. 2025). L'étude a été réalisée avec un appareil de la marque Thermo Scientific, modèle Nicolet 6700. L'appareil est couplé à un ordinateur équipé d'un logiciel spécialisé dans l'acquisition et le traitement des résultats dans la plage de $400\text{ à }4000\text{ cm}^{-1}$.

2.3 Préparation des solutions

La poudre de norfloxacin (numéro de lot LRAD2292) achetée chez Sigma Aldrich a été dissoute dans de l'eau nanopure à une concentration de $3.31 \times 10^{-6}\text{ M}$ en tenant compte de la solubilité de la NOF dans des conditions ambiantes. La structure moléculaire de la NOF est caractérisée par deux constantes d'acidité $\text{pK}_{a1} = 6.34$ et $\text{pK}_{a2} = 8.75$ (Wang, Cheng et al. 2020). En fonction du pH du milieu, la NOF peut ainsi exister sous différentes formes ioniques représenter dans le **schéma 2.5**. Ces caractéristiques structurales et acidobasiques influencent directement le comportement de la NOF en solution, notamment sa solubilité et sa réactivité. La solubilité de la NOF est ainsi rapportée à 450 mg/L à 25 °C et pH 7.5 (Loh, Tan and Peh 2016), et peut atteindre 1.01 g/L selon les conditions du milieu (Zhao, Li et al. 2022).

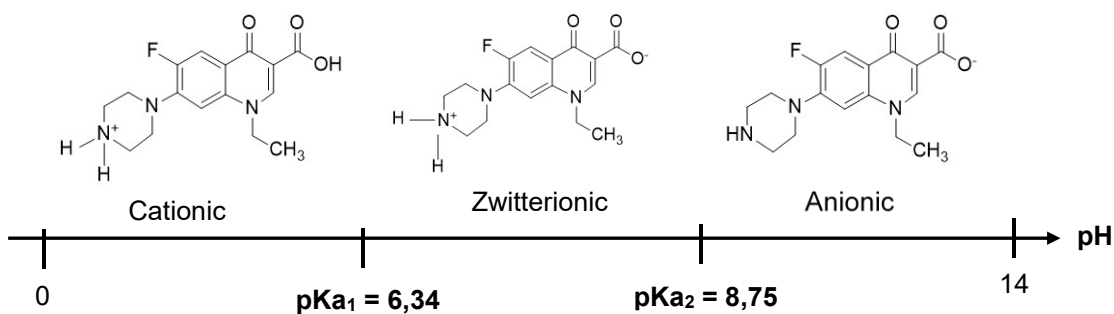


Schéma 2.5. Structure chimique et spéciation ionique de la norfloxacin en fonction du pH.

2.3.1 Tests d'ozonation

Afin de simuler l'oxydation partielle de la NOF dans l'environnement en présence et absence de suspension argileuse. Des tests d'ozonation non catalytique ont été réalisés par barbotage d'ozone (600 mg.h^{-1}) à pH intrinsèque ou modifié et température ambiante pendant différentes durées d'ozonation (0.166 à 30 minutes). L'ozone a été produit par un générateur d'ozone (A₂Z Ozone Inc., USA) dans des tubes en verres ($25 \times 200 \text{ mm}$) contenant des aliquotes de 25 mL de la même solution de NOF de départ. Des tests d'ozonation catalytique ont été effectués en présence de 50 mg de catalyseur argileux sec (taille des particules $\leq 0.1 \text{ mm}$) dans 25 mL d'aliquotes de la même solution de NOF de départ selon une procédure similaire. L'ozone est produit sur place grâce à ce générateur qui transforme l'oxygène de l'air par décharge électrique à haute tension en ozone. Pour éviter l'effet de turbidité, les mélanges ozonés résultants ont été centrifugés jusqu'à atteindre des surnageants parfaitement clairs.

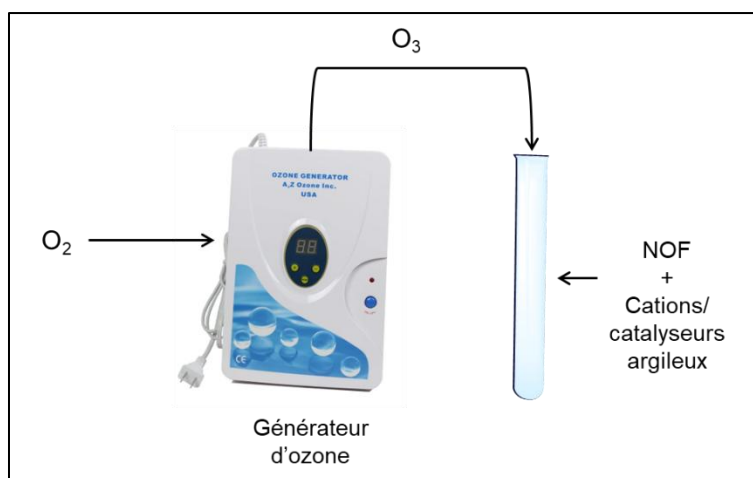


Schéma 2.6. Procédure expérimentale des expériences d'ozonation.

2.3.2 Tests d'adsorption

Les expériences d'adsorption ont été réalisées en mettant en contact 50 mg de montmorillonites échangées par des cations métalliques (M^{2+}/Mt) ou de montmorillonites modifiées par des métaux à valence zéro (M^0/Mt) avec 25 mL d'une solution de NOF à pH intrinsèque. Les suspensions ont été maintenues sous agitation vigoureuse à différents temps à température ambiante. Après filtration immédiate à travers une membrane de 0.45 μm , les échantillons sont conservés en l'absence d'oxygène sous atmosphère d'azote pour être analysés.

2.4 Analyse des produits de transformation

Tous les échantillons ozonés ou adsorbés sont filtrés et par la suite analysés par la spectrophotométrie UV-Vis et par HPLC-DAD pour évaluer l'avancement de la réaction. L'analyse par chromatographie liquide couplé à la spectrométrie de masse à temps de vol LC-Tof-MS a été également effectuée pour identifier les produits de dégradation.

2.4.1 Spectrophotométrie UV-Vis

C'est une méthode analytique simple, peu coûteuse, flexible et non destructive pour l'étude de composés organiques, elle est basée sur la transition des électrons de l'état fondamental vers l'état excité par l'absorption d'un photon. La solution est placée dans une cuve en quartz de longueur l . Elle est ensuite exposée à un rayonnement d'intensité notée I_0 . Une partie de cette lumière incidente est absorbée par le milieu et l'autre partie, notée I , est transmise. Le rayonnement incident absorbée par une substance de concentration C est proportionnel au nombre de molécules absorbantes (Rocha, Gomes et al. 2018). La quantité de radiations absorbée par l'analyte est mesurée et tracée par rapport à la longueur d'onde afin d'obtenir le spectre. Le spectre UV-Vis donne des bandes caractéristiques qui peuvent informer sur les groupements chromophores. La détermination de la concentration d'une espèce en solution réside en l'utilisation la loi de Beer-Lambert

$$A = -\log T = \log I_0 / I = \epsilon lc \quad (2.4)$$

où les symboles sont définis par les suivants : (A) absorbance, (T) transmittance, (I_0) intensité de la lumière incidente sur l'échantillon, (I) intensité de la lumière sortante de l'échantillon, (ϵ . L.mol⁻¹.cm⁻¹) absorptivité molaire, (l, cm) longueur du trajet de l'échantillon, et (c, mol/L) la concentration molaire de l'analyte.

Le spectrophotomètre UV-Vis contient une source de lumière monochromatique (visible: Lampe à incandescence à Tungstène et iode) et (UV : lampe à arc à Deutérium ou à Xénon, ou mercure), un monochromateur et un détecteur qui converti les signaux lumineux en signaux électriques.

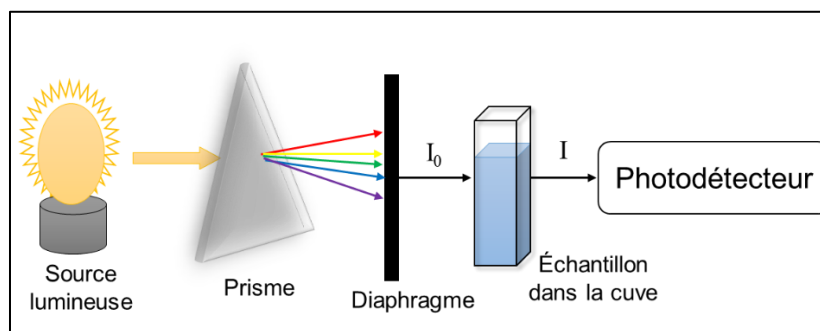


Schéma 2.7. Principe de la spectrophotométrie UV-visible (Conception propre).

2.4.2 Chromatographie Liquide couplée à la Spectrométrie de Masse (LC-MS)

La spectrométrie de masse est une méthode analytique qui permet à la fois de séparer et d'identifier les composés présents dans un échantillon complexe, même à l'état trace. Elle combine la capacité de séparation de la chromatographie liquide (LC) avec la puissance d'identification de la spectrométrie de masse (MS). La phase chromatographique permet d'isoler les molécules en fonction de leurs propriétés physico-chimiques, tandis que le spectromètre de masse fournit des informations structurales et moléculaires précises à partir du rapport masse sur charge (m/z).

Un spectromètre de masse est composé de trois modules principaux, une source d'ionisation, un analyseur de masse et un détecteur d'ions. D'abord, les molécules à analyser sont ionisées par électro-nébulisation (ESI). Cette ionisation est douce et qui génère des ions en les nébulisant sous haute tension et qui donne la possibilité de mesurer les molécules protonées et déprotonées (selon le mode d'ionisation choisi). Ensuite les ions sont focalisés et transmis à l'analyseur où ils sont séparés en fonction de leur rapport m/z . Dans cette étude un analyseur à temps de vol (TOF) est utilisé, les rapports m/z des ions sont déterminés en fonction du temps que ces derniers requièrent à parcourir une distance donnée. Ils sont enfin collectés par le détecteur qui convertit le signal électrique en données exploitables.

2.4.3 Chromatographie Liquide à Haute Performance (HPLC)

C'est une technique de séparation basée sur la répartition des solutés entre deux phases, phase mobile et phase stationnaire. L'échantillon est injecté et transporté par une phase mobile liquide qui le transporte à travers une colonne chromatographique sous haute pression. La colonne cylindrique contient une phase stationnaire, un matériau solide très poreux souvent composé de C18. Le mélange est séparé en fonction des interactions et l'affinité pour les deux phases. Les molécules du mélange passent, en moyenne, des durées différentes dans la phase stationnaire donc ils sortent de la colonne à des temps différents (temps de rétention), permettant leur séparation. Un détecteur, tel qu'un détecteur UV, analyse ensuite les composés sortants. Chaque soluté génère un pic sur un chromatogramme, et l'aire de ce pic est proportionnelle à la concentration de ce composé (Fornstedt, Forssén and Westerlund 2015).

2.4.4 Spectrométrie d'émission optique avec plasma à couplage inductif (ICP-OES)

L'ICP-OES est l'une des techniques les plus puissantes pour l'analyse des éléments traces dans des matrices complexes. Cette méthode permet de mesurer simultanément la concentration de jusqu'à 70 éléments tout en prenant en compte des interférences (Caruso, Mantellato et al. 2017).

Lors de l'injection l'échantillon sous forme liquide est transformé en un aérosol fin à l'aide d'un nébuleur. Cet aérosol est transporté dans la torche à plasma d'argon dans la chambre de pulvérisation double passe en verre cyclonique. Le plasma est généré par un champ électromagnétique à haute fréquence. Les atomes qui composent l'échantillon sont ionisés et excités. En revenant à leur état fondamental, ces atomes relâchent de l'énergie sous forme de rayonnement électromagnétique. Chaque émission correspond à une longueur d'onde spécifique mesurée par le détecteur (**Schéma 2.8.**). L'identification des éléments repose sur la position spectrale du rayonnement émis, tandis que leur concentration est déterminée à partir de l'intensité de ce rayonnement (Khan, Sharma et al. 2022).

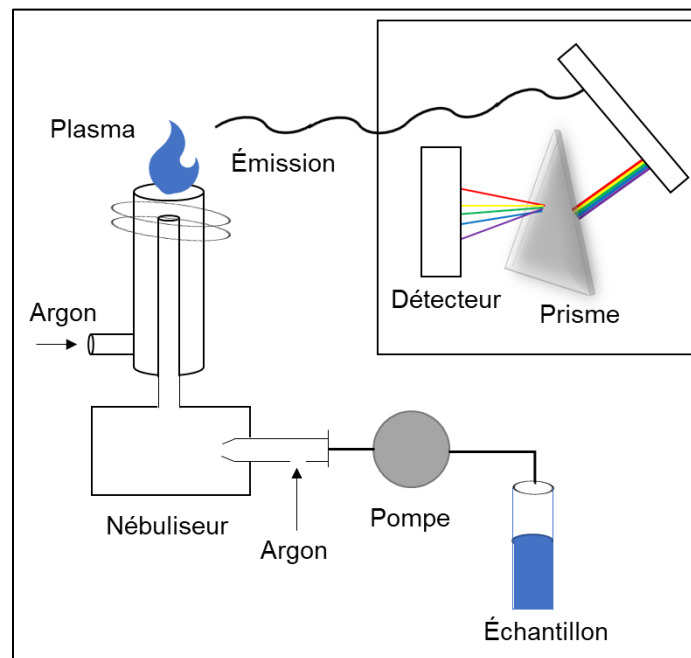


Schéma 2.8. Principe de l'ICP-OES inspiré de (Cherevko and Mayrhofer 2018).

2.5 Analyses éco-toxicologiques

Tout le matériel utilisé dans cette étude a été préalablement lavé avec du détergent, puis rincé à l'aide de l'éthanol à 95 % suivi de plusieurs rinçages à l'eau Nanopure. L'ensemble du matériel a ensuite été stérilisé par autoclavage pendant 45 minutes.

2.5.1 Culture de *Lemna minor*

La plante *L. minor* a été obtenue de la part du laboratoire Pr. Juneau (UQAM). La solution nutritive utilisée pour la croissance était un milieu nutritif préparé selon l'Institut suédois des normes (SIS) préparé selon les directives l'Organisation de Coopération et de Développement Économiques (OECD, 2006) contenant les nutriments figurés dans le tableau suivant.

Tableau 2.1. Composition du milieu de croissance pour *Lemna minor* établi par la norme suédoise (Swedish standard (SIS)).

Stock solution	Chemicals	Concentration in		Manufacturer
		Stock solution (g/L)	SIS medium* (mg/L)	
I	Na NO ₃	8.500	85.000	Anachemia, ACS grade Anachemia
	KH ₂ PO ₄	1.340	13.400	
II	MgSO ₄ .7H ₂ O	15.000	75.000	Anachemia
III	CaCl ₂ .2H ₂ O	7.200	36.000	Anachemia, ACS grade
IV	Na ₂ CO ₃	4.000	20.000	Fisher Scientific
V	ZnSO ₄ .7H ₂ O	0.050	0.050	Anachemia, ACS grade
	H ₃ BO ₃	1.000	1.000	Anachemia, ACS grade
	MnCl ₂ .4H ₂ O	0.200	0.200	Anachemia, ACS grade
	Na ₂ MoO ₄ .2H ₂ O	0.010	0.010	Fisher Chemicals
	CuSO ₄ .5H ₂ O	0.005	0.005	Anachemia, ACS grade
	Co(NO ₃).6H ₂ O	0.010	0.010	Anachemia, ACS grade
VI	FeCl ₃ .6H ₂ O	0.170	0.840	Anachemia
	Na ₂ -EDTA.2H ₂ O	0.280	1.400	Anachemia
VII	MOPS (buffer)	490.000	490.000	Anachemia

Le milieu SIS a été préparé en ajoutant 10 mL de la solution I, 5 mL des solutions II, III et IV, 1 mL de la solution V, 5 mL de la solution VI et 1 mL de la solution VII à 900 mL d'eau nanopure. Le pH du milieu a été ajusté à 6.5 ± 0.2 à l'aide de solutions de HCl ou de NaOH à 0.1 M ensuite soumis à une stérilisation sous vide pendant une durée d'exposition de 30 min à une température de 121 °C à l'aide d'un stérilisateur à vapeur sous vide modèle LSS 275. Des Erlenmeyer de 250 mL ont été utilisés pour la croissance des lentilles d'eau.



a

b

Figure 2.2. Cultures de la plante aquatique *Lemna minor* (a) et (b).

Les spécimens de plantes cultivés (**Figure 2.2**) ont été maintenus dans des conditions contrôlées de lumière de 16 h (intensité lumineuse de $100 \pm 10 \mu\text{mol de photons m}^{-2}\text{s}^{-1}$) et de cycle d'obscurité de 8 h, avec une température constante de $24 \text{ °C} \pm 2 \text{ °C}$ sous une humidité relative de $60 \pm 5\%$. La culture a été renouvelée chaque 7 jours pour préserver la qualité de la croissance.

2.5.2 Exposition de *Lemna minor* aux solutions contaminées

La toxicité intrinsèque de la Norfloxacin ainsi que les métaux étudiés (Na^+ , Fe^{2+} , Ni^{2+} , Cu^{2+} et Co^{2+}) a été évaluée en mettant en contact trois plantes vertes saines de *Lemna minor* à triple fronde à une gamme de concentrations de concentrations de NOF, préalablement préparées dans de l'eau Nanopure (0.001, 0.01, 0.1, 1.0, 10 et 100 mg L^{-1}) pendant une durée de 7 jours. Les solutions métalliques, préparées dans de l'eau Nanopure à partir des mêmes sels métalliques que ceux utilisés pour la préparation des catalyseurs à base d'argile, couvraient une gamme de concentrations comprise entre 2.5×10^{-4} et 25 mg/L . La toxicité de NOF après ozonation ou adsorption a également été évaluée en exposant trois plants de *L. minor* à triple fronde à un milieu constitué de 10 mL de surnageant issu du mélange réactionnel et de 10 mL de milieu SIS.

La toxicité a été évaluée au moyen du taux d'évolution de la biomasse exprimé en termes de nombre de frondes et de poids frais, de la teneur en chlorophylle, l'efficacité de l'appareil photosynthétique ainsi que le niveau d'espèces réactives de l'oxygène (ROS) et comparés à des échantillons à blanc constitués de plantes non exposées dans 20 mL du milieu SIS.

2.5.3 Mesures de l'inhibition de croissance

Le nombre de frondes et le poids frais ont été estimés dans chaque flacon après 7 jours d'exposition et comparés à des échantillons témoins constitués de plantes en milieu SIS.

Le pourcentage d'inhibition du taux de croissance peut ensuite être calculé selon la formule suivante :

$$\% \text{ inhibition} = 100 \times \left(1 - \frac{u_{\text{échantillon}}}{u_{\text{control}}} \right) \quad (2.5)$$

Où : % inhibition est le pourcentage d'inhibition du taux de croissance spécifique moyen; μ_C est la valeur moyenne de μ dans le groupe témoin; $\mu_{\text{échantillon}}$ est la valeur moyenne de μ dans le groupe traité. Le poids frais de chaque échantillon, y compris l'échantillon témoin a été séché et pesé à l'aide d'une balance analytique.

2.5.4 Mesure de la teneur des pigments chlorophylliens

Après sept jours d'exposition, les teneurs en chl *a* et chl *b* des plantes ont été analysées. Les deux chlorophylles ont été extraites en broyant les feuilles de *Lemna minor* dans un mortier en présence de 5 mL d'éthanol (95 %) à température ambiante. L'extrait a ensuite été centrifugé pendant 10 minutes. À l'aide d'un lecteur à microplaques (Infinite M200®, Tecan, Männedorf, Suisse), l'absorbance du surnageant a été mesurée à 665 nm (A665) pour Chl *a* et à 648 nm (A648) pour Chl *b*. Les teneurs en Chl *a* et Chl *b* ont été estimées l'aide des équations décrites par Lichtenthaler (Lichtenthaler and Buschmann 2001). Les résultats ont été exprimés en µg/mg de poids frais.

2.5.5 Mesure des espèces réactives d'oxygènes

La génération de ROS a été déterminée dans l'ensemble de la biomasse de spécimens de *Lemna minor* déjà en contact avec des mélanges ozonées ou adsorbées pendant 7 jours, collectés et séchés sur un papier kimwipe en utilisant une méthode fluorométrique et la sonde fluorogène CellROX green à 5 µM (maximum d'absorption/émission à 485 /520nm).

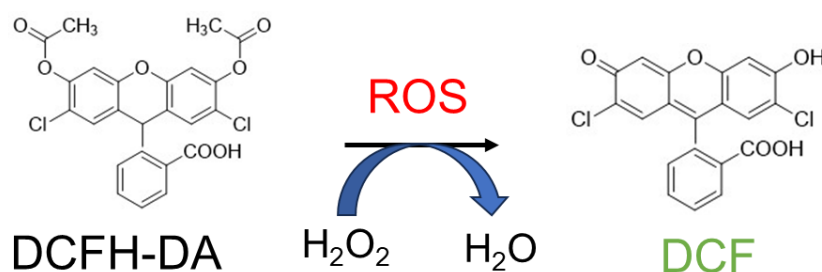


Schéma 2.9. Détection des espèces réactives de l'oxygène par la sonde 2',7'-dichlorodihydrofluorescéine diacétate.

Les spécimens de plantes ont été incubés pendant 30 min à 25 °C, puis lavés avec une solution saline tamponnée au phosphate pendant 10 min à température ambiante. L'émission relative de fluorescence a été mesurée à 520 nm sous une longueur d'onde d'excitation lumineuse à 485 nm

avec un lecteur de microplaques (Infinite M200, Tecan) ayant un gain de 200. Les données de fluorescence ont été normalisées en fonction du poids frais. Les valeurs moyennes et d'écart type ont été calculées pour chaque groupe de tous les tests effectués en triplicata.

CellROX Green est largement employée pour sa spécificité accrue vis-à-vis des radicaux superoxydes ($O_2^{\cdot-}$) et sa forte stabilité fluorescente une fois liée à l'ADN. Bien que sa structure chimique exacte ne soit pas divulguée par le fabricant (Thermo Fisher Scientific), cette sonde appartient à la famille des colorants à base de cyanine et fonctionne selon un mécanisme d'activation par oxydation, suivi d'une fixation au matériel nucléaire, ce qui permet une détection ciblée et sensible du stress oxydatif cellulaire (Scientific). À titre de comparaison, la sonde 2',7'-dichlorodihydrofluorescéine diacétate (H_2DCFDA) non fluorescent traverse facilement la membrane cellulaire. Une fois à l'intérieur de la cellule, elle est hydrolysée par des enzymes intracellulaires (esterases) qui retirent les groupes acétate pour former le H_2DCF . En présence de ROS et de peroxydases cellulaires, le H_2DCF est transformé en un composé hautement fluorescent, le 2',7'-dichlorofluorescéine (DCF) (Oukarroum, Barhoumi et al. 2015).

2.5.6 La fluorescence de la chlorophylle *a*

La fluorescence de la chlorophylle *a* constitue un outil non invasif largement utilisé pour évaluer l'efficacité de l'appareil photosynthétique (Lazár 1999). Le photosystème II (PSII) absorbe l'énergie lumineuse et initie le transfert des électrons à travers différents transporteurs de la chaîne de transport d'électrons. La lumière absorbée par les molécules de chlorophylle non utilisée pour la photochimie ou dissipée sous forme de chaleur, est réémise sous forme de fluorescence (Kumar, Dahms et al. 2014). Lors de l'induction de fluorescence, les plantes présentent une augmentation rapide appelée la courbe O-J-I-P correspondant à des étapes spécifiques de la chaîne de transport d'électrons. O (Origine) Tous les centres réactionnels du PSII sont ouverts et aucun transfert d'électrons n'a encore eu lieu; J (2 ms) intensité de fluorescence à 2 ms reflétant la réduction de l'accepteur primaire d'électrons (QA^-); I (30 ms) intensité de fluorescence à 30 ms indiquant l'hétérogénéité de la plastoquinone PQ pendant le transfert des électrons de QA^- vers QB; P (Pic) tous les accepteurs d'électrons en aval du PSII sont entièrement réduits interrompant temporairement la photochimie et tous les centres réactionnels sont fermés (Lazár 1999, Singh, Kumar and Soni 2022). Toutes perturbations des processus biochimiques et physiologiques associé

à la photosynthèse se traduit par une modification du rendement et de la dynamique de la fluorescence ce qui en fait un indicateur sensible des stress environnementaux (Kumar, Dahms et al. 2014).

Avant les mesures, les feuilles de *L. minor* ont été placées dans l'obscurité pendant 30 minutes afin de favoriser l'oxydation complète de la chaîne de transport d'électrons et d'assurer l'ouverture complète des centres réactionnels du PSII. Par la suite, les plantes ont été soumises à une impulsion lumineuse (intensité de 3000 μmol de photons $\text{m}^{-2}\cdot\text{s}^{-1}$, émission maximale à 650 nm) durant une seconde à l'aide du fluorimètre Handy-PEA (Plant Efficiency Analyser, Hansatech Instruments Ltd, Royaume-Uni). Cet appareil a été conçu pour la mesure rapide et à haute résolution de la fluorescence de la chlorophylle émise par le Photosystème II. À partir des différentes phases de la cinétique de fluorescence, plusieurs paramètres peuvent être estimés pour refléter la dynamique du transport électronique photosynthétique. Pour calculer les différents paramètres liés à la fluorescence chlorophyllienne, tels que PI_{ABS} , F_v/F_m , V_J et M_0 les formules suivantes ont été utilisés (Singh, Kumar and Soni 2022).

$$\frac{F_v}{F_p} = \frac{F_p - F_{20\mu s}}{F_p} \quad (2.6)$$

$$M_0 = \frac{(F_{300\mu s} - F_{20\mu s})}{(F_p - F_{20\mu s}) \times 0.25} \quad (2.7)$$

$$V_J = \frac{F_{2ms} - F_{20\mu s}}{F_p - F_{20\mu s}} \quad (2.8)$$

$$PI_{ABS} = \frac{1 - \left(\frac{F_0}{F_m}\right)}{\frac{M_0}{V_J}} \times \frac{(F_m - F_0)}{F_0} \times \frac{(1 - V_J)}{V_J} \quad (2.9)$$

Avec :

F_0 : Fluorescence minimale mesurée sur un échantillon adapté à l'obscurité.

F_p : Fluorescence maximale au niveau de transtion P.

$F_{20\mu s}$, F_{2ms} , $F_{300\mu s}$: Niveaux de fluorescence aux temps spécifiques pendant la phase d'induction O-J-I-P.

F_V/F_M : Rendement quantique maximal.

V_J : Fluorescence variable liée à la réduction de QA.

CHAPITRE 3

Clay-catalyzed ozonation of Norfloxacin - Effects of metal cation and degradation rate on aqueous media toxicity towards *Lemna minor*

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Preparation of experimental protocols. Preparation, purification, and ion-exchange modification of clay materials. Execution of ozonation, and catalytic ozonation experiments for norfloxacin, as well as ecotoxicological assays. Processing and analysis of experimental data, interpretation of results and drafting of the manuscript.

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Revision and optimization of experimental and ecotoxicological protocols. Verification of preliminary experiments, review and validation of ecotoxicological data, contribution to data interpretation and correction of the final manuscript.

Abdelkrim Azzouz

Conceptualization and design of the research project on the catalytic ozonation of organic pollutants. Supervision of experimental work, validation and interpretation of results and critical revision and finalization of the manuscript.

Abstract

Norfloxacin was ozonized in aqueous montmorillonite suspensions and the resulting toxicity on *Lemna minor* was investigated for understanding the impact of natural partial oxidation of antibiotics on clay-containing ecosystems. Ion-exchanged montmorillonites (Mt) were used as catalysts because of their large occurrence in soils and aquatic media, while *Lemna minor*, an aquatic macrophyte is regarded as a bioindicator highly responsive to ecotoxicity change in the environment. NOF solutions exhibit intrinsic toxicity on *L. minor* expressed in terms of fresh mass, frond number, chlorophyll content and production of reactive oxygen species. This toxicity was found to trigger through oxidative stress and was enhanced by ozonation. UV-Vis spectrophotometry and liquid chromatography coupled to mass spectrometry (LC-MS) showed that the toxicity specifically evolves in time according to the clay exchangeable cations, oxidation advancement and derivatives distribution, and confirmed the unavoidable formation of hydroxylated and acidic intermediates. The cleavage of the phenyl and pyridinyl groups appear to occur even in non-catalytic ozonation and generate potentially more toxic derivatives than the parent molecule with excessive oxidative stress and changes in the distribution of the photosynthetic pigments. Addition of Fe(II)Mt and Cu(II)Mt induced a more effective ozonation with, but with much less toxicity with Fe²⁺ exchanged Mt catalyst. This research provides valuable insights into the environmental fate of antibiotics under aerobic conditions, and allows understanding their impact evolution on biodiversity, envisaging strategies targeting optimized water treatments with complete mineralization of organic pollutants.

Highlights

- Norfloxacin ozonation induces changes in toxicity upon *Lemna minor*.
- Clay catalysts influence the antibiotic oxidation processes and toxicity.
- Cu²⁺-exchanged montmorillonite enhances NOF and derivatives conversion.
- Fe²⁺-exchanged catalyst also enhances NOF conversion but with less derivatives.
- The ecotoxicity strongly correlates to the degradation rate, pH and cations.

Keywords: Norfloxacin; Clay catalysts; Montmorillonite; Ozonation; *Lemna minor*; Toxicity

3.1 Introduction

Water contamination by organic pollutants is a major environmental issue for aquatic ecosystems. Antibiotics are particularly harmful to biodiversity and even human health because of their intrinsic capacity to promote bacteria resistance by adaptation and mitigation of the drug efficiency. In addition, antibiotics release through household and farm wastewaters unavoidably leads to their partial oxidation into a variety of undesired intermediates sometimes more toxic than the parent molecules (Liu, Nanaboina et al. 2012, Benghaffour, Foka-Wembe et al. 2022, Benghaffour, Azzouz and Dewez 2023, Benghaffour, Dewez and Azzouz 2023, Foka Wembe, Benghafour et al. 2023). Among antibiotics, fluoroquinolones (FQs) are commonly used in human and animal healthcare, being released in domestic sewage in concentrations ranging from 489 to 7560 ng/L (Liu, Nanaboina et al. 2012, Zhang, Li et al. 2023). FQs can induce negative impact on the antioxidation defense systems of aquatic organisms, while their oxidized derivatives unavoidably affect the very enzyme activity. Exposure to FQs may alter the growth processes of aquatic flora and fauna (Shen, Hu et al. 2023) at concentrations of 1-50 mg/L (Migliore, Civitareale et al. 1997, Hong, Huang et al. 2022, Shen, Hu et al. 2023). Among FQs, Norfloxacin (NOF) is particularly interesting compound that displays toxicity towards aquatic species, including fish females (Liang, Wang et al. 2015) and zooplankton (Migliore, Civitareale et al. 1997).

NOF ranks among the five most consumed antibiotics (Zhang, Li et al. 2023), more specifically for treating enterocolitis, dysentery and urinary-tract infections (Loh, Tan and Peh 2016). The molecular structure of NOF (**Scheme S1**) confers some specific features (**Table S1**), with a long half-life due to a high stability at acidic and basic pH, in darkness and in the absence of oxygen (Nangia, Lam and Hung 1991, Wang, Gong et al. 2020, Chen and Wang 2021). The stability towards pH fluctuations favors easy transitions between the cationic, zwitterionic and anionic forms (**Scheme S2**), and can be explained by the occurrence of two pKa values in slightly acidic (pKa₁ 6.34) and alkaline media (pKa₂ 8.10) (Wang, Cheng et al. 2020). Nonetheless, the presence of functionalized aromatic and heteroatomic cycles should react to UV-Vis radiations and oxidizing agents (Shahidi, Roy and Azzouz 2015), more particularly in acidic media around pH 2 (Mishra, Thaker et al. 2023).

Thus, once, released in nature, NOF like any organic molecules can easily and readily undergo unavoidable natural oxidation, photodegradation and photooxidation processes on sun-exposed surfaces (Ahmad, Bano et al. 2015). The use of ozone is particularly interesting for simulating in accelerated mode the natural oxidative degradation of organic molecules on both liquid and solid surfaces exposed to air and solar radiations (Benghaffour, Foka-Wembe et al. 2022, Benghaffour, Azzouz and Dewez 2023, Benghaffour, Dewez and Azzouz 2023, Foka Wembe, Benghafour et al. 2023). In this regard, the ozonation of trimethoprim (TMP) in the presence of ZnO (Taie, Fadaei et al. 2021), ciprofloxacin with anatase (TiO₂) (Wajahat, Yasar et al. 2019) and NOF with UV radiation and activated carbon catalysts have particularly focused interest (Rivas, Encinas et al. 2011). The piperidine (N-ring) and piperazinyl group (2N-ring) of NOF are expected to display high reactivity towards ozone (O₃). In the presence of Cu-CuFeO₄ based catalyst, NOF ozonation appears to trigger via defluorination through a first cleavage of the C–F bond and opening of the piperazinyl and quinolone sections (Ling, Ben et al. 2018, Huang, Fang et al. 2020, Zhang, Li et al. 2023).

The occurrence of metals in cationic, oxide or zero-valent forms in NOF-contaminated media is expected to significantly influence the evolution in time of NOF oxidation and ecotoxicity. Metal oxides such as MnOx/SBA-15 were found to improve NOF degradation due to an additional contribution of hydroxyl groups of the mesoporous silica (Chen, Li et al. 2017). NOF removal rate of 90.6% was reported for the catalytic system MnFe₂O₄/H₂O₂ after 3h (Wang, Zhao et al. 2018), and even 97.7% after 5 h electro-Fenton treatment (Özcan, Özcan and Demirci 2016), where the parent molecule and intermediates showed toxicity on biodiversity.

In aerobic conditions like on outermost layers of soils and aqueous media, metals cations play a significant role in the oxidative degradation of organic pollutants. As compared to the wide variety of cations in nature, free and supported Fe²⁺ have larger occurrence and lower toxicity in natural media, as reported by ample literature. Fe²⁺ exchanged montmorillonite in optimum concentration already showed catalytic activity in the ozonation of various organic pollutants (Azzouz, Kotbi et al. 2010, Shahidi, Roy and Azzouz 2014, Shahidi, Moheb et al. 2015, Shahidi, Roy and Azzouz 2015, Larouk, Ouargli et al. 2017, Boudissa, Zekkari et al. 2020, Benghaffour, Foka-Wembe et al. 2022, Benghaffour, Azzouz and Dewez 2023, Benghaffour, Dewez and Azzouz 2023, Foka Wembe, Benghafour et al. 2023).

Here, the pH of the aqueous medium and molecule deprotonation were found to significantly influence the ozonation pathway and water interactions with the protonated/deprotonated forms of the organic molecules and solid surfaces bearing OH groups displaying a pH-dependent behavior. For instance, increase in pH from 6.3 to 8.3 in NOF ozonation was found to raise the specific reaction rate constants by approximately 10 and 1000 times for the monoprotonated and deprotonated forms, respectively (Ling, Ben et al. 2018). Similar beneficial pH effect was also observed in NOF ozonation in the presence of highly dispersed Co_3O_4 particles in a Co_3O_4 -Carbon matrix (Chen, Zhang et al. 2021). Metal combination appears as an essential factor in redox process improvement, since Co-Mn/ CeO_2 based catalyst showed higher effectiveness in NOF ozonation as compared to CeO_2 alone and non-catalytic processes. This was explained in terms of beneficial contributions of Mn and Co incorporation (Li, Xiong et al. 2022). $\text{Co}_3\text{O}_4\text{-C@FeOOH}$ composite resulted in even higher catalytic activity in NOF ozonation due a higher surface area as compared to Co_3O_4 -Carbon and a possible Fe-Co synergy supposedly involving simultaneous contributions of Co(III)/Co(II) and Fe(III)/Fe(II) couples (Chen and Wang 2021) in agreement with other works (Zhang, Li et al. 2023).

Clay catalysts are probably much more suitable for simulating in accelerated mode the natural degradation of organic pollutants, due to their large occurrence in most natural media that usually such as soils and aquatic media, that usually host ecosystems undergoing organic pollution. Previous works already demonstrated a narrow correlation of the degradation rate and ecotoxicity evolution in time of some pesticides and drugs with the catalytic activity of montmorillonites (Benghaffour, Foka-Wembe et al. 2022, Benghaffour, Azzouz and Dewez 2023, Benghaffour, Dewez and Azzouz 2023, Foka Wembe, Benghafour et al. 2023). The role of Fe^{2+} species, selected for its large occurrence in soils and aquatic media will be examined in comparison with other exchangeable cations.

A special interest was herein devoted to montmorillonite-catalyzed NOF ozonation and resulting toxicity on *Lemna minor*. This aquatic macrophyte from the Lemnaceae family is easily cultivated and highly responsive to ecotoxicity change in the environment (Lee, Depuydt et al. 2021). Therefore, this research aims to provide valuable insights into the environmental fate of antibiotics under aerobic conditions and their degradation products in natural clay-containing matrices. Such a study is expected to be somehow biased by: *i.* the reciprocal effects of microorganisms which can

also reduce the amounts of antibiotics in polluted media and conversely (Ling, Ben et al. 2018, Ricky and Shanthakumar 2023), *ii.* appearance of more or less toxic intermediates and consecutive secondary reactions with other derivatives, *iii.* and unavoidable ecotoxicity fluctuations (Chen, Li et al. 2017). In this regard, the discussion was only focused on early ozonation when NOF toxicity still correlates with ROS production and detection of primary NOF intermediates.

3.2 Experimental

3.2.1 Catalyst preparation and characterization

A crude bentonite (Sigma-Aldrich, USA) with a 1.58 silica/alumina weight ratio and a montmorillonite content of 84 wt.% was previous purified using a procedure fully described elsewhere (Bouazizi, Barrimo et al. , Azzouz, Messad et al. 2003, Azzouz, Platon et al. 2013, Boudissa, Arus et al. 2023). Thus, 400 g of bentonite and 50 g of NaCl were introduced into 2 L of distilled water and then heated at 80 °C under vigorous stirring for 7 hours. After cooling for 24 hours, the clay suspension was repeatedly centrifuged and washed with distilled water. Repeated cycles involving and alternate settlings upon ultrasound exposure and centrifugation steps for removing dense phase fractions gave rise in a Na⁺-montmorillonite-rich material (95–96% NaMt) with a Si/Al mole ratio of 2.5-2.43. The latter was then repeatedly dialyzed overnight in deionized water (40–50 °C) to remove the physically sorbed salt excess until no chloride detection by the aqueous AgNO₃ test. NaMt was recovered by centrifugation and air-drying at 40 °C overnight (Benghaffour, Foka-Wembe et al. 2022) and further calcined at 400 °C for the elimination of organic impurities. The predominance of montmorillonite was supported by two characterization data. Fourier Transform Infrared spectroscopy (KBr IR cell and a Model IR 550, Magna Nicolet instrument) revealed characteristic vibration bands at 3628 cm⁻¹ (Al-OH stretching), 1200 cm⁻¹ (Si-O and Si-O-Si stretching), 989 cm⁻¹ (Si-O stretching), 908 cm⁻¹ (Al-OH bending) and 797 cm⁻¹ (Si-O stretching). X-ray diffraction (Siemens D5000, CuKα at 1.54051 Å) showed a special feature of homo-cationic smectites reflected by a sharper 001 XRD reflexion as compared to bentonite and a parallel layout of the clay sheets, as previous reported (Bouazizi, Barrimo et al. 2017, Arus, Nousir et al. 2018, Benghaffour, Azzouz and Dewez 2023).

Other clay catalysts, namely Fe(II)Mt and Cu(II)Mt were prepared by impregnating 40 g of dry NaMt in 1 L of 1 M solutions of nitrate of the respective bivalent cation salt solution, at 70–80 °C under vigorous stirring for 4–5 h. The resulting suspensions were then repeatedly dialyzed in fresh deionized water at room temperature, and finally centrifuged and air dried overnight at 60°C (Azzouz, Kotbi et al. 2010, Shahidi, Moheb et al. 2015).

Clay behaviour in aqueous media was assessed through measurements of the zeta potential (ZP) and particle size (PS) at room temperature by dispersing 50 mg of clay catalyst in 25 mL of pure water or aqueous solutions of NOF. The PS and ZP were measured using Zetasizer model Malvern, Zetasizer Ultra Red Label (Malvern Panalytical, UK) (**Table S2**). The two analyzes were carried out at room temperature on 50 mg of clay powder previously dispersed in 25 mL of water/aqueous NOF solution.

3.2.2 Ozonation tests and product analysis

An aqueous solution of Norfloxacin (NOF) purchased from Sigma Aldrich (Lot No LRAD2292), was previously prepared by dissolution in Nanopure water at specific concentrations considering the solubility of NOF at ambient conditions and occurrence in most wastewaters ($3.31 \cdot 10^{-6}$ M). Calibration curves for UV-Vis spectrophotometry (UV-Vis) were plotted for the three UV-Vis bands of NOF using various solutions prepared by successive dilutions of the mother-solution, at intrinsic pH and room temperature (**Fig. S1**). For this purpose, a UV-Vis spectrophotometer Agilent-Cary 60 instrument (1 cm quartz cell) was used.

Diverse non-catalytic ozonation tests were performed by ozone bubbling ($600 \text{ mg}\cdot\text{h}^{-1}$) at intrinsic pH and room temperature for various ozonation times (0.50 to 9 minutes). Ozone was produced by an A₂Z generator (A₂Z Ozone Inc. USA) in a series of glass tubes (25 × 200 mm) containing 25 mL aliquots of the same starting NOF solution. Catalytic ozonation tests were carried out in the presence of 50 mg of dry clay catalyst (Particle size $\leq 0.1 \text{ mm}$) 25 mL in aliquots of the same starting NOF solution in a similar procedure. To avoid the effect of turbidity, the resulting ozonized mixtures were centrifuged until reaching perfectly clear supernatants. The latter were further analyzed through UV-Vis for a qualitative assessment of the evolution in time of the relative absorbance of the different UV-Vis bands of NOF as reported to those of the non-ozonized NOF

mother-solution ($(A/A_0) \times 100\%$). Additional analyses for product identification were achieved for the different ozonation mixtures (10 s, 30 s, 1 min, 3 min and 6 min) by Liquid Chromatography coupled to Time-of-Flight Mass Spectrometry (LC-MS) in an Agilent 1260 Series LC System (Agilent Technologies, Santa Clara, CA) under specific conditions (**Table S3**). LC-MS analysis of the aqueous 3.31×10^{-6} M NOF solution prepared in Nanopure water revealed a NOF purity of 97.86 mol.%, and the presence of two major impurities with m/z values of 306 g ($C_{15}H_{20}FN_3O_2$) and 294 g ($C_{14}H_{16}FN_3O_3$) and proportions of 1.69 and 0.45 %, respectively (**Table S4**).

3.2.3 Toxicity tests

Lemna minor was cultured in an aqueous medium prepared with a specific nutrient composition according to the Swedish Institute for Standards (SIS) (**Table S5**). The medium pH was adjusted to 6.5 ± 0.2 , and the resulting SIS medium was prone to a sterilization under vacuum conditions for an exposure time of 30 min at temperature of 121 °C using a vacuum steam sterilizer model LSS 275. The plants were maintained under controlled conditions of a 16 h light exposure (light intensity of $100 \pm 10 \mu\text{mol of photons m}^{-2}\text{s}^{-1}$) and 8 h dark cycle, with a constant temperature of $24 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ and a relative humidity of $60 \pm 5\%$.

The intrinsic toxicity of NOF was assessed by contacting healthy green three triple-fronded plants of *L. minor* with five NOF concentrations previously prepared in Nanopure water ($0.0001\text{-}100 \text{ mg L}^{-1}$) for 7 days. Each essay was performed in a 50 mL Erlenmeyer. In a second step, the toxicity of 10 mL of the supernatant of ozonized NOF solutions was evaluated after 7 days in contact with 10 mL of SIS medium containing similar three triple-fronded *L. minor* plants. The toxicity evolution, expressed in terms of number of fronds and fresh weight, chlorophyll content and distribution, along with the level of reactive oxygen species (ROS) were measured after 7 days in contact and compared to blank samples consisting of non-exposed plants in 20 mL of SIS medium. The four toxicity parameters considered in this work are widely used in most studies when dealing with *Lemna minor* and counterparts. In some cases, only some of these parameters are investigated (Oukarroum, Barhoumi et al. 2015, Nunes, Veiga et al. 2019, Lee, Depuydt et al. 2021, Hong, Huang et al. 2022, Zhao, Li et al. 2022, Kochi, Kitamura et al. 2023, Sikorski, Bęś and Warmiński 2023, Elbasan, Arikan-Abdulveli et al. 2024, Sikorski and Bęś 2024) but they are seldom studied together (Jmii and Dewez 2021, Benghaffour, Azzouz and Dewez 2023). As compared other

toxicity criteria, they involve convenient and relatively fast experimental procedures that provide fairly reliable data for toxicity comparison.

Both chlorophylls were extracted by grinding *L. minor* leaves in a mortar in the presence of 5 mL of ethanol (95 %) at room temperature. The extract was then centrifuged for 10 min. Using a micro-plate reader (Infinite M200®, Tecan, Männedorf, Switzerland), the supernatant absorbance was measured at 665 nm (A665) for Chl *a*, and at 648 nm (A648) for Chl *b* according to a procedure fully described elsewhere (Lichtenthaler and Buschmann 2001). The generation of ROS was determined in the entire biomass of *Lemna minor* specimens already in contact with ozonized mixtures for 7 days, collected and dried on a Kim wipepaper using a fluorometric method and the fluorogenic probe CellROX green at 5 µM (absorption/emission maxima at 485/520 nm). The plant specimens were incubated for 30 min at 25 °C, then washed with phosphate-buffered saline solution for 10 min at room temperature. The relative fluorescence emission was measured at 520 nm under a light excitation wavelength at 485 nm with a micro-plate reader (Infinite M200, Tecan) having a gain of 200. The mean and standard deviation values were calculated for each group of all assays performed in triplicate.

3.3 Results and Discussion

3.3.1 Intrinsic ecotoxicity of NOF

As a general tendency, the intrinsic toxicity of NOF after 7 days of contact time with *L. minor* in SIS media turned out to increase with increasing nominal concentration in the starting NOF solutions from 0.0001 to 100 mg/L. The results clearly showed a dose-dependent relationship between the toxicity parameters considered (frond number, fresh weight, and ROS production) and NOF concentration. Indeed, increasing NOF concentration up to 10 mg/L induced a pronounced decay in both the frond number and fresh weight by ca. 76 and 80 %, respectively, followed by a plateau for higher concentrations (**Fig. 3.1a**). This was accompanied by a marked increase of the relative ROS amount by more than 7 times with increasing NOF concentration up to 10 mg/L and an attenuation beyond this value (**Fig. 3.1b**). This indicates that the toxicity of NOF is proportional to ROS production within this concentration range, i.e. as long as increasing NOF concentration induces no suppression of the plant metabolism. This toxicity appears to shade those of the main

impurities with m/z of 320, 306, 294 detected with increasing amounts in the reaction mixture during short ozonation times (**Table S4**). Therefore, these results provided clear evidence that NOF exhibits intrinsic toxicity leading to impaired growth through an enhancement of cellular oxidative stress in *L. minor* plant system. Such strong increase in ROS production with NOF exposure indicated that oxidative stress represented the primary mechanism of NOF toxicity. Here, the molecular structure and/or induced pH in NOF solution are expected to govern not only in the molecule oxidation in the presence of reactive oxidizing agents but also in the intermediate distribution and toxicity, as already observed for the effect of various organic pollutants on living species (Benghaffour, Foka-Wembe et al. 2022, Benghaffour, Azzouz and Dewez 2023, Benghaffour, Dewez and Azzouz 2023, Foka Wembe, Benghafour et al. 2023).

Visible symptoms of toxicity such as necrosis followed by chlorosis appeared in *L. minor* plants upon exposure to concentrations of 1 and 10 mg/L, indicating severe physiological stress and damage to the plant tissues. In the meantime, the content of chlorophyll *b* (Chl *b*) increased at the expense of that of chlorophyll *a* (Chl *a*) at the highest tested concentration of NOF (**Fig. S2a**). A clear correlation can be established for both Chl *a* and Chl *b* fluctuations, reflected by a visible decrease of the chlorophyll *a/b* ratio (Chl *a/b*), despite the weak precision of the spectrophotometry measurements (**Fig. S2b**). The decrease in the Chl *a/b* ratio with increasing NOF concentration indicated a shift in chlorophyll composition, suggesting an impact on the photosynthetic efficiency of the plant.

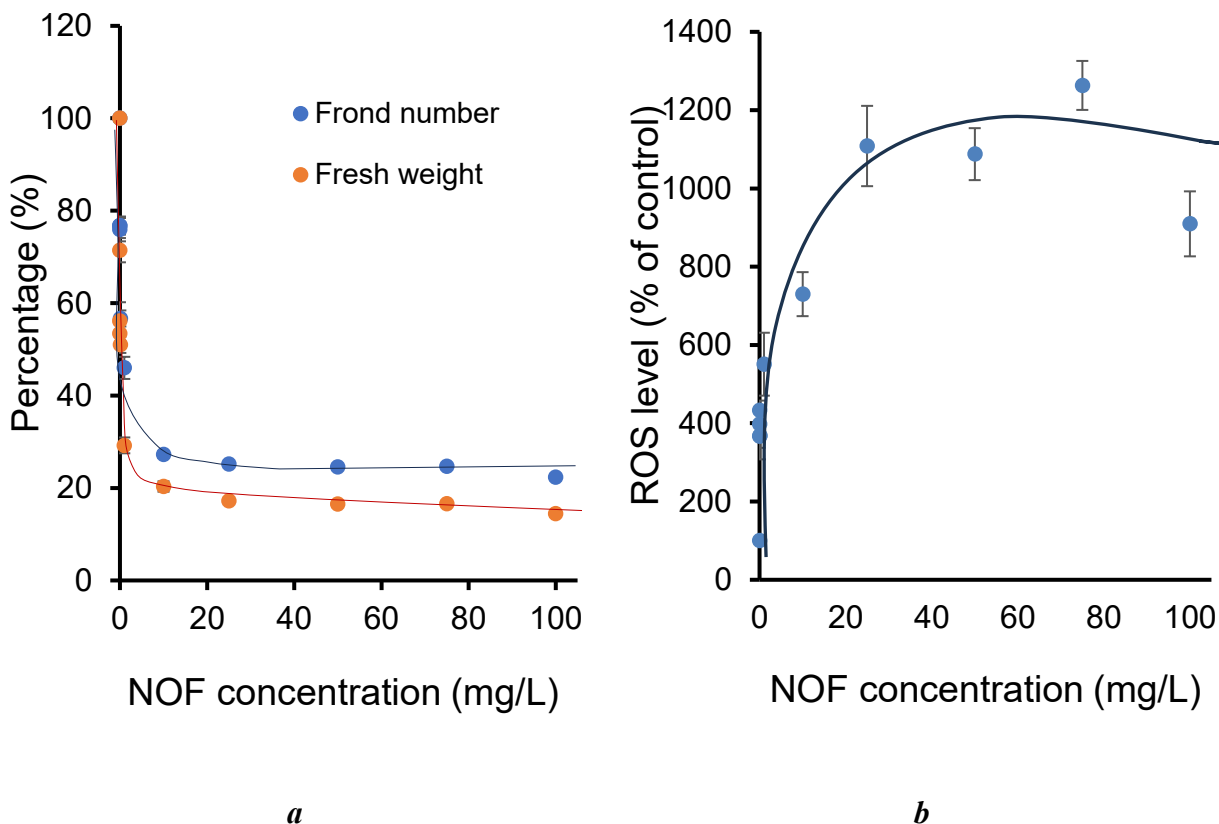


Figure 3.1. Effect of NOF concentration on *L. minor* relative frond number and fresh weight (a) and ROS relative amount (b) as reported to the blank sample (Control). These toxicity parameters were measured after 7 days of exposure in SIS medium containing NOF solutions at concentrations ranging from 0.001 to 100 mg/L. The results are expressed as percentages relative to the control sample (antibiotic-free SIS medium). Triplicate measurements were achieved for all concentrations considered including the antibiotic-free control sample). The control consisted of plants grown in SIS medium without NOF, an average fresh weight of 91.3 mg and 61.6 fronds.

This detrimental effect of NOF concentration on the Chl *a/b* ratio and subsequently on the photosynthetic apparatus of *L. minor* agrees with other data related to *L. minor* and *L. gibba* exposure to fluoroquinolone and more particularly to ciprofloxacin (CIP) (Richards, Wilson et al. 2004, Nunes, Pinto et al. 2014). This must undoubtedly be attributed to ROS production in excess with respect to the intrinsic ROS level of non-exposed *L. minor* to NOF. This is supported by the fact that an increase in ROS level was already reported to cause harmful effects on *L. minor* through

photosynthesis attenuation, due to cellular H₂O₂ production as compared to control (Gomes, Kitamura et al. 2022, Sikorski and Bęś 2024) and even on the respiratory metabolism (Gomes, Gonçalves et al. 2017). Quinolones are also known to inhibit DNA gyrase, an essential enzyme for bacterial replication (Bhatt and Chatterjee 2022), to disturb the respiratory system via mitochondrial damage and to affect chloroplasts as well (Aristilde, Melis and Sposito 2010).

3.3.2 Toxicity changes upon non-catalytic ozonation

The decay the frond number and fresh weight by approximately 70 % and 85 %, respectively compared to the control after 7 days of exposure to ozonated reaction mixtures (**Fig. 3.2a**) appears to be proportional to the pronounced increase in the ROS production during the first minute of ozonation (**Fig. 3.2b**). This accounts for the rise of NOF intermediates that induce oxidative stress and adversely affect plant growth and biomass accumulation. The discussion was focused on the range of ozonation time 0.5-9 min, because for reaction mixtures resulting from longer ozonation times *i.* this narrow dependence between the NOF toxicity and ROS production seems to fade, and *ii.* the main NOF intermediates become undetectable by HPLC-UV and LC-MS.

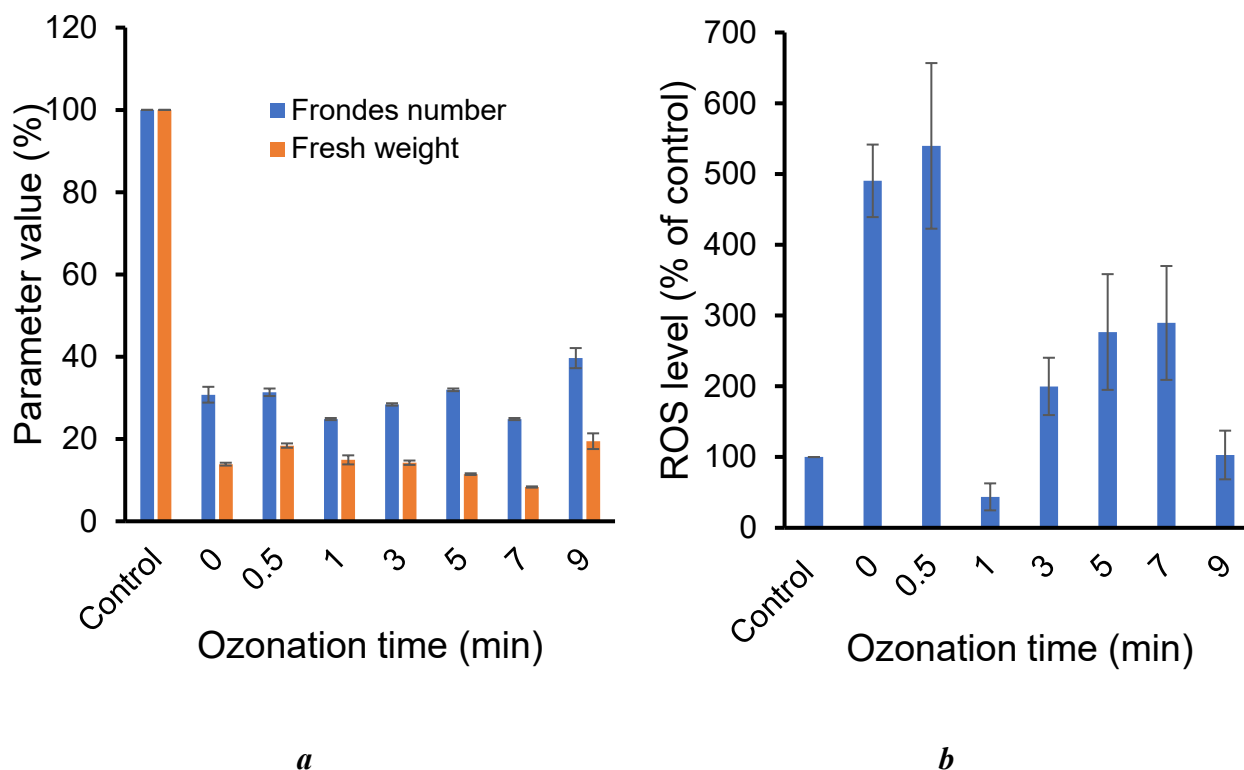


Figure 3.2. Effect of the ozonation time on the toxicity of reaction mixture through the frond number and fresh weight of *L. minor* (a) and of ROS amount (b) as reported to the blank sample (Control). These toxicity parameters were measured after 7 days of exposure in SIS medium not in contact with NOF mixtures after different ozonation times. Triplicate measurements were achieved for all concentrations considered including the antibiotic-free control sample. The results are expressed as percentages relative to the control sample (antibiotic-free SIS medium). The control consisted of fresh weight of 91.3 mg of plant with an average number of 61.6 fronds in SIS medium in the absence of NOF (0 mg/L NOF).

Moreover, the fluctuations of the ROS production account for successive variations of the toxicity on plant growth and progressive changes in intermediate distribution (Benghaffour, Foka-Wembe et al. 2022, Benghaffour, Azzouz and Dewez 2023, Benghaffour, Dewez and Azzouz 2023, Foka Wembe, Benghafour et al. 2023). This dynamic toxicity profile was illustrated by two bumps that suggest the formation of at least two successive intermediates (**Fig. 3.2b**). Here, different intermediates are supposed to be produced at different stages, contributing to the overall toxicity impact observed on plant growth.

Exposure to ozone alone was found to induce a pronounced and almost uniform intensity increase for the 200 nm band (**Fig 3.3a**). This must be due to the formation of hydroxylated NOF intermediates and rise of H-bridge interactions in the aqueous media (Ling, Ben et al. 2018). This appears to be a common feature of organic products during ozonation, regardless to the parent molecule (Shahidi, Moheb et al. 2015, Larouk, Ouargli et al. 2017, Boudissa, Mirilà et al. 2019, Boudissa, Zekkari et al. 2020, Benghaffour, Foka-Wembe et al. 2022, Zekkari, Ouargli-Saker et al. 2022, Benghaffour, Azzouz and Dewez 2023, Benghaffour, Dewez and Azzouz 2023, Foka Wembe, Benghafour et al. 2023). This was accompanied by a surprisingly pronounced intensity decay for the 275 nm and 315 nm absorption bands (**Fig 3.3b**). This contrasts with the barely detectable decrease in the peak height ratio (I/I_{max}) as compared to clay-catalyzed ozonation (**Fig. 3.4b**) and cannot be explained by an intense ozonation but rather by a specific selectivity towards degradation reactions involving ring opening. Therefore, these results have important toxicological implications since the ozonation process can lead to the formation of more hydrophilic and potentially more reactive intermediates of NOF. In fact, such changes in the chemical structure and properties of NOF had a significant toxicity impact on treated *L. minor* plants (**Fig. 3.2**).

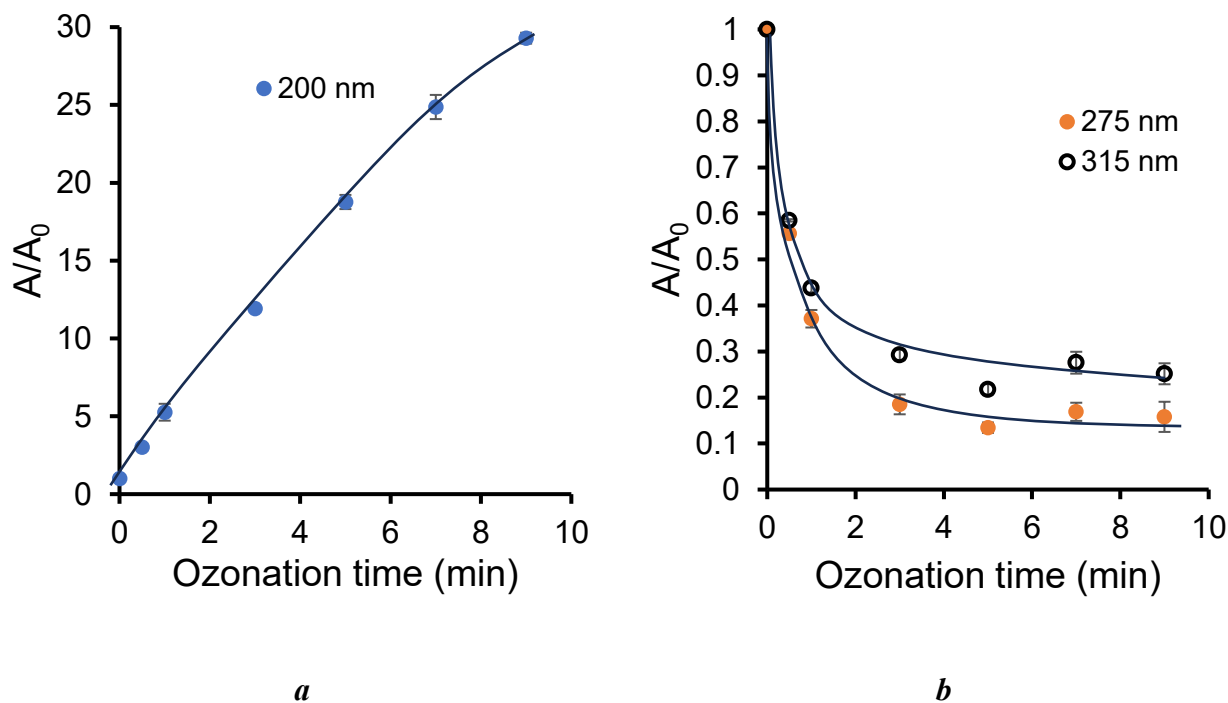


Figure 3.3. Evolution of the relative absorbance at 200 nm of NOF (a) and at 275 nm and 315 nm (b) during non-catalytic ozonation. $T = 25\text{ }^{\circ}\text{C}$. $\text{pH} = 5.28$. Quartz cell = 1 cm. O_3 throughput: 600 mg h^{-1} . Sample volume = 25 mL. NOF initial concentration: $3.31 \times 10^{-6}\text{ M}$.

3.3.3 Effect of clay-catalysts on ozonation

The UV-Vis spectrum of NOF is characterized by three absorption bands at 200, 275 and 315 nm (**Fig. S3**). A weak intensity band observed at 275 nm corresponds to the electronic transition $n \rightarrow \pi^*$, which is likely associated to the interaction between the free electron pairs on the oxygen atoms of the hydroxyl groups and the π unsaturated system within the molecule (Boudissa, Zekkari et al. 2020). The 200 nm and 315 nm bands were ascribed to the electronic transition $\pi \rightarrow \pi^*$ within the aromatic ring system (El Hassani, Kalnina et al. 2019, Zekkari, Ouargli-Saker et al. 2022).

Clay catalyst addition induced even more marked changes in the UV-Vis spectrum as compared to non-catalytic ozonation (**Fig S1-4**). A noticeable hypsochromic shifts of the 200 nm band towards lower wavenumbers was observed during NaMt-catalyzed ozonation, presumably due to water H-bridge interaction with hydroxylated groups of NOF derivatives. This was reflected by the

pronounced intensity increase of the 200 nm band during ozonation in the presence of NaMt and bentonite, slightly attenuated after 6-7 min process (**Fig. 3.4a**). This is expected to shade the advanced NOF degradation reflected by the noticeable decrease of the relative peak height (I/I_{max}) upon ozonation in the presence of NaMt and bentonite (**Fig. 3.4b**). This similarity in the behavior of both catalyst is mainly due to the preponderance of montmorillonite and sodium as the main component and exchangeable cation (Arus, Nousir et al. 2018) and almost the same Si/Al ratio (Bouazizi, Barrimo et al. 2018, Boudissa, Arus et al. 2023).

Here, the presence of low amounts of heavier metal cations in bentonite may explain the lower A/Ao of the 200 nm band for bentonite as compared to NaMt. This is agreement with the much lower but almost linearly increasing intensity ratio for the 200 nm band, suggesting lower amounts of hydroxylated intermediates for ozonation in the presence of Cu(II)Mt and Fe(II)Mt. This cannot be explained by a possible strong chelation of these transitional metal cations by fluoroquinolone-like derivatives, if any (El Khateeb, Razek and Amer 1998) but rather by selectivity changes and an enhanced ozonation that shortens the persistence of hydroxylated intermediates (Shahidi, Moheb et al. 2015, Boudissa, Mirilà et al. 2019). An enhanced ozonation was confirmed by the pronounced decrease in the relative absorbance for the 275 nm and 315 nm bands below the unity for Cu(II)Mt and Fe(II)Mt (**Fig. 3.5**).

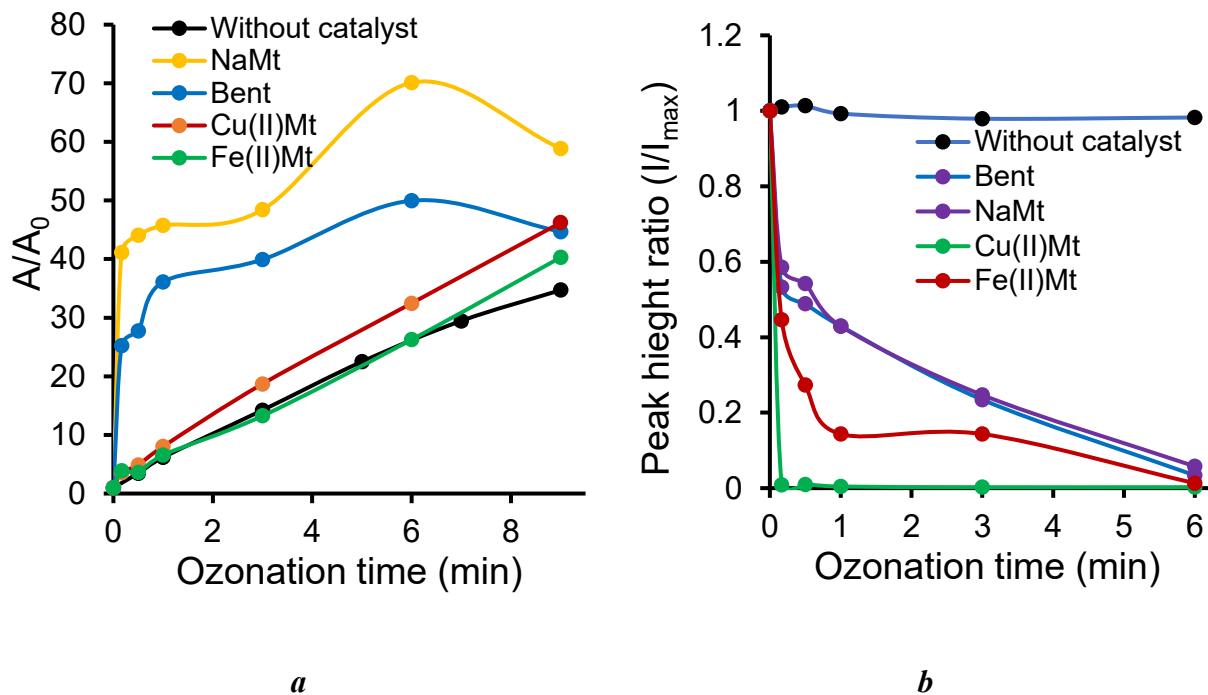


Figure 3.4. Effect of catalyst addition on the relative absorbance at 200 nm (a) and LC-MS peak ratio of NOF during ozonation (b). $T = 25\text{ }^{\circ}\text{C}$. $\text{pH} = 5.28$. Quartz cell = 1 cm. O_3 throughput: 600 mg h^{-1} . Sample volume = 25 mL. NOF initial concentration: $3.31 \times 10^{-6}\text{ M}$. Catalyst amount = 50 mg (2 g/L). The relative peak height was reported the instant/Initial height ratio as measured through LC-MS for assessing the conversion grade of NOF (%) through the equation $(1 - I/I_{\max}) \times 100\%$.

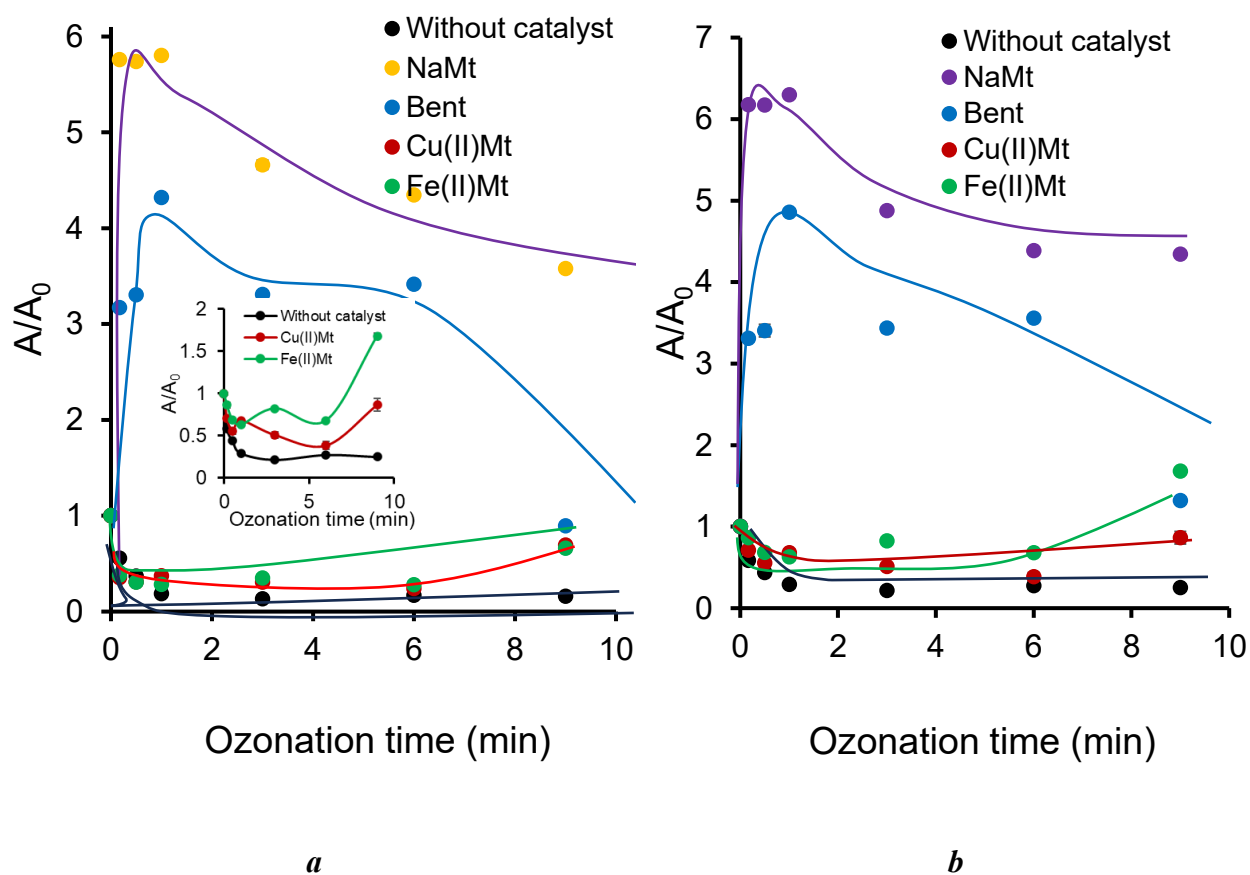


Figure 3.5. Evolution of the relative absorbance of the 275 nm with close-up on the A/A_0 value range 0-2 (a) and 315 nm (b) bands during clay-catalyzed ozonation. $T = 25\text{ }^{\circ}\text{C}$. Initial $\text{pH} = 5.28$. Quartz cell = 1 cm. O_3 throughput: 600 mg h^{-1} . Sample volume = 25 mL. NOF initial concentration: $3.31 \times 10^{-6}\text{ M}$. Catalyst amount = 50 mg (2 g/L).

Indeed, the I/I_{max} decrease accounts for almost total consumption of NOF after only 10-30 s of Fe(II)Mt-catalyzed ozonation, ca. 95-99 % degradation after 6 min process in the presence of the other clay catalysts and less 1-2 % after 6 min of non-catalytic ozonation. This clear improvement of the ozone efficiency in the presence of clay catalysts was already attributed to additional contributions of pathways involving adsorbed ozone, NOF and/or intermediates to those occurring through the direct action of undissolved gaseous at the bubble-liquid interface and dissolved molecules within the bulk solution (Azzouz, Kotbi et al. 2010, Azzouz 2012, Azzouz, Mirila et al. 2019). Therefore, it appears that even a weakly effective non-catalytic ozonation takes place via

degradation reactions on the very structure of the phenyl and pyridinyl groups with possible ring cleavages. Their preponderance must vary according to the selectivity of each clay catalyst, thereby strongly influencing the product distribution in the ozonation mixture (**Fig. S6-S7**).

Understanding the effect of the clay catalysts Cu(II)Mt and Fe(II)Mt on NOF ozonation is essential for evaluating its chemical behavior in aquatic environment. The obtained results showed that Cu(II)Mt and Fe(II)Mt are more effective catalysts in NOF ozonation than NaMt and bentonite. Given the unavoidable contribution of adsorption in the ozonation process, this could be due at least partly to a beneficial effect of their high dispersion in the aqueous NOF solution reflected by smaller particle sizes of 2133 and 2409 nm, respectively versus 2614 and 5759 nm for NaMt and bentonite (**Table S2**). However, the redox Cu(II)/Cu(I) and Fe(III)/Fe(II) cycling should have a much greater contribution in the enhancement of the oxidative degradation of NOF.

3.3.4 Effect of clay-catalyzed ozonation on toxicity

The LC-MS analysis revealed that early stage of NOF ozonation result in the formation of a variety of intermediates, with product distribution strongly depending on the type of catalytic system used. Interestingly, NOF ozonation during 9 min in the presence of NaMt and Fe(II)Mt induced weaker decays of the frond number (from 100 % to 37-38 % with NaMt, and 60 % with Fe(II)Mt) and fresh weight (down to 59 % and 62 %, respectively) as compared to other ozonation process (**Fig. 3.6a**).

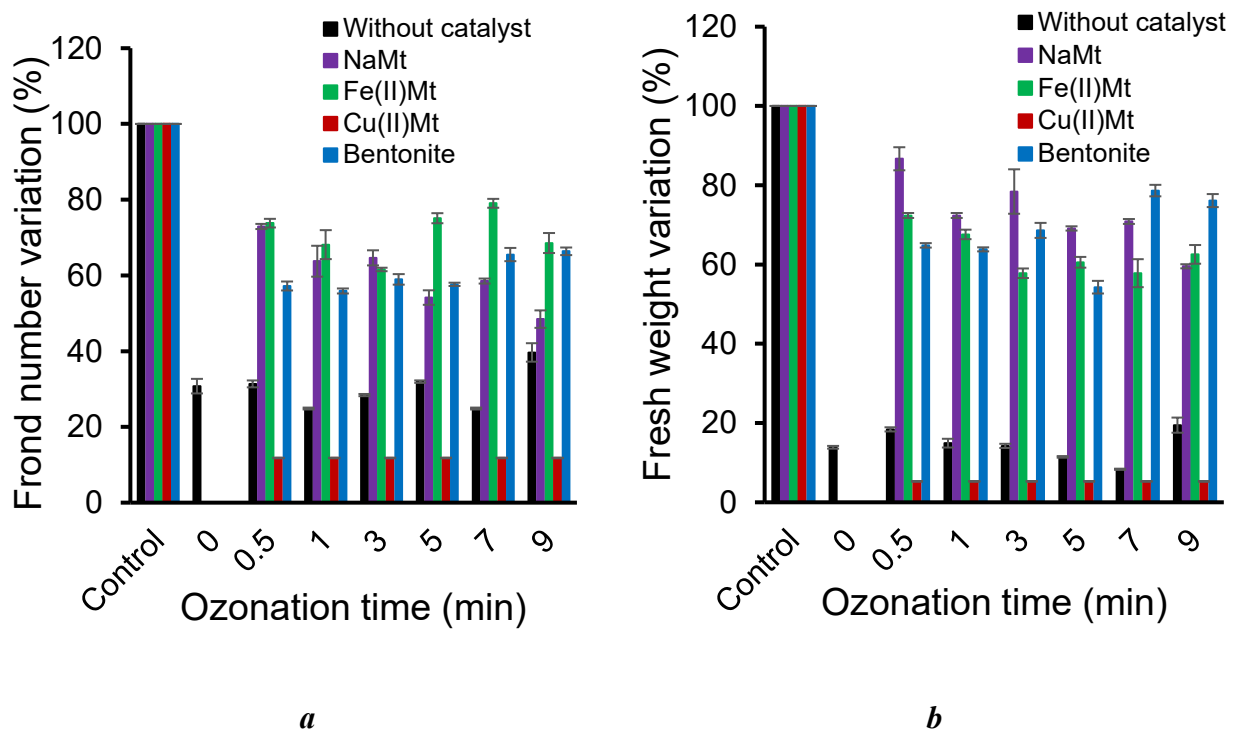


Figure 3.6. Effect of the ozonation times in the presence of different catalysts (NaMt, bentonite, Cu(II)Mt, Fe(II)Mt) on the toxicity of the reaction mixture expressed in terms of frond number (a) and fresh weight of *Lemna minor* (b) as reported to the blank sample (Control). The results are expressed as percentages relative to the control sample (antibiotic-free SIS medium). These data account for the mean of triplicate measurements after plant exposure for 7 days including the control sample and to ozonized reaction mixtures. T = 25 °C. Initial pH = 5.28. Quartz cell width = 1 cm. O₃ throughput: 600 mg h⁻¹. Sample volume = 25 mL. NOF initial concentration: 3.31×10⁻⁶ M. Catalyst amount = 50 mg (2 g/L).

These weak influences must be due to the harmless character of NaMt and the beneficial effect of Fe²⁺ cations that are supposed to act as nutrient for *L. minor* (Rout and Sahoo 2015, Schmidt, Thomine and Buckhout 2020). This effect seems to be attenuated in time by the ozonation progress more particularly for the frond number in NaMt-catalyzed ozonation and for the fresh weight in the process catalyzed by both NaMt and Fe(II)Mt. A possible explanation should consist in the rise of more toxic intermediates and in an unavoidable pH decrease.

Stronger toxicity with 2-3 fold more pronounced depletion of the frond number of ca. 18-23 % was registered for ozonation without catalyst and of approximately 9 % in the presence of Cu(II)Mt. Similar effect was noticed for the fresh weight (19 % and 5 %, respectively) (**Fig. 3.6b**) and chlorophyll *a/b* ratio (**Fig. 3.7a**). These changes in the chlorophyll *a/b* ratio reflect the impact of ozonation process on the photosynthetic efficiency of *Lemna minor*. The more significant decrease in the chlorophyll *a/b* ratio for ozonation with Cu(II)Mt and without catalyst indicates that these processes produce intermediates that are more disruptive to the photosynthetic system of the plant. This result is of great importance, because it provides clear evidence that at least some early NOF derivatives are more harmful for *L. minor* than the parent molecule, which was already found to exhibit noticeable toxicity for high concentrations (**Fig. 3.1a**).

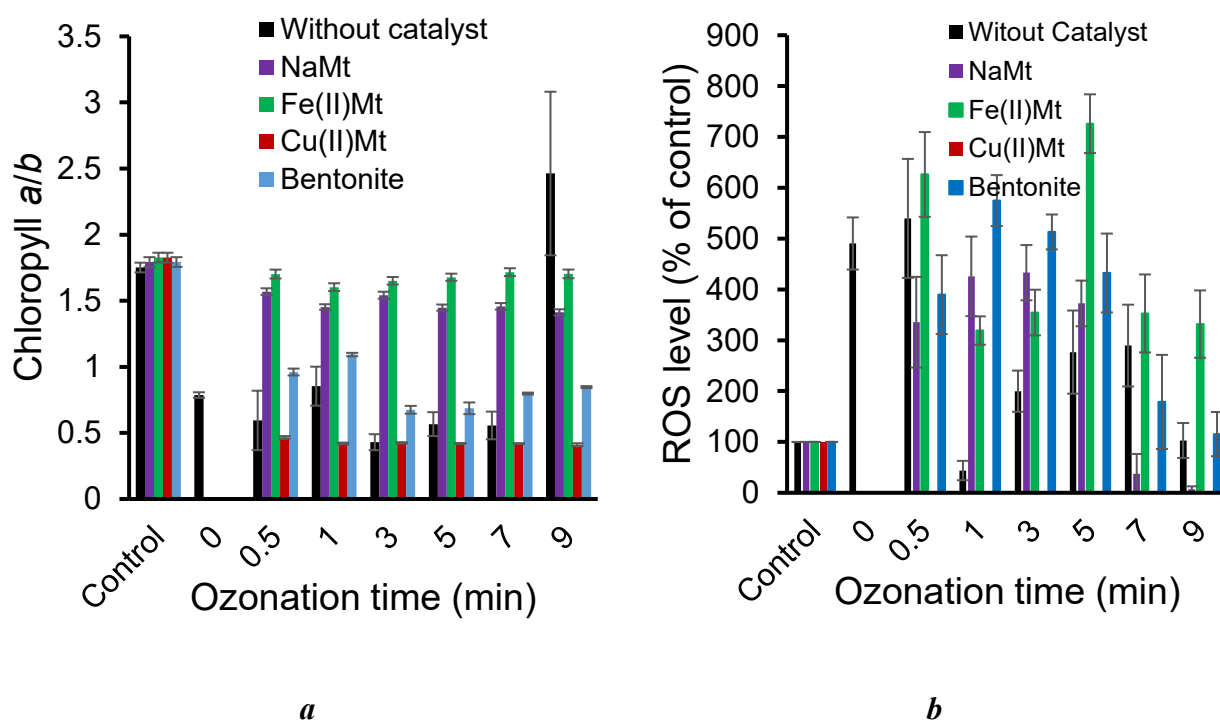


Figure 3.7. Evolution of the toxicity of the reaction mixture through changes in the chlorophyll *a/b* ratio (a) and ROS amount (b) during clay-catalyzed ozonation. These data account for the mean of triplicate measurements after plant exposure for 7 days including the control sample (*Lemna minor* in SIS medium without NOF ozonized) and to ozonized reaction mixtures, T = 25 °C. Initial pH = 5.28. Quartz cell = 1 cm. O₃ throughput: 600 mg h⁻¹. Sample volume = 25 mL. NOF initial concentration: 3.31×10⁻⁶ M. Catalyst amount = 50 mg (2 g/L).

Unlike the non-catalytic ozonation, addition of clay catalyst resulted in a pronounced production of ROS that varies in time according to the catalyst (**Fig. 3.7b**). ROS amount fluctuations during ozonation can be attributed to change in NOF derivatives distribution. The fact that the ROS totally disappeared during Cu(II)Mt-catalyzed ozonation must be due to total alteration and depletion of *L. minor*. Fe(II)Mt and to a lesser extent NaMt gave various ozonized mixtures in time with increased ROS production beyond the natural level (control), seemingly without leading to the plant death at within this time range not exceeding 9 min.

The obtained results clearly showed that the exchangeable cation of the clay catalyst significantly influences the toxicity of the ozonated NOF solutions: NaMt and Fe(II)Mt resulted in less toxic intermediates, while Cu(II)Mt and non-catalytic ozonation produced more harmful NOF derivatives. This was confirmed by the changes in the tested biological parameters in *Lemna minor* (frond number, fresh weight, Chl *a/b*, and ROS production) reflected by different levels of toxicity. This suggest that various catalysts promote different degradation pathways and intermediate distributions.

3.3.5 Potential ozonation pathways

As previously stated, the intensity increase for the 200 nm band at the early stages of ozonation (**Fig. 3.3a**) is a precise indicator of the formation of hydroxylated NOF intermediates and rise of H-bridge interactions in the aqueous media (Ling, Ben et al. 2018). Such derivatives were identified by LC-MS analysis in the early step of ozonation in both the absence and presence of clay-catalysts through *m/z* values of 334, 336 and 348 (**Table S5**). The already assumed occurrence of a structure alteration of at least on the phenyl ring moieties was supported by the preponderance of NOF intermediates with *m/z* values of 322, 296 and 294. Such processes also appear to take place in clay-catalyzed ozonation, as supported by similar main intermediates. Most of these derivatives identified by LC-MS during ozonation with and without catalyst were already reported for diverse other oxidative processes (Hubicka, Źmudzki et al. 2013, Carneiro, Aquino et al. 2020, Wu, Li et al. 2020, Chen and Wang 2021, Lu, Li et al. 2021, Zhao, Wang and Wang 2022, Wang, Gao et al. 2023, Wang, Zhao et al. 2023, Zhao, Sun et al. 2023, Liu, Wang et al. 2024). Nonetheless, the intermediate distribution appeared to strongly vary after catalyst addition and according to the catalyst selectivity (Wu, Li et al. 2020, Chen and Wang 2021, Liu, Wang et al. 2024). This suggests

that organic pollutant oxidation takes place specifically by resulting in specific intermediate distribution according to the composition of the natural aqueous media and their solid suspensions.

Deep analysis of LC-MS data revealed different chronological successions of NOF intermediates based on different successions of onsets and starting slopes in the evolution of the product distribution during ozonation in both the absence (**Fig. S6**) and presence of clay catalysts (**Fig. S7**). As a common feature, these successions suggest the simultaneous and similar occurrence of NOF hydroxylation and pyridinyl ring opening as the first steps of ozonation. (**Scheme 3.1-3.2**).

The first pathway branch (I) involves the formation of compound $m/z = 296$ via quinolone ring opening then the appearance of compound $m/z = 278$ after water molecule loss. The latter only appeared after 3 min and disappeared rapidly (Zhao, Sun et al. 2023). The second pathway branch (II) revealed intermediates $m/z = 336$ (a) formed via hydroxylation of NOF near the N-4 piperazine atom, which persists throughout the ozonation process. This was followed by the formation of compound $m/z = 322$ resulting from piperazine ring opening and formation of compound $m/z = 294$ by the elimination of an ethylene moiety (Tegze, Sági et al. 2019) and of compound $m/z = 251$ through deamination (Hubicka, Źmudzki et al. 2013). The amount increases of compound $m/z = 294$ must be due to its production via different pathways. Another intermediate detected with the same $m/z = 336$ (b) is probably an isomeric form which leads to the formation of compounds $m/z = 292$ and $m/z = 310$ by decarboxylation and opening of the two rings (Liu, Wang et al. 2024). On the other hand, hydroxylation of compound $m/z = 336$ (b) could giving rise to product $m/z = 354$ due to the intense attack by reactive radicals on the quinolone moieties on the C=C double bond (Wang, Gao et al. 2023). Product $m/z = 336$ (a) could also generate $m/z = 348$ through oxidation to oxo-form (Wang, Liu et al. 2022). Intermediate $m/z = 334$ and $m/z = 306$ (Methyl loss) could directly arise from NOF (Wang, Cheng et al. 2020, Zhao, Sun et al. 2023, Leite, Colombo et al. 2024). Compound $m/z = 362$ may originate from N-acetylated norfloxacin (Kim, Heinze et al. 2011). Therefore, the Scheme 1 represent an overview of the complex degradation pathways of NOF during non-catalytic ozonation, suggesting the formation of various intermediates through different reaction mechanisms, including hydroxylation, ring-opening, and decarboxylation.

The evolution in time of each NOF derivative was found to vary according to each clay catalyst (**Fig. S5**). For instance, bentonite and to a lesser extent NaMt (**Fig. S6 a-b**) generated much higher numbers of intermediates through ozonation as compared to Fe(II)Mt and Cu(II)Mt, which showed stronger catalytic activities. One of these intermediates ($m/z = 318$) is known to be quite unstable (Wang, Cheng et al. 2020), appearing only in the presence of bentonite after 3 minutes of ozonation and then disappears.

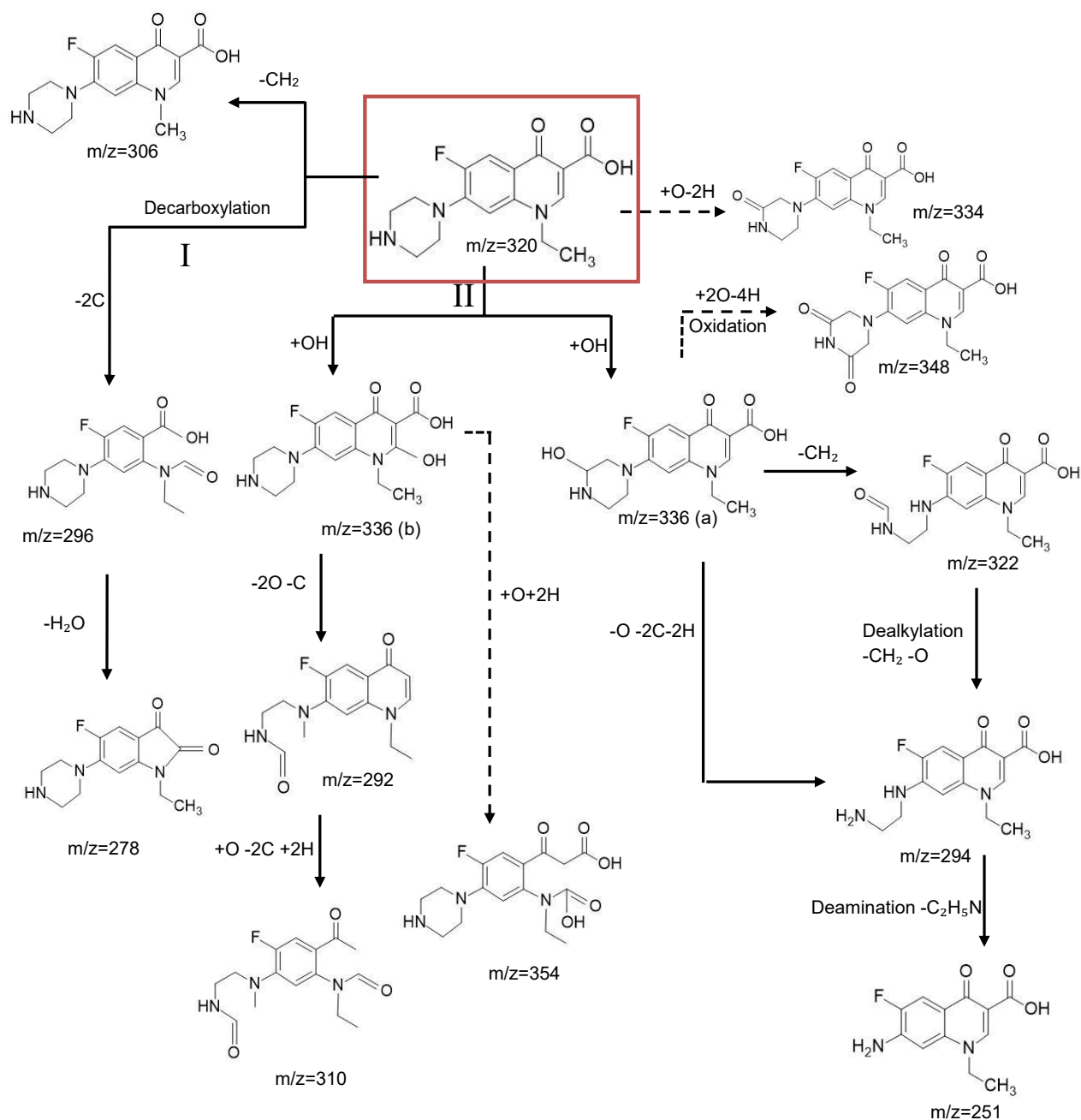
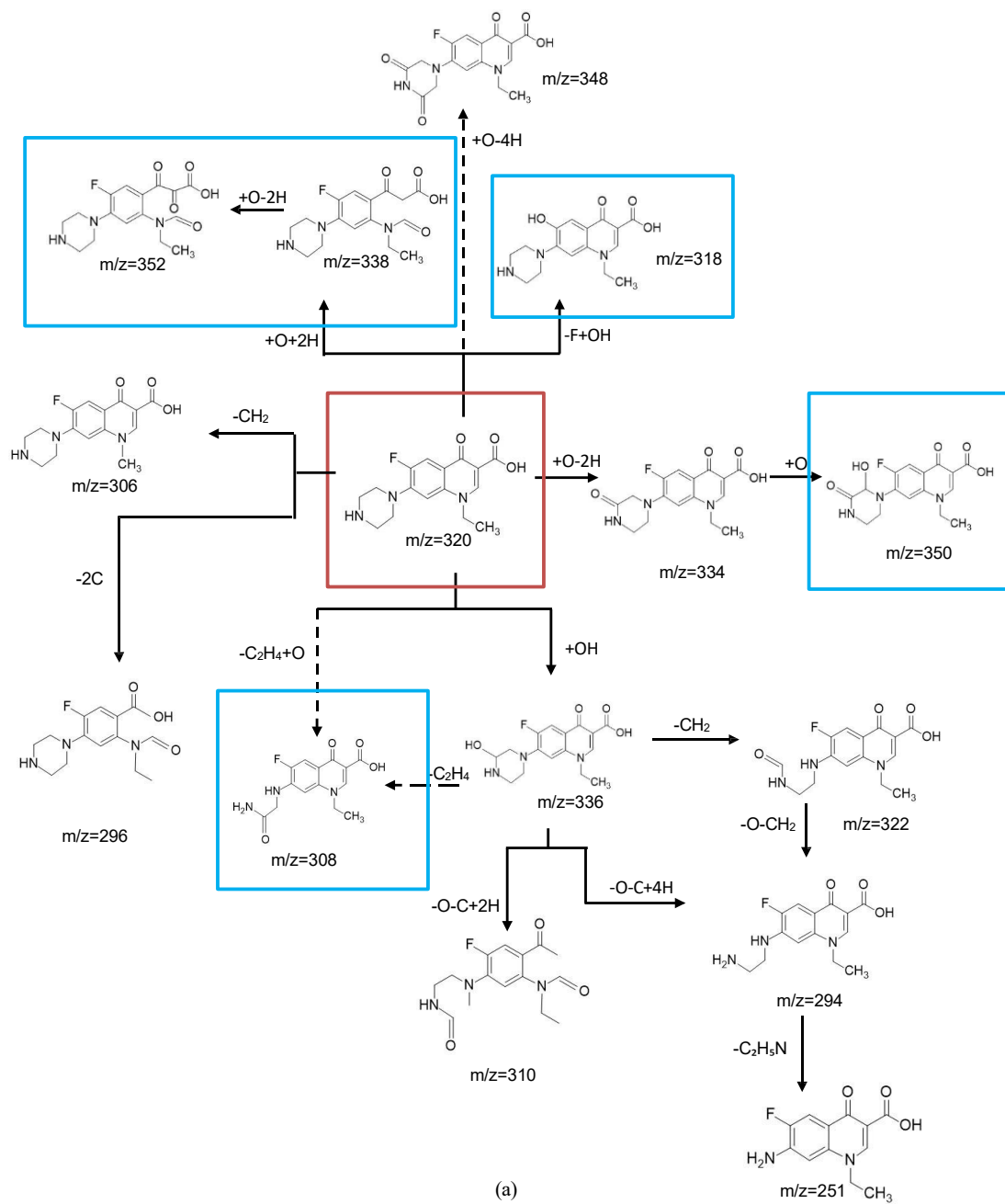
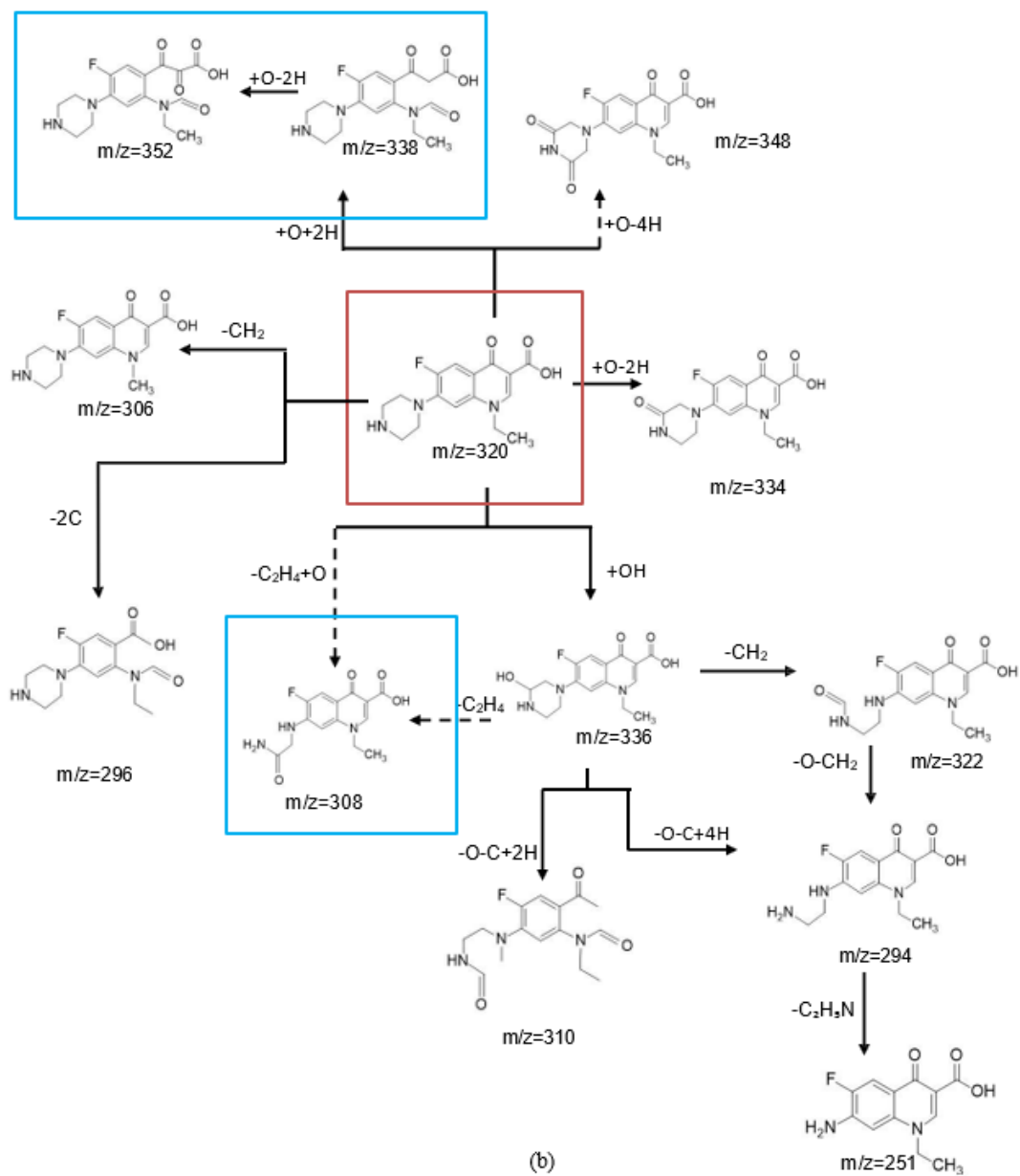


Schéma 3.1. Proposed pathway for Norfloxacin (NOF) degradation upon non-catalytic ozonation. The degradation process involves multiple steps, including hydroxylation, ring-opening, and decarboxylation. All intermediates were identified by LC-MS. The red rectangle represents NOF molecule with $m/z = 320$. The dashed arrows are based on LC-MS measurements that suggests hypothetical parallel degradation pathways with unknown starting compounds.



(a)



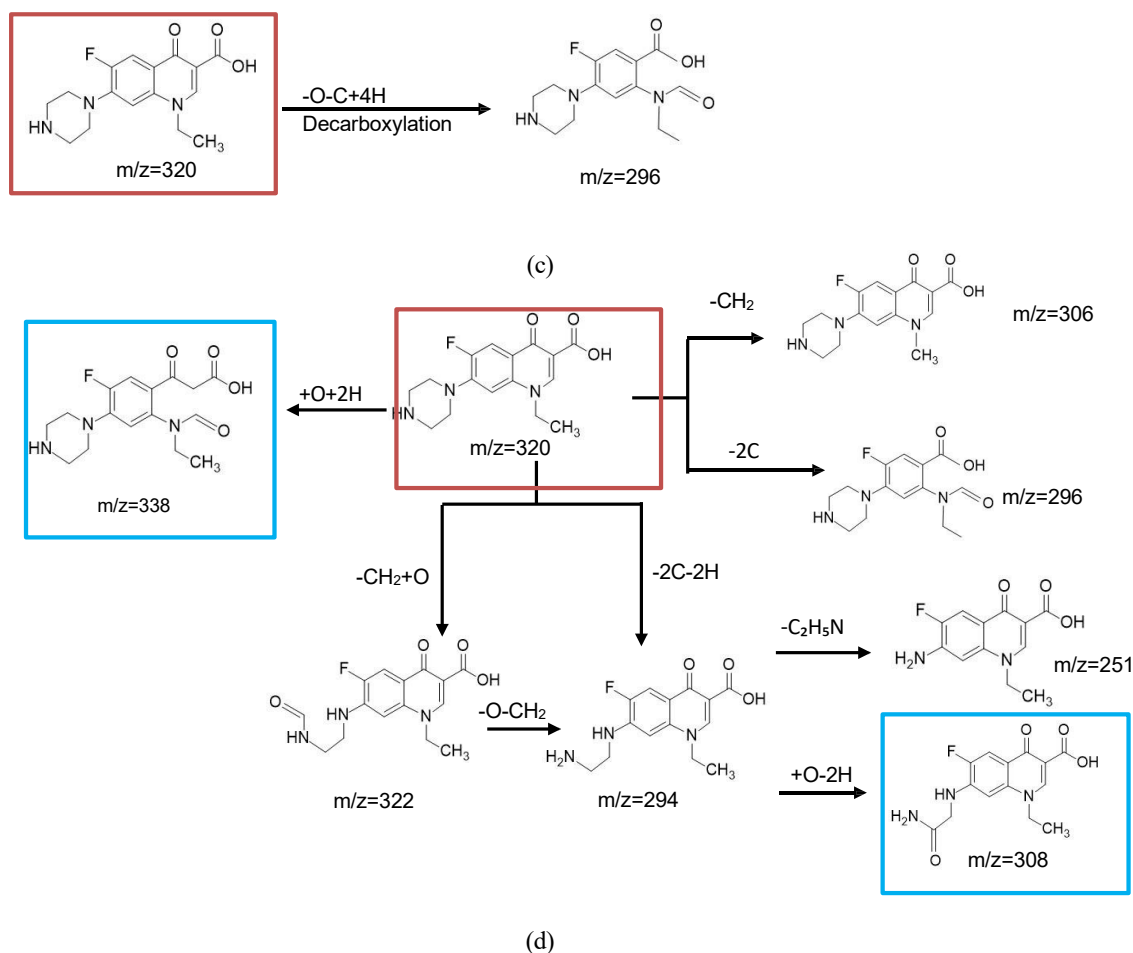


Schéma 3.2. Proposed pathway for Norfloxacin (NOF) degradation upon clay-catalyzed ozonation: Bentonite (a), NaMt (b), Fe(II)Mt (c) and Cu(II)Mt (d). All intermediates were identified by LC-MS. The red rectangle represents NOF molecule with $m/z = 320$. The dashed arrows are based on LC-MS measurements that suggests hypothetical parallel degradation pathways with unknown starting compounds. The blue rectangles highlight new intermediates detected only in clay-catalyzed ozonation, not observed in non-catalytic ozonation.

As expected, Fe(II)Mt and Cu(II)Mt-catalyzed ozonation gave rise to lower amounts of hydroxylated intermediates as compared to bentonite and NaMt, which is in agreement with the lower A/Ao of the 200 nm band (**Fig. 3.4a**). This was already explained in terms of strong catalytic activity of Fe(II)Mt and Cu(II)Mt, as illustrated by LC-MS analysis that showed a marked NOF depletion during ozonation. This result provides confirmation that clay catalysts exhibit specific selectivity that governs the oxidation of organic molecules (Wu, Li et al. 2020, Chen and Wang 2021, Liu, Wang et al. 2024). Overall, the Scheme 2 provides a detailed overview of the complex

degradation pathways of NOF in clay-catalyzed ozonation by highlighting the formation of unique products not observed in non-catalytic ozonation (see the blue rectangles).

These ozonation pathways are not only explained by the identification of the generated intermediates but also correlated with similar derivatives identified by reported works and both the reaction schemes and radical mechanisms proposed by the literature. This strategy along with the evolution in time of the derivative distribution agrees with this literature, which allows proposing process pathways by analogy. Given the objectives of the present work, these schemes are probably not sufficiently accurate but fairly useful to understand, at least partly, the specific contribution of clay catalysts in modulating the antibiotic oxidative degradation and the resulting toxicity.

3.3.6 Correlation between fluctuations in derivative distribution and toxicity

Attempts to plot each toxicity criterion as a function of each NOF derivative distribution revealed no clear correlations for some NOF derivatives. Nevertheless, visible decreases in the frond number were noticed with increasing proportions of compounds $m/z = 362$, $m/z = 348$ and $m/z = 336$ and its isomeric form along with $m/z=322$ if one excludes the Cu(II)Mt data (**Fig. S8**). This result demonstrates a direct proportionality between these compound contents in the ozonation reaction mixture and their toxicity. NOF derivatives were already found to display higher toxicity towards aquatic species regardless to the oxidizing agents (Médice, Afonso et al. 2021). Pesticide hydroxylated and dealkylated degradation products turned out to be even more harmful than their parent molecules (Benghaffour, Azzouz and Dewez 2023). Other attempts to plot all the toxicity criteria with NOF conversion grade also resulted in no clear correlation, most likely due the presence of a wide variety of compounds displaying diverse toxicity. Slight increase tendencies in the frond number and fresh weight were observed upon increasing NOF conversion, i.e. decreasing residual NOF amount (**Fig. S9**). Such a correlation remains valid if one excludes the contribution of Cu(II)Mt and Fe(II)Mt ozonation processes, where previous data already showed strong catalytic activities (**Fig. 3.4**). This result agrees with the intrinsic toxicity of non-ozonized NOF (**Fig. 3.1**).

As already reported, compound $m/z = 296$ showed higher toxicity to green algae than the parent molecule (Chen and Wang 2021). Compound $m/z = 322$ was already found to be harmful (Chen and Chu 2015) while compounds $m/z = 294$ and $m/z = 318$ were found to exhibit toxicity to *fathead*

minnow with LC50 of 2.24 mg/L (Zhao, Wang and Wang 2022). The mere fact that NOF ozonation generates toxic species agrees with the toxicity persistence of antibiotics oxytetracycline and doxycycline during ozonation, resulting in harmful oxidation products towards fish, daphnid and freshwater algae (Park, Pineda et al. 2023). This appears to be a common feature of most organic pollutants released in natural aqueous media, such as pesticides atrazine, diazinon and their intermediates (Lee, Depuydt et al. 2021).

3.3.7 Conclusion

The data obtained herein revealed that NOF ozonized mixtures exhibit significant toxicity on *L. minor* via an oxidative stress and an alteration of the chlorophyll content. The toxicity evolution in time appears to strongly correlates with the oxidation advancement which determines the product distribution. Catalyst addition induces specific enhancement of the ozonation process and changes in both the intermediates distribution and toxicity evolution according to the exchangeable cation. NaMt and Fe(II)Mt catalysts exhibited different effects on antibiotic ozonation and differential impacts on the toxicity parameters. Even less effective non-catalytic ozonation seems to occur through degradation reactions on the very structure of the phenyl and pyridinyl groups leading to ring cleavages and formation of intermediates potentially more toxic than the parent molecule. The predominance of these reactions varies with the selectivity of each clay catalyst, and significantly influences the product distribution in the ozonation mixture. It results that norfloxacin ozonation generates specific toxicity towards *L. minor* in specific clay-containing host media. These findings allow envisaging strategies for predicting how antibiotics or their degradation products impact the environment based on given clay-containing natural media under oxidation conditions. Deeper understanding of the fate of antibiotics in the environment is undoubtedly useful for designing effective nature-inspired water treatments with judiciously tailored catalytic systems that favor total mineralization of the organic compound or at least the formation of less persistent and potentially less toxic intermediates.

CHAPITRE 4

Reciprocal effects of acidity evolution on metal cations and clay catalyst dispersion in Norfloxacin oxidative degradation and toxicity in aqueous media

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Development of the methodology and execution of the experimental work, including adsorption, ozonation and catalytic ozonation studies as well as ecotoxicological assays. Processing and analysis of experimental data, interpretation of results and drafting of the manuscript. Investigation and preparation of the original manuscript draft.

David Dewez

Revision and optimization of experimental and ecotoxicological protocols. Verification of preliminary experiments, review and validation of ecotoxicological data, contribution to data interpretation and correction of the final manuscript.

Abdelkrim Azzouz

Conceptualization and design of the research project on the catalytic ozonation of organic pollutants. Supervision of experimental work, validation and interpretation of experimental results, supervision of the research work, and participation in manuscript writing, reviewing and editing.

Abstract

The ozonation of norfloxacin (NOF), a widely used fluoroquinolone antibiotic, in the presence of Na^+ , Fe^{2+} , Cu^{2+} , Ni^{2+} , and Co^{2+} cations and their montmorillonite-supported counterparts was investigated. The NOF degradation and the toxicity of the ozonized mixtures towards an aquatic organism (*Lemna minor*) were evaluated in terms of changes in its frond number, chlorophyll content, photosynthesis efficacy, and production of reactive oxygen species (ROS). The evolution over time of the NOF degradation grade and the toxicity were discussed in terms of *i.* the observed changes in the interactions of the cation and clay catalyst with NOF molecules; *ii.* the pH decay, during ozonation. Ion-exchange and Lewis acid–base interactions appear to govern NOF adsorption and clay catalyst dispersion in correlation with the progressive formation of acidic species in the aqueous media. These findings reveal promising prospects for tailoring optimum oxidative water treatments with minimum toxicity and for predicting their environmental impacts on aquatic media.

Keywords: metal cations; norfloxacin; montmorillonite; ozonation; *Lemna minor*; toxicity

4.1 Introduction

Antibiotic overuse and release in nature, even at low concentrations, are now recognized as pollution sources with negative impacts on biodiversity, particularly in aquatic environments (Ma, Gao et al. 2024). Antibiotics can not only accumulate in ecosystems, giving rise to antibiotic-resistant bacteria, but also undergo unavoidable decomposition into persistent derivatives that are harmful to aquatic life (Salma, Thorøe-Boveleth et al. 2016, Arora, Thangavelu and Karanikolos 2020). Fluoroquinolones (FQs) are the third-largest class of antibiotics and are commonly employed for both human and animal infection issues, and they have been ubiquitously detected in aquatic environments at various concentrations. In surface waters, ciprofloxacin and norfloxacin are frequently reported at levels ranging from ng/L to $\mu\text{g/L}$ (Herrero-Villar, Mateo-Tomás et al. 2022, Fang, Chen et al. 2023, Ariyani, Jansen et al. 2024).

In aquatic ecosystems, plants and algae are vital to biodiversity equilibrium contributing through photosynthesis and oxygen production, nutrient cycling and as food sources. They can also act as

bioindicators for monitoring water quality due to their sensitivity and rapid response to pollutants. In plant metabolism, FQs were found to trigger the production of reactive oxygen species (ROS) that inhibit *C. ovalisporum* biomass growth and reduce the chlorophyll *a* content and photosynthetic capacity (Chen, Zhang et al. 2020). The resulting Chl triplet state can react with $^3\text{O}_2$ yielding additional ROS inhibiting the chlorophyll synthesis (Sharma, Jha et al. 2012). FQs quinolone ring structure gives them appreciable chemical resistance to hydrolysis and high thermal resistance (Tian, Wang et al. 2024). Norfloxacin (NOF) is the most frequently detected FQs in wastewaters, groundwaters and even in drinking water supplies (Wang, Li et al. 2019). NOF chemical structure (**Scheme. S1**) includes an electron-rich piperazine moiety, which displays reactivity, more particularly towards ozone and other oxidizing agents (Mutke, Swiderski et al. 2024).

Among many oxidative removal routes of organic pollutants (Wen, Chen et al. 2023, Li, Zhang et al. 2024, Tian, Wang et al. 2024), ozonation in clay suspensions appears as the most suitable route for achieving a nature-inspired oxidative technique which can mimic the catalytic activity of clay-rich media (Benghaffour, Azzouz and Dewez 2023). The latter are supposed to govern not only the ionic force and pH but also the complex [clay:substrate:water:cation] interactions and subsequently the very kinetics of the oxidative process (Novikau and Lujanienė 2022). For instance, maximum adsorption of ciprofloxacin (CIP) onto Montmorillonite K-10 and kaolin was observed at pH 7.4, presumably due to may promote strong electrostatic interactions of the zwitterionic form of CIP with the clay surfaces (Hamilton, Hutcheon et al. 2014). Maximum adsorption involving π - π stacking interactions and H-bridges between NOF zwitterions and polydopamine microspheres was also reported at optimal pH 6.6 (Wan, Liu et al. 2018).

Montmorillonite (Mt) is a 2:1 clay mineral with aluminosilicate lamellae that can reciprocally interact and exhibit various interactions with species dispersed in aqueous media according to the pH level. Each lamella has two tetrahedral silica layers that sandwich an octahedral one, where Mg^{2+} substitutes for Al^{3+} generating a permanent negative charge balanced by exchangeable cations (Lin, Jiang and Zhan 2018, Mishra, Mehta and Basu 2018). The lamella structure is also expected to bear both in-plane and out-of-plane silanols, respectively located between exchangeable cations and in surface edges (Pei and Zhang 2021). Both silanols induce additional pH-dependent charges and hydrophilic character unlike silica-rich islands that favor hydrophobic

interactions with organic molecules through their siloxy groups. Besides, the lattice oxygen atoms act as Lewis bases through their free electron pairs that promote Lewis acid–base interactions with metals cations and H-bridges with water and OH-functionalized molecules (El Baktaoui, Hadj-Abdelkader et al. 2022). All these interactions are known to govern *i.* the clay sheet dispersion, interlayer water content and exchangeable cation mobility, *ii.* modulate the accessible surface *i.* and favor adsorption and subsequently catalytic activity, as already reported for clay-ozonation of organic pollutants (Shahidi, Roy and Azzouz 2014, Shahidi, Roy and Azzouz 2015, Boudissa, Mirilà et al. 2019, Boudissa, Zekkari et al. 2020, Wen, Chen et al. 2023).

Adsorption plays a crucial role in heterogeneous catalysis, more particularly when metals are involved. Except alkalis, alkaline earths along with iron in natural amounts, most metals are harmful for biodiversity beyond certain tolerated doses. Industrial and mining activities usually release cationic forms of heavy metals such as Pb, Hg, Cd, Cr, Ni and/or As which are highly toxic to living organisms (Sasmaz, Dogan and Sasmaz 2016). Other metals (Co, Fe, Cu, Mn, and Zn) are essential micronutrients for plants and animals at optimum concentrations, but can become toxic beyond certain levels (Van Dyck, Vanhoudt et al. 2023). When released in wastewaters, metals can interact with organic pollutants, thereby influencing their degradation and toxicity evolution. For instance, Fe^{2+} cation is known to act as catalyst in oxidative degradation of organic molecules (Boudissa, Arus et al. 2023). Besides, addition of $\text{Fe}_3\text{O}_4\text{--MnO}_2$ significantly was found to enhance bisphenol A degradation efficiency, more particularly in the presence of hydroxyl radicals ($\text{HO}\cdot$) (Zhang, He et al. 2020).

Nonetheless, advanced degradation of organic molecules is often accompanied by toxicity changes due to the production of intermediates that could be more or less harmful than the parent molecule and that specifically interact with cations and adsorbent surface, if any. In other words, changes in product distribution unavoidably results in toxicity evolution during the degradation process in clay-containing media. In addition, metal cations unavoidably interact with π electron-rich groups (carboxylic, carbonyl and/or piperazinyl groups) of the molecules, thereby modifying their chemical properties and reciprocally reducing their bioavailability to living organisms and consequently their respective toxicity (Serafin and Stańczak 2009, Chen, He et al. 2024, Li, Jiang and Wei 2024, Cao, Zhang et al. 2025).

The use of Fe^{2+} , Zn^{2+} , Cu^{2+} , Ce^{3+} , and Co^{2+} cations in oxidative degradation techniques must take into account the potential risk of metal leaching and of secondary contamination of the environment (Zhang, Li et al. 2023). Cu^{2+} cation was already found to inhibit the Na^+/K^+ -ATPase enzyme in fish metabolism, resulting in reduced intracellular sodium absorption, cardiovascular collapse and respiratory deficiency (Macoustra, Jolley et al. 2020, Liao, Zhu et al. 2023). Reportedly, high thallium (Tl^+) concentrations induce an increase of oxidative stress level and alteration of photosynthesis activity of algal *Microcystis aeruginosa* (Tsai 2024). Hexavalent chromium at certain pHs showed highest toxicity with increased oxidative stress on plants *L. minor*, reducing the contents in sugar, chlorophylls and carotenoids (Aslanzadeh, Saboori and Moradlou 2024). Exposure to Co^{2+} cation induced oxidative damage to *L. minor* with a deactivation of superoxide dismutase SOD (Begović, Mlinarić et al. 2016). The presence of Zn^{2+} in plants may lead to a substitution of the central Mg^{2+} ion in chlorophyll, thus altering their photosynthetic core and system (Tarrahi, Khataee et al. 2018). *L. minor* exposure to Ag^+ affects the growth rate and protection system that controls the oxidative stress (Pereira, Jesus et al. 2018). The toxicity of CuO is mainly due to Cu^{2+} cation release in media contacting the plant roots (Song, Hou et al. 2016, Yue, Zhao et al. 2018). Nickel appears to inhibit the photosystem and induction of oxidative stress of microalgae *Phaeodactylum tricornutum* (Guo, Lu et al. 2022). However, current toxicity risk assessments often overlook mixture interactions, underestimating true ecological impacts. A comprehensive understanding of the combined toxicity effect of metals with NOF derivatives from oxidative degradation on aquatic organisms is useful in this regard.

This research targets knowledge advancement that provides foundations for designing practical applications involving nature-inspired and clay-catalyzed oxidative water treatments. This is useful for a better understanding of the role of clay-containing media in nature self-regeneration. A previous study demonstrated the proof of concept that clay minerals exhibit specific catalytic activity and selectivity in the oxidative degradation of antibiotics, and that the distribution of the products governs the toxicity evolution. The aim of the present study resides in correlating the role of metal cations to the catalytic activity and selectivity in NOF oxidative degradation by ozonation and demonstrating that the process evolution modifies the dispersion, activity, and selectivity of the clay catalyst behavior in aqueous suspensions and, consequently, the toxicity of the resulting mixture on aquatic ecosystems. For this purpose, *Lemna minor*, a macrophyte with rapid growth

and easy cultivation (Ebert, Bachmann et al. 2011), was used as a reliable bioindicator of environmental pollution (Horvat, Vidaković-Cifrek et al. 2007, Benghaffour, Azzouz and Dewez 2023). Different criteria, such as the frond number (FN), chlorophyll content, and ratio, along with the ROS level, should provide valuable data regarding the decay in plant growth and photosynthetic efficiency (de Morais, Barbosa-Neto et al. 2019, Elbasan, Arian-Abdulveli et al. 2024, Guzman-Tordecilla, Pacheco-Bustos et al. 2024, Kumar, Sarkar et al. 2024).

4.2 Experimental

4.2.1 Clay material's preparation and dispersion in aqueous suspension

A montmorillonite-rich sample (NaMt) (cation exchange capacity = 99 ± 3 meq/100 g; specific surface area = $34 \text{ m}^2/\text{g}$, silica/alumina weight ratio = 1.58; montmorillonite content = 84 wt%) was prepared from a crude bentonite supplied by Sigma Aldrich (Burlington, MA, USA) according to a procedure described previously (Foka Wembe, Benghafour et al. 2023, Djidja, Dewez and Azzouz 2025). Other ion-exchanged counterparts (Fe(II)Mt, Ni(II)Mt, Co(II)Mt, and Cu(II)Mt) resulted from the ion exchange of 9 g of dry NaMt powder in 6 g/L of each corresponding aqueous solution of metal salts,

$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 7\text{H}_2\text{O}$, supplied by Fisher Scientific, Pittsburgh, PA, USA (99% purity), and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (99.995% purity, Sigma Aldrich, Burlington, MA, USA), in Nanopure water. The mixture was stirred at 80°C for 7 h and cooled overnight at room temperature (RT). The resulting suspension was repeatedly washed with distilled water, centrifuged, dried at RT and characterized by X-ray diffraction (D8 Advance Brücker, Madison, WI, USA, $\text{CuK}\alpha$ at $1.54051 \text{ \AA}$). All the clay catalysts showed sharp 001 X-ray diffraction lines (Figure S1) and perfectly parallel clay sheet layouts. Na^+ exchange induced a shift of the 001 line towards lower 2-Theta due to an enlargement of the interlayer space (Benghaffour, Foka-Wembe et al. 2022, Djidja, Dewez and Azzouz 2025). Additional characterization by Fourier Transform Infrared spectroscopy (Thermo Scientific, Nicolet 6700 instrument, Madison, WI, USA) revealed characteristic vibration bands at 3626 cm^{-1} (Al–OH stretching), 985 cm^{-1} (Si–O stretching), 908 cm^{-1} (Al–OH bending), and 796 cm^{-1} (Si–O stretching), as others already reported (Benghaffour, Dewez and Azzouz 2023, Djidja, Dewez and Azzouz 2025).

The clay material dispersion in aqueous media was evaluated through the measurement of the particle size (PS) and zeta potential (ZP), which provides valuable data on the pH's effect on the particle suspension (Lunardi, Gomes et al. 2021). This was achieved with 50 mg of clay powder, previously dispersed in 25 mL of Nanopure water or aqueous NOF solution, after each catalytic ozonation test. The two analyses were carried out using a Malvern Zetasizer device Ultra Red Label (Malvern Panalytical, Malvern, UK) at 25 °C, while the data acquisition was achieved with a Zeta potential analyzer, Ver.5.68 software, and plastic cuvettes (DTS1070, Malvern instruments LTd, Worcestershire, UK).

4.2.2 Adsorption tests

NOF powder (NOF, Lot No LRAD2292) purchased from Sigma Aldrich was dissolved in nanopure water at specific concentrations considering the solubility of NOF at ambient conditions (3.31×10^{-6} M). Adsorption experiments were carried out by contacting 50 mg of dry clay mineral with 25 mL of NOF solution at intrinsic pH under vigorous stirring at RT in 50 mL erlenmeyer flask. The samples were analyzed immediately after filtration through 0.45 mm membrane. Insights in NOF adsorption kinetics were achieved through measurements of the instant and equilibrium amounts of adsorbate (q_t and q_e , respectively) for each sample. The pseudo-first and pseudo-second order models were then plotted as functions of time using Equations (1) and (2), respectively:

$$\text{Log}(q_e - q_t) = \text{Log}(q_e) - \frac{K_1}{2303} t \quad (1)$$

$$\frac{t}{q_t} = \frac{1}{K_2 \times q_e^2} + \frac{t}{q_e} \quad (2)$$

allowed calculating K_1 and K_2 , the rate constants of pseudo-order 1 and 2 respectively.

4.2.3 Ozonation tests

Ozonation tests were performed by ozone bubbling (600 mg/h) in a series of glass tubes (25 mm × 200 mm) containing 25 mL samples of NOF solution (3.31×10^{-6} M), either without a catalyst, in

the presence of 50 mg of a dry clay catalyst, or in the presence of Fe(II), Ni(II), Cu(II), and Co(II) cations at a 2.5×10^{-3} mg/L concentration to evaluate their effect at RT.

The concentration of dissolved ozone was determined using the iodometric method (Chasanah, Yulianto et al. 2019). Fully described procedures have been added in the Supporting Information File (SI). For this purpose, the A2Z-AQUA-6 portable ozone generator (A2Z Ozone Inc., Louisville, KY, USA) was pre-calibrated using another portable ozone generator (A2Z-MP 8000, Louisville, KY, USA) equipped with a built-in internal air compressor and buttons for adjusting the ozone concentration and bubbling time. This pre-calibration also confirmed the value of the gas ozone throughput of 600 mg/h provided by the original factory calibration and allowed us to determine the time evolution of the O₃ concentration in water. The results support the assumption of an pseudo-linear relationship between the bubbling time and the dissolved ozone concentration during the initial phase (10–15 min) (Benghaffour, Foka-Wembe et al. 2022). Regardless of the ozone concentration in the carrier gas or the operational conditions (600 mg/h versus 8000 mg/h), the dissolved ozone concentration remained limited to approximately 0.4 mmol/L, due to physicochemical constraints (Zekkari, Ouargli-Saker et al. 2022). This value is much lower than the reported 20.8 mmol/L in water at 0 °C (Battino, Rettich and Tominaga 1983) or 11.9 mmol/L in water at 20 °C (Shahidi, Roy and Azzouz 2015). Even under optimal conditions, the solubility of ozone in water remains limited (Roth and Sullivan 1981, Egorova, Voblikova et al. 2015). The resulting O₃/NOF molar ratio of ca. 121 ensured a sufficient excess of ozone after 10 min ozonation.

The possible release of metal cations upon clay mineral leaching in NOF solution and during ozonation was investigated through Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES) using an Agilent Technologies-5100 device (Santa Clara, CA, USA) with an axial plasma configuration and a concentric quartz nebulizer under specific conditions (Table S1). For the digestion method, 2 mL samples of the ozonized mixture were mixed into 2 mL of HNO₃ (Fisher Scientific, purity 70%), and the glass tubes were heated in a sand bath at 90 °C for 1 h. After digestion, each sample was acidified with HNO₃ to obtain a final concentration of 5% HNO₃ prior to the analysis.

After ozonation and catalyst removal by centrifugation and filtration, the supernatant was analyzed through UV–Vis spectrophotometry (Agilent-Cary 60 instrument, Santa Clara, CA, USA, 1 cm

quartz cell) and High-Performance Liquid Chromatography coupled to an ultraviolet-visible detector (HPLC-DAD), Agilent Technologies model 1290 equipment under specific conditions (Table S2). The ozonation process was investigated at either the initial intrinsic or at the initial pH of the reaction mixture, which was adjusted by adding a few drops of 0.1 M sodium hydroxide (NaOH) or hydrochloric acid (HCl). The pH was measured before and after ozonation using an Accumet[®] model 15 pH-meter (Fisher Scientific, Pittsburgh, PA, USA), with an accuracy of ± 0.01 units.

4.2.4 Toxicity tests on *Lemna minor*

Lemna minor used as a bioindicator was cultivated in a nourishing medium previously prepared according to the Swedish Institute for Standards (SIS) containing (mg/L): NaNO₃, 85; KH₂PO₄, 13.4; MgSO₄·7H₂O, 75; CaCl₂·H₂O, 36; Na₂CO₃, 20; H₃BO₃, 1; MnCl₂·4H₂O, 0.20; Na₂MoO₄·5H₂O, 0.010 ; ZnSO₄·7H₂O, 0.050 ; CuSO₄·5H₂O, 0.005 ; Co(NO₃)₂·6H₂O, 0.010; FeCl₃·6H₂O, 0.84 ; Na₂-EDTA·2H₂O, 1.4; buffer MOPS, 490. The medium pH was adjusted to 6.5 ± 0.2 , and the resulting SIS medium was prone to a sterilization at 121 °C under vacuum in a steam sterilizer model LSS 275 for 30 min. The stock culture of *L. minor* specimens was maintained under standardized growth conditions in cotton-sealed erlenmeyer flasks to reduce evaporation and prevent contamination. The plant cultivation was achieved under 16 h light exposure (light intensity of $100 \pm 10 \mu\text{mol of photons m}^{-2}\text{s}^{-1}$ with white fluorescent lamps) and 8 h dark cycles for 7 days at $24 \text{ °C} \pm 2 \text{ °C}$ and a relative humidity of $60 \pm 5\%$.

In a first step, the intrinsic toxicity of metals cations (Na⁺, Fe²⁺, Ni²⁺, Cu²⁺ and Co²⁺) towards *Lemna minor* was investigated by contacting for 7 days healthy green three-fronds specimens of *L. minor* in 50 mL erlenmeyers containing different cation concentrations (2.5×10^{-4} – 25 mg/L) prepared in nanopure water using the same metal salts as for clay catalyst preparation. These initial concentrations were selected covering the range of natural to highly contaminated conditions (Monroy, Maceda-Veiga and de Sostoa 2014, Elkhatat, Soliman et al. 2021). In a second step, the toxicities of the cations alone and adsorbed on montmorillonite were evaluated in the very supernatant of NOF solutions before and after ozonation. For this purpose, in addition to the toxicity criteria already investigated in previous work (Benghaffour, Dewez and Azzouz 2023, Djidja, Dewez and Azzouz 2025), such as the FN, FW, chlorophyll content and relative amount of

reactive oxygen species (ROS), the photosynthesis efficiency was measured. The methods used for assessing ROS and other parameters are described elsewhere (Jmii and Dewez 2021, Djidja, Dewez and Azzouz 2025). This provides an insight into the impact of metal cations on toxicity of oxidative NOF degradation. The number of fronds and fresh weight were estimated in each flask after 7 days of exposure and compared to control samples consisting of plants in SIS medium non exposed to ozonation mixtures. The growth rate inhibition was then calculated in relation to the control using Equation (3), where μ is the specific growth rate for the FN.

$$\% inhibition = 100 \times \left(1 - \frac{u_{sample}}{u_{control}}\right) \quad (3)$$

The photosynthetic efficiency of *L. minor* was determined by using the chlorophyll fluorimeter Handy-PEA (Plant Efficiency Analyser, Hansatech Instruments Ltd, UK), which is designed for the rapid and high-resolution measurement of chlorophyll fluorescence from the Photosystem II (PSII). The chlorophyll fluorescence kinetics provide key insights into PSII functionality under varying conditions. Prior to measurements, *L. minor* fronds were submitted to 30 min of dark adaptation to quench fluorescence and favor full oxidation of the electron transport chain. Further, the plants were exposed to a light pulse (intensity of 3000 μmol of photons $\text{m}^{-2}\text{s}^{-1}$, maximal emission at 650 nm) during one second.

From the different phases of the fluorescence kinetic, several parameters can be estimated reflecting the photosynthetic electron transport dynamics. However, this study was restricted to the Performance Index (PI_{ABS}), which is a holistic metric quantifying the efficiency of the photosynthetic biological system by integrating multiple functional aspects of PSII energy fluxes and offering a sensitive diagnostic tool for plant stress physiology (Strasser, Tsimilli-Michael and Srivastava 2004, Stirbet 2011, Jmii and Dewez 2021). The parameter PI_{ABS} was calculated as $\text{PI}_{\text{ABS}} = \text{RC}/\text{ABS} \times \phi\text{Po}/(1 - \phi\text{Po}) \times \psi\text{Eo}/(1 - \psi\text{Eo})$, where: 1. RC/ABS indicates the density of active reaction centers (RC) per energy dissipation from chlorophyll antenna; 2. ϕPo , estimated as the quantum yield of primary photochemistry, reflects the efficiency of light energy conversion into redox energy, $\phi\text{Po} = 1 - \text{Fo}/\text{Fm}$. The Fo represents the fluorescence intensity at 20 μs and Fm is the maximum fluorescence intensity; 3. ψEo is the efficiency of electron transport beyond the quinone A (Q_A) in the PSII, derived from $\psi\text{Eo} = 1 - [(\text{Fj} - \text{Fo})/(\text{Fm} - \text{Fo})]$. The Fj represents the fluorescence

intensity at 2 ms. The other parameters of the photosynthetic system efficiency were also assessed using their corresponding equations and summarized in the supporting information file (SI).

For toxicity tests, For toxicity tests, the means and corresponding standard deviations were determined for triplicate measurements (**Tables S3 and S4**). The differences between the treated and control samples were tested using a one-way ANOVA followed by a Tukey's post-hoc test for $p < 0.05$, using OriginPro 2025 software version 10.2.0.188.

4.3 Results and Discussion

4.3.1 Effect of adjusted initial pH on non-catalytic ozonation

This research was first focused on the influence of the medium pH, regarded as a key-factor in the ozonation process, before investigating its effect on metal cations which already govern the clay catalyst structure (**Figure S1**). It clearly appears that pH changes strongly influence the process evolution and selectivity, most likely due to changes in ozonation action mechanisms from molecular to radical forms and conversely (Azzouz 2012 2nd quarter, Shahidi, Roy and Azzouz 2015). Deeper insights in this regard through additional non-catalytic attempts during 10 min ozonation at adjusted initial pH showed a beneficial effect of acidic pH on the oxidative degradation process (**Figure 4.1a**).

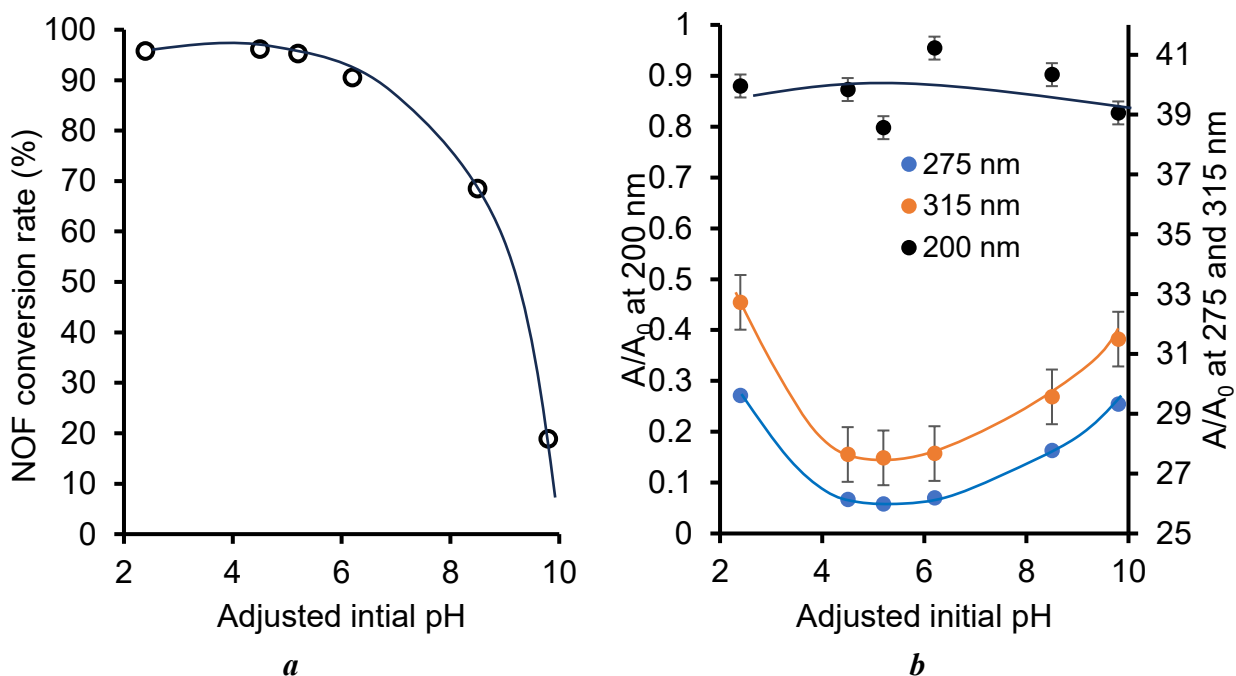


Figure 4.1. Effect on adjusted initial pH on relative UV-Vis band of NOF (a) and NOF conversion rate as assessed through HPLC-UV after 10 min of non-catalytic ozonation (b). The adjusted initial pH was measured with a ± 0.2 accuracy that is overlapped by the mark width.

Indeed, lowering initial pH from 9.8 down to 5.2-6.2 resulted in a pronounced improvement of NOF conversion from 18.92% up to a maximum plateau at 95.25-96.19% with no changes below pH 5.2 down to 2.4. A possible explanation should consist in that non-catalytic ozonation of NOF is rather favored by acidic initial pH that promote the action of molecular ozone than by neutral to basic media that favor hydroxyl radical-based pathway (Azzouz 2012 2nd quarter, Shahidi, Roy and Azzouz 2015, Azzouz A., Mirilà D. et al. 2019). pH changes within this range turned out to greatly influence the process selectivity as clearly illustrated by specific evolutions of the main UV-Vis bands of the ozonized NOF mixture (**Figure 4.1b**).

The pH changes seem to weakly influence the formation of OH-bearing species including acids and at most their interactions in water, given the barely detectable bump on the 200 nm band intensity between pH 4 and 6. By comparison, the pronounced minima of the 275 and 315 nm bands noticed in the same pH range must arise from weak alteration of the other moieties of NOF molecules including ring cleavage. This is probably due to a transitional pH range between decreasing action of molecular ozone on the one hand and increasing hydroxyl radical contribution on the other hand and conversely (Azzouz 2012 2nd quarter, Shahidi, Roy and Azzouz 2015,

Azzouz A., Mirilà D. et al. 2019). Such transitions induced by pH changes unavoidably control the contributions of the different potential reaction involved in the global ozonation process.

The non-catalytic ozonation of NOF induced a significant intensity increase for the 200 nm UV-Vis band (**Figure S2a**) and a decay for those appearing in the 250-370 region after only 10 min (**Figure S2b**). pH decrease during ozonation is a precise indicator of the process advancement, providing evidence of the conversion of the organic substrates and formation of acidic species but not necessarily on the process selectivity (Shahidi, Moheb et al. 2015). This is a common feature of all oxidative degradation reactions of organic molecules regardless of the absence or presence of catalysts, the latter being mainly known to govern the process kinetics and selectivity. Therefore, one must expect that for a given imposed initial pH, the unavoidable pH decrease during ozonation should specifically will influence the evolution of the process selectivity according to the presence/absence of catalysts, process progress in time and intermediate distribution. This will be examined further.

4.3.2 pH changes during cation-catalyzed ozonation

Ozone induced pH decrease in both pure water and NOF solutions, even more pronounced in the presence of dissolved cations (**Tableau 4.1**). In pure water, this pH decrease must arise mainly from a 2-steps reaction of molecular ozone with hydroxyl radicals ($O_3 + HO^\bullet \rightarrow O_2 + HO_2^\bullet \leftrightarrow O_2^{\bullet-} + H^+$) produced by direct ozone reaction with water ($O_3 + H_2O \rightarrow 2HO^\bullet + O_2$) (Kalandarov, Zhunussov et al. 2024). In the absence of chemical species including organic impurities, metal cations, carbonates and others that consume ozone, pure dionized water is known to produce the most pronounced pH decrease during ozonation. Indeed, nanopure water ozonation gave a stronger pH decrease expressed by a much higher pH difference (2.9) as compared to those registered for cation solutions, which range from 0.66 for Fe^{2+} to 1.39 for Na^+ .

The ozonation of NOF-containing media generates acidic species that are responsible of the pH decrease, which appears to be enhanced by the conversion rate of the organic substrate in the presence of dissolved cations (**Figure S3a**) and dispersed clay catalyst suspension (**Figure S3b**). As expected, this process appears to be enhanced by the catalytic role of the dissolved cation, as already reported for other organic substrates (Azzouz, Kotbi et al. 2010, Shahidi, Roy and Azzouz

2014, Shahidi, Moheb et al. 2015, Larouk, Ouargli et al. 2017, Boudissa, Mirilà et al. 2019, Boudissa, Zekkari et al. 2020, Mirilă, Boudissa et al. 2020, Boudissa, Arus et al. 2023). Cations act as Lewis acids and once dissolved in water they unavoidably undergo hydrolysis resulting in an acidic solution. Hydrated cations will then act as Bronsted acids, releasing more or less hydronium ions (H_3O^+) according to their polarizing power (PP) (Brown 1981). That is why the initial pH of the cation solutions increased almost linearly ($\text{Ni}^{2+} < \text{Cu}^{2+} < \text{Co}^{2+} < \text{Fe}^{2+} < \text{Na}^+$) with decreasing PP in the sequence $\text{Ni}^{2+} > \text{Cu}^{2+} > \text{Co}^{2+} > \text{Fe}^{2+} > \text{Na}^+$. Slight pH increase occurred after NOF addition, but the sequence was maintained. This is rather due to partial cation capture via two mechanisms: *i.* by chelation by NOF atoms bearing accessible electron pairs and *ii.* cation exchange given the presence of negative charges on NOF molecules at these pH levels.

Tableau 4.1. Initial features of cation-containing NOF solution with pH before and after 10 min ozonation

Metal cation	Dissolved cation ^a		Aqueous medium	Initial NOF ^c	charges	of	pH	
	PP ^a	Lewis acidity ^b					Initial _d	Final ^e
				R ₂ N	-CO ₂ ⁻			
None	X	X	Water	X	X	6.50	3.60	2.9
			NOF _(aq) _c	+++	-	5.28	2.41	2.87
Na ⁺	1.0	0.16	Water	X	X	7.49	6.10	1.39
			NOF _(aq) _c	++	--	6.13	4.67	1.46
Fe ²⁺	3.3	0.34	Water	X	X	6.16	5.50	0.66
			NOF _(aq) _c	+	---	6.8	3.15	3.65
Co ²⁺	3.6	0.40	Water	X	X	6.04	4.88	1.16
			NOF _(aq) _c	++	---	6.29	3.14	3.15
Ni ²⁺	4.2	0.50	Water	X	X	5.24	4.50	0.74
			NOF _(aq) _c	+++	--	5.81	3.09	2.72
Cu ²⁺	3.8	0.45	Water	X	X	5.83	4.52	1.31
			NOF _(aq) _c	++	---	6.26	3.18	3.08

^a PP: Polarizing power of cations as expressed in (Charge)/(Ionic radius in Å)²; ^b Lewis acidity strength as expressed in valence units (v.u.) as defined in (Brown 1981, Hawthorne and Schindler 2008); ^c NOF_(aq)* : solution concentration:

$3.31 \cdot 10^{-6}$ M; ^e Arbitrary charge scale based on pKa values of 8.75 and 6.34 for ammonium and carboxyl groups of NOF, respectively: 1. +++ or --- $\rightarrow$ 50%, 2. ++ or -- $\sim$ 50%, 3. + or - $<$ 50%; ^d Initial pH before ozonation; ^e Final pH after 10 min ozonation. All pH values were measured with an average standard deviation of 0.01. ; ^f Diff.: pH difference between the initial and final values during ozonation. X: feature not applicable.

In NOF ozonation, this pH decay was found to decrease in the following sequence $\text{Fe}^{2+}(3.65) > \text{Co}^{2+}(3.15) > \text{Cu}^{2+}(3.08) > \text{water}(2.87) > \text{Ni}^{2+}(2.72) > \text{Na}^+(1.46)$. and should be preponderantly due to the production of acidic ozonation product. Unlike Na^+ cation, nanopure water seems to behave similarly as the other cations as reflected by similar decays in the 275 nm band absorbance (**Figure 4.2a**) and residual HPLC peak ratio of NOF during ozonation (**Figure 4.2b**).

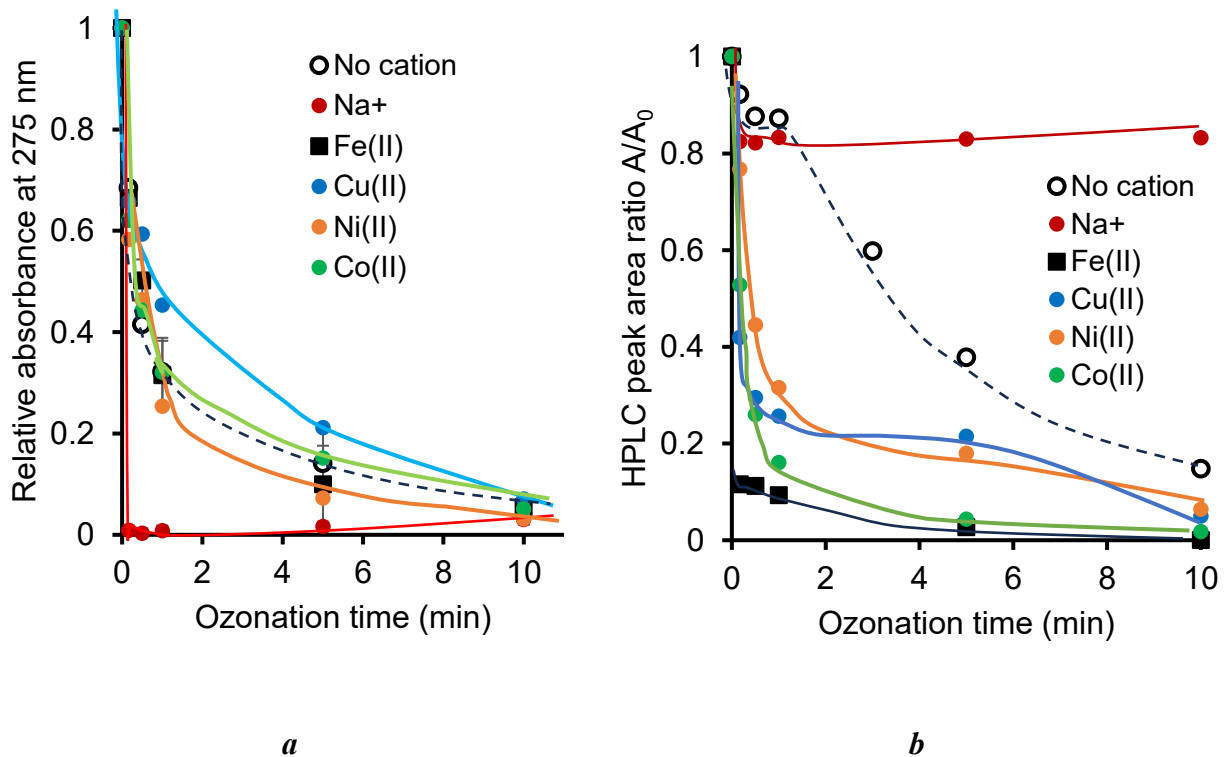


Figure 4.2. Time evolution of the main UV-Vis band of NOF (a) and NOF conversion yield as determined by HPLC-V during cation-catalyzed ozonation (b). The adjusted initial pH was measured with a ± 0.2 accuracy that is overlapped by the mark width. The HPLC-UV peak area ratio was evaluated with a standard deviation of ± 0.02 . The dashed lines account for process evolutions during ozonation in cation-free media.

Here, Na^+ cation seems to mitigate NOF oxidative degradation by ozone, as supported by the plateau of the residual NOF HPLC peak ratio after a weak depletion by less than 20 % after a few seconds of ozonation. In the meantime, this specific behavior of Na^+ cation appears to favor the selectivity towards certain NOF reactions as reflected by the sharp decay and almost total disappearance of the relative absorbance of the 275 nm band of NOF. Besides, Na^+ cation is expected to promote an indirect ozonation of NOF through a higher hydroxyl radical production than any other transitional metal cation, more particularly at this starting neutral to alkaline pH levels (Dmitrieva, Tyutereva and Voitsekhovskaja 2024).

This is presumably due to a complete chelating process with NOF molecules, as reflected by the total disappearance of the UV-Vis spectrum of NOF during in NaCl solution (**Figure S4**). Nonetheless, this was accompanied by a sharp intensity increase and broadening for the 210-220 nm band of NOF as like with any other cation. Hence, it clearly appears that dissolved cations influence the course and selectivity of NOF ozonation, which allows predicting also an influence on the distribution of the ozonation products and toxicity for the targeted bioindicator. This will be investigated further.

4.3.3 pH changes during clay-catalyzed ozonation

As previously stated, significant changes in the UV-Vis spectrum were observed after only 10 min of NOF in water (**Figure S2**). Simultaneously, the initial pH decreased from 5.28 to approximately 3.65 after only 1-2 min (**Figure 4.3a**) for a NOF conversion not exceeding 10-12%, most likely due to an early formation of acids. A correlation attempt revealed that the pH of the ozonation mixture remains constant till certain threshold values of the NOF conversion yield (**Figure 4.3b**). Non-catalytic ozonation showed a long and slightly inclined plateau to pH 3.2 and an abrupt drop down to 2.44 for NOF conversions of ca. 84.12 % and 95.96%, respectively. The shape of the pH profile suggests the occurrence of a 2-step ozonation process with NOF conversion mainly into acid species sandwiching an intermediate step with less acid production.

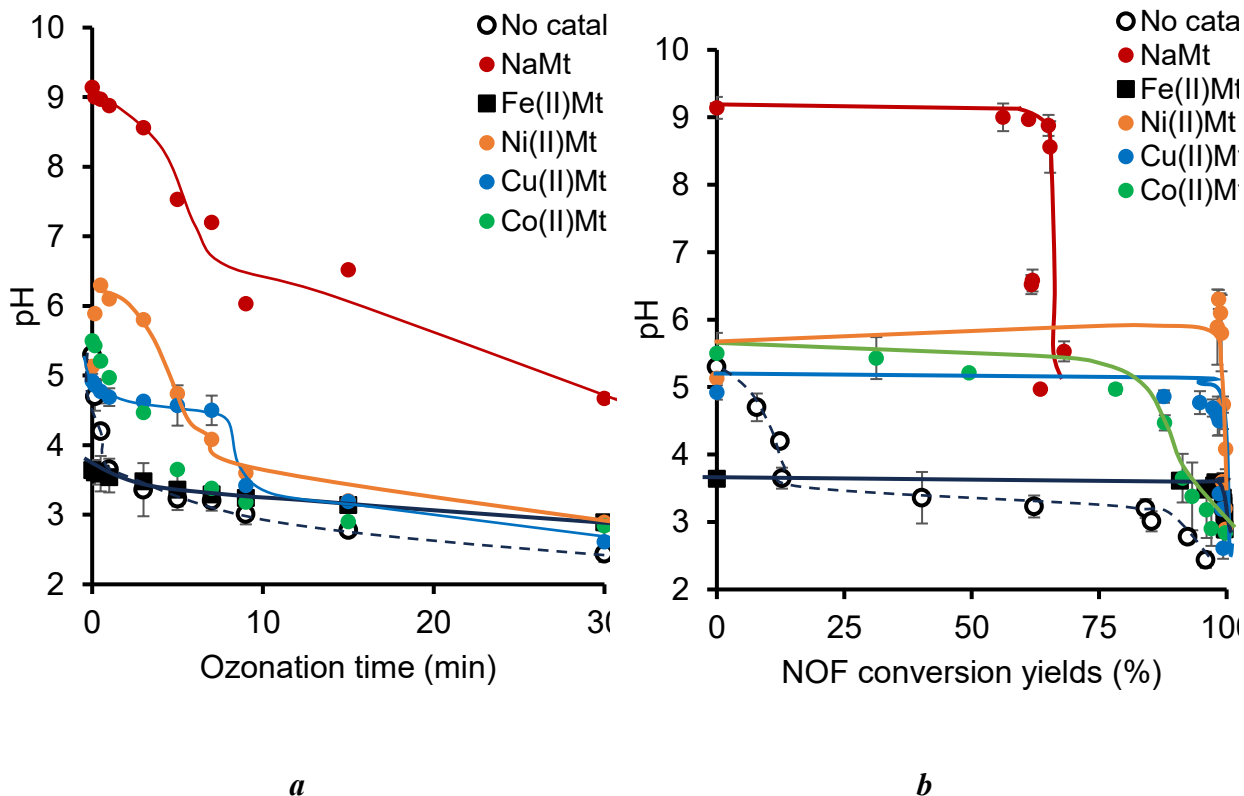


Figure 4.3. Evolution of instant pH (a) and correlation as a function of NOF consumption during ozonation (b). Ozone flow rate: 600 mg/h. Catalyst amount: 2 g/L. Sample volume: 25 mL. NOF concentration: 3.31×10^{-6} M. NOF conversion yield was calculated using the relationship $(1 - (A/A_0)) \times 100\%$ and measurement of the instant/initial peak area ratio (A/A_0) by HPLC-UV. The instant pH was measured with variable standard error (± 0.2 - 0.5) during ozonation.

The pH decline (**Figure 4.3a**) seems to be non-dependent on the NOF degradation increase, as illustrated by a specific plateau for each clay catalyst, which was slightly shorter for NaMt (Figure 3b). This must be due to the occurrence of buffering effects involving the progressively generated acidic species and their instant and unavoidable reaction with the metal cations of the clay catalysts into carboxylate salts. The continuous formation of oxidized species was supported by the intensity increase for the 200 nm UV-Vis band, which resulted from clay catalyst addition (Figure S5). Metal cation exchange between the clay catalysts and NOF molecules and then the generation of acidic species were demonstrated by the ICP-OES analysis via the changes in the cation amounts in the NOF solution before and after ozonation (Figure S6). The NOF molecules, and then most of its oxidized derivatives, bear Lewis's base sites and carboxyl groups, which exert competitive chelation and the ion exchange of metal cations at the expense of clay catalysts, depending on the

pH level. Both phenomena prevent the metal cation from being released into the bulk solution, thereby explaining why no significant iron loss was detected by the ICP-OES during ozonation, at least up to certain pH levels (Cromley and O'Connor 1976, Beltrán, Rivas and Montero-de-Espinosa 2005, Sallanko, Esko and Röpelinén 2006, Hopwood, Santana-González et al. 2020, Benghaffour, Foka-Wembe et al. 2022). However, the excessive pH decrease during ozonation and the increasing number of re-use cycles promote irreversible cation loss. Unless altered upon acid attack, the clay structure can be revived through periodic impregnations in fresh metal cation solution (Shahidi, Roy and Azzouz 2014).

In the meantime, a marked NOF depletion occurred during the first 2–7 min of ozonation, as illustrated by the pronounced intensity decrease of the HPLC-DAD peak ratio of NOF by more than ca. 90%, which was much weaker (ca. 63–65%) with NaMt or in the absence of catalyst, even for longer ozonation of up to 30 min (**Figure S7**). In all cases, this was accompanied by a fast production of acidic species, as supported by the noticeable pH decrease.

NaMt showed the most attenuated pH decrease, which barely reached 4.67 after 30 min of ozonation (**Figure 4.2a**), and a weaker NOF depletion not exceeding 64% (**Figure S7a**), as compared to the other clay catalysts (>98%) and even to ozone alone (96%). These opposite data, obtained with NaMt and Fe(II)Mt, can be regarded as the limits of the variation range for the catalytic activity of the clay catalysts and, consequently, of the other exchangeable cations.

4.3.4 Effect of cation on clay catalyst dispersion

Such a weaker catalytic activity of NaMt contrasts with its higher dispersion in the aqueous media reflected by its lower particle size (1608 nm) as compared to the other catalyst (**Tableau 4.2**). However, NaMt displayed a particle size (PS) almost twice higher in NOF solution than in pure water (846 nm) with no noticeable difference in Zeta potential values. This can be explained in terms of partial clay lamellae aggregation induced by repulsive [NOF:Clay] interactions. In this case, NOF adsorption on NaMt, if any, should have a reduced contribution to the heterogeneous catalytic process of ozonation due to the basic character of NaMt (Mirilă, Boudissa et al. 2020).

The effect of cations is mainly related to their polarizing power (PP), usually and often expressed in (Charge)/(Ionic radius in Å)², being directly proportional to the charge on a cation and inversely proportional to the size of the cation. This factor determines the Lewis acidity strength of the cation and thereby its capacity to be neutralized by hydration water molecules that act as Lewis bases and undergo dissociation with proton release (Brown 1981, Hawthorne and Schindler 2008). This explains why the lowest PP of Na⁺ cation (1.0) promotes almost no interaction with water molecules and no acidity in aqueous media.

Tableau 4.2. Initial physico-chemical features of the aqueous clay suspensions before ozonation

Clay-catalyst	Aqueous medium	ZP (mV) ^a	Standard deviation	PS ^b (nm)	Standard deviation	Initial charges				pH		
						NOF ^c		Silanols ^d		pH		
						R ₂ N	-CO ₂ ⁻	Out	In	Initial ^e	Final ^f	Diff. ^g
None	NOF _(aq) [*]					++	-	X	X	5.28	2.44	2.84
NaMt	Water	-23.45	1.663	846	25.65			---	---	9.19	4.03	5.16
	NOF _(aq) [*]	-23.36	0.5761	1608	101.7	+	----	---	---	9.72	4.67	5.05
Fe(II)Mt	Water	-30.5	0.7215	2515	232.6			+/-	+/-	3.88	3.19	0.69
	NOF _(aq) [*]	-28.71	0.876	2409	702.4	+++	-	+/-	+/-	4.29	2.89	1.4
Co(II)Mt	Water	-19.54	0.9465	1972	397.5			--	-	5.31	3.50	1.81
	NOF _(aq) [*]	-17.93	1.059	1875	184	+++	--	--	-	5.83	2.84	2.99
Ni(II)Mt	Water	-23.54	0.3172	2147	338.5			---	--	5.89	3.29	2.6
	NOF _(aq) [*]	-29.97	0.8394	2362	98.46	+++	---	--	-	6.31	2.89	3.42
Cu(II)Mt	Water	-14.95	0.4041	5068	472.5			--	-	5.02	3.46	1.56
	NOF _(aq) [*]	-13.36	0.4912	1858	69.51	++	--	--	-	4.71	2.61	2.1

^{*} NOF_(aq)^{*} : solution concentration: 3.31.10⁻⁶ M; ^a ZP: Zêta potential; ^b PS: particle size; ; ^c Arbitrary charge scale based on pKa values of 8.75 and 6.34 for ammonium and carboxyl groups of NOF, respectively: 1. +++ or --- :> 50%, 2. ++ or -- :~ 50%, 3. + or - :< 50% ; ^d Arbitrary charge scale based on pKa values of 5.6 and 8.5 for in-plane (In) and out-of-plane (Out) silanols, respectively: 1. --- :> 50%, 2. ++ or -- :~ 50%, 3. + or +/- :< 50% with possible rise of positive charges around pH 2-4; ^e Initial pH before ozonation; ^f Final pH after 30 min ozonation. All pH values were measured with an average standard deviation of 0.01; ^g Diff.: pH difference between the initial and final values during ozonation. . X: feature not applicable.

NaMt even induced an alkaline initial pH of the solution of 9.40. This pH is higher than the pKa of both ammonium (8.75) and carboxyl groups (6.34), which will bear no charges due to protonation and negative charges due to deprotonation, respectively. As a result, repulsive interactions towards both deprotonated silanols that hinder an electrostatic adsorption of NOF on NaMt, unlike on the other clay catalysts. The latter induce acidic pHs below the pKa of R₂N groups (8.75), thereby promoting ammonium protonation and interaction with silanols. Silanols still bear negative charges below their pKa values (5.6 for out-of-plane silanols and 8.5 for in-plane silanols)

(Sulpizi, Gaigeot and Sprik 2012, **Boudissa, Mirila et al. 2019, Zekkari, Ouargli-Saker et al. 2022**) and above their pH-Zero Charge (pH_{PZC}) around pH 2–4 depending on the silica type (Bolt 1957, Sögaard, Funehag and Abbas 2018).

4.3.5 Correlation induced pH-cation polarizing power

The reverse proportionality between the PP and induced pH previously observed for dissolved cations (**Tableau 4.1**) appears to be somehow altered in the presence of clay catalysts (**Tableau 4.2**). Indeed, Fe(II)Mt and Ni(II)Mt induced the lowest and highest initial pH respectively, which contrasts with the sequence observed for dissolved cations. In other words, this sequence, i.e. pH decrease with increasing PP, appears to even be reversed for the initial pH and at least markedly attenuated for the final pH in clay-catalyzed ozonation of both water and NOF solution (**Figure 4.4**).

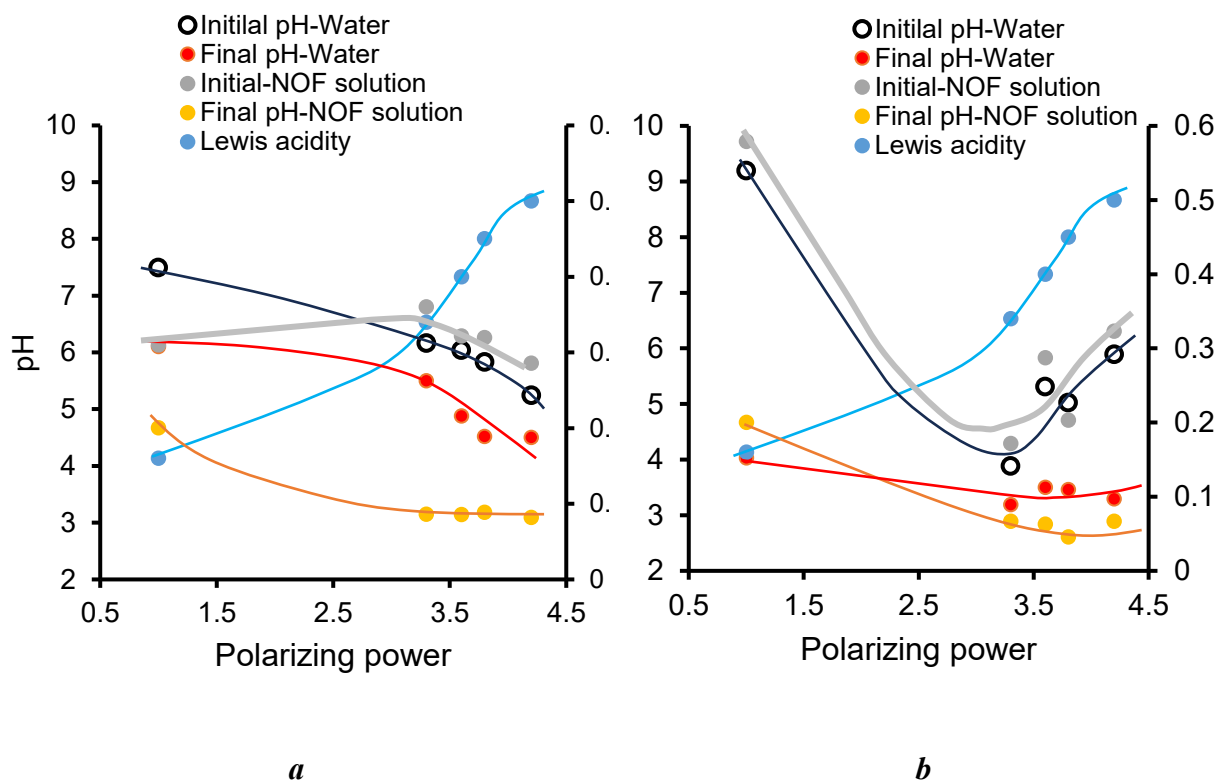


Figure 4.4. Effect of cation polarizing power on the pH before and after ozonation in the presence of dissolved cation (a) and clay catalysts (b). Ozone flow rate: 600 mg/h. Catalyst amount: 2 g/L.

Sample volume: 25 mL. NOF concentration: 3.31×10^{-6} M. NOF conversion yield was calculated using the relationship $(1-(A/A_0)) \times 100\%$ and measurement of the instant/initial peak area ratio (A/A_0) by HPLC-UV. The instant pH was measured with variable standard error (+ 0.2-0.5) during ozonation.

Here, the formation of Fe^{3+} as a more acidic exchangeable cation at the expense of Fe^{2+} is more probable given the lower oxidation capacity of Ni^{2+} . Nonetheless, the most plausible explanation should rather consist in that the presence of a negatively charged clay surface (Mt^-) modifies the sequence of the Lewis and Bronsted acidity strengths of free (dissolved) cations. This must be due to changes in the Lewis basicity of their corresponding counterions, since cation interaction with a chloride counterion for instance is expected to differ from that involving Mt^- polyanion (Brown 1981, Hawthorne and Schindler 2008). This finding is of great importance, because it provides clear evidence that the presence of a clay surface modifies the cation behavior in aqueous media.

4.3.6 Evolution of catalyst dispersion during ozonation

The decrease in pH during ozonation was found to significantly influence the surface charges of NOF molecules and clay lamellae interactions that govern the clay sheet aggregation/dispersion in water. This was illustrated by noticeable changes in ZP (**Figure S7a**) and particle size in aqueous media (**Figure S7b**), in agreement with the literature (Foka Wembe, Benghafour et al. 2023, Pavan, Escolano-Casado et al. 2023). These changes are expected to determine the contact surface available for NOF adsorption and degradation.

There is no clear trend in the effect of instant pH on the individual evolution of dispersion of each catalyst, but all the PS values of all of the clay catalysts investigated seem to correlate well with all the measurements of the instant pH induced by NOF ozonation. However, the general tendency is a decrease in PS of the clay catalyst with increasing pH induced by the ozonation progress (**Figure 4.5a**).

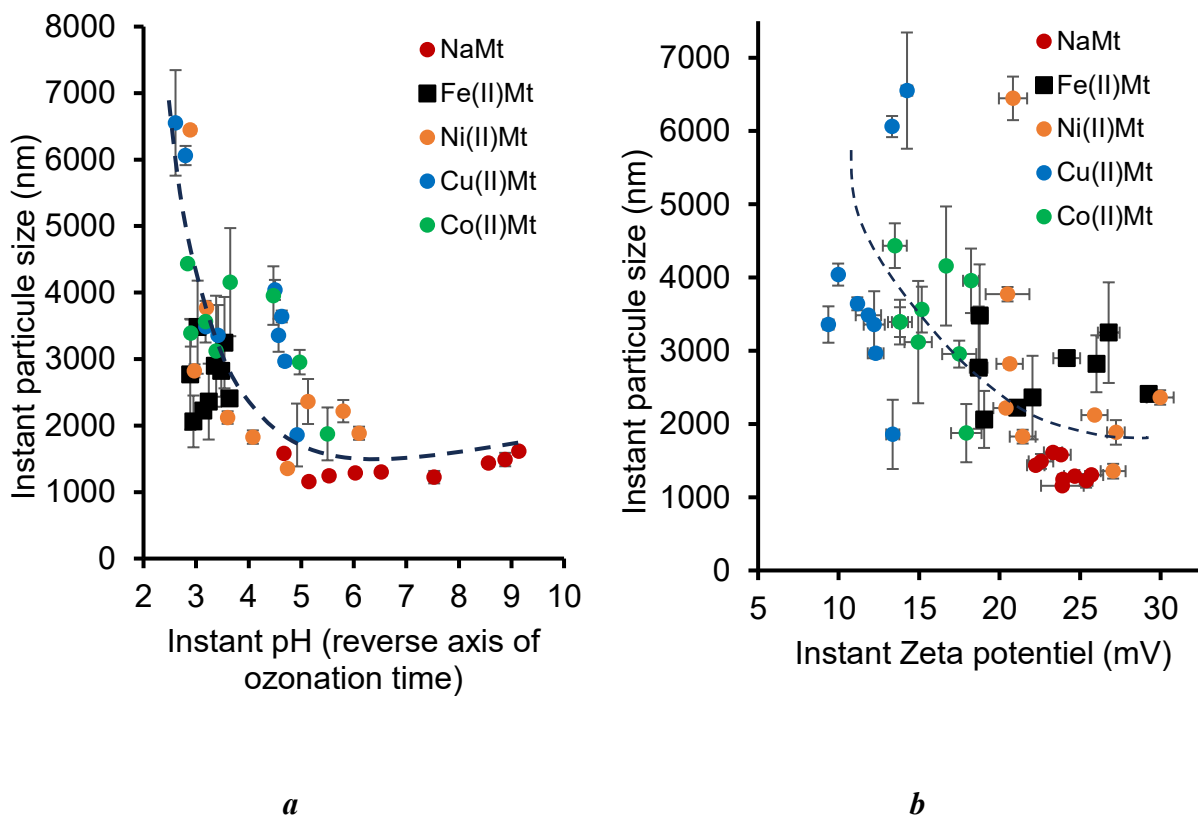


Figure 4.5. Correlation of the catalyst particle size with changes in pH (a) and Zeta potential absolute value (b) during NOF ozonation. Ozone flow rate: 600 mg/h. Catalysts amount: 50 mg. Sample volume: 25 mL. These experimental data were obtained at different ozonation time from 0 min without ozone to 30 min ozonation, respectively corresponding to the last mark at the left side and first mark at the right side of each colored series. The pH was measured with variable standard error (± 0.2 - 0.5) during ozonation.

This phenomenon is barely visible for NaMt most likely due to a shading effect of its basic character and to potential [NOF:NaMt] interactions in agreement with our previous statement. However, a much more important finding undoubtedly resides in the general tendency that the particle size increases with the unavoidable pH decrease during ozonation. This phenomenon was markedly more pronounced in the presence of clay catalysts exchanged with transition metal cations. The clay catalyst dispersion appears to strong depend on the exchangeable cations, which were found to induce specific pH and Zeta potential (ZP) (**Tableau 4.1**).

4.3.7 Electrostatic interaction changes during ozonation

The initial pH and Zeta potential (ZP) evolves during ozonation and greatly influence the [Clay:Clay] and [NOF:Clay] interactions and their contributions to the catalytic process. Here also, no clear correlation between the instant PS and ZP can be established for each clay catalyst. This is due essentially to the PP of each exchangeable cation that governs its Lewis acidity, and consequently number of hydration molecules and their dissociation into free protons (Brown 1981, Hawthorne and Schindler 2008). Yet, there exists a vague reverse proportionality between both parameters when correlating all the data for all catalysts together (**Figure 4.5b**). Indeed, the clay particle size appears to somehow increase from with increasing ZP during NOF ozonation, as a result of pH decay.

As previously stated, for all clay catalyst, a part of the negative charges are permanently compensated by cations (Tombácz and Szekeres 2006). ZP changes arise from silanol deprotonation for pH above of the pzc for the silica part between pH 2-4 (Bolt 1957, Sögaard, Funehag and Abbas 2018). This process is enhanced at pH higher than $pK_a = 5.6$ for the "out-of-plane" silanols and even more beyond $pK_a = 8.5$ for "in-plane" silanols (Sulpizi, Gaigeot and Sprik 2012, **Boudissa, Mirila et al. 2019, Zekkari, Ouargli-Saker et al. 2022**). Besides, bivalent cations are known to have the capacity to be sandwiched between clay lamellae, inducing stronger interactions that promote flocculation into larger particles and surface contact decay (Zhu, Marchuk and Bennett 2020). The consecutive clay aggregation and compaction is expected to hinder the effectiveness of the clay material by lowering the accessible adsorption and catalytic sites.

In the presence of NaMt suspension, the decrease in pH (from 9.14 to 4.64) in NOF solution after ozonation process paradoxically induced a decrease in particle size from 1612 nm to 1156 nm (**Fig. S7b**). This can be explained by possible depletion the repulsive [NOF:Clay] interactions due to a progressive protonation of both NOF carboxyl and silanols with pH decrease (Benghaffour, Foka-Wembe et al. 2022). This phenomenon seems to be somehow attenuated by excessive pH decrease (from 3.64 to 2.89) in the presence of Fe(II)Mt, which leads to NOF amino group protonation and rise of positive charges that attract deprotonated silanols. Similar but less or more pronounced

phenomena were noticed for the other clay-supported metal cations according to their specific features in aqueous media (Rengasamy, Tavakkoli and McDonald 2016).

4.3.8 Chemical species interactions during NOF adsorption

In the absence of ozone, Fe(II)Mt and Ni(II)Mt showed highest NOF adsorption yields reaching equilibrium at ca. 99 % and 99.4 %, respectively after only 1 min contact time (**Figure 4.6**). Fe(II)Mt showed a slightly slower NOF adsorption reaching surface saturation at approximately 97-97% after 2 min contact time. Deeper insights in the adsorption mechanisms were achieved applying kinetic models, which revealed that the process occurs mainly via the pseudo-second order based on the closest correlation coefficients values (R^2) to unity (**Tableau 4.3**).

This result provides confirmation that metals can retain antibiotics and clear evidence that NOF adsorbs preponderantly via chemical interactions with the clay catalyst surface, most likely through ion-exchange. Such an adsorption process should involve direction interaction of the negatively charged clay surface with the positive charges of the amino groups of NOF. These positive charges are induced by protonation at the pH range induced by clay suspensions below the pKa of the amino group, this process being more attenuated for NaMt (**Tableau 4.2**).

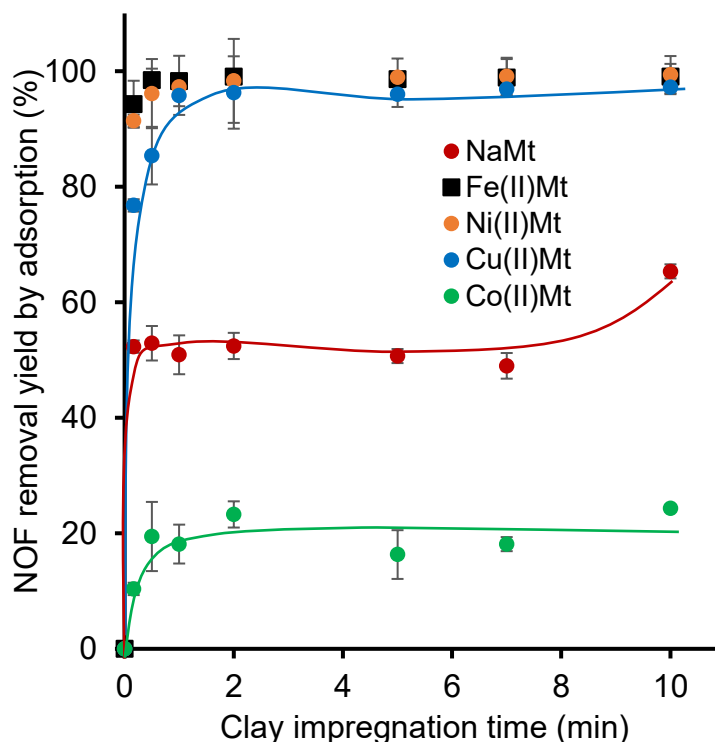


Figure 4.6. Adsorption isotherms of NOF on different clay catalysts. Sample volume = 25 mL. NOF initial concentration = 3.31×10^{-6} M. catalyst amount = 2 g/L. T = 25 °C. The relative peak area (A/A_0 calculated as the instant/initial HPLC-UV peak area ratio of NOF. The adsorption yield was defined as $(1-(A/A_0)) \times 100\%$, where A is the instant HPLC-UV peak area and A_0 the initial HPLC-UV peak area.

Tableau 4.3. Adsorption kinetics constants and correlation coefficients as assessed with HPLC-DAD data

Clay material	Pseudo-1 st order		Pseudo-2 nd order	
	K_1	R_1^2	K_2	R_2^2
NaMt	1.165	0.486	13.944	0.966
Fe(II)Mt	4.048	0.205	444.532	0.999
Co(II)Mt	2.851	0.386	32.303	0.949
Ni(II)Mt	3.122	0.544	123.296	0.999
Cu(II)Mt	2.689	0.574	58.055	0.999

R_1^2 and R_2^2 are correlation factors for the two different mathematical models.

Hence, electrostatic adsorption seems to prevail notwithstanding that NOF adsorption occurs on different adsorption through its different functional groups (Hamilton, Roberts et al. 2019). This turned out to be a common feature FQs adsorption on cation-exchanged montmorillonite (Wang,

Li et al. 2010). Adsorption should also involve chelation through Lewis acid-base interactions between cations and NOF moieties bearing non-binding electron pairs. Reportedly, ciprofloxacin (CIP) can chelate metals ions via oxygens of carbonyl or carboxylic groups, and similar interactions were observed in presence of humic substance (Cuprys, Pulicharla et al. 2018). Coordination interaction was also reported between some FQs and Fe^{3+} and Cu^{2+} cations (Cao, Zhang et al. 2025). Coordination reactions between three others FQs and two ions Fe(III) and Cu(II) was observed with UV-Vis at different pH in previous work (Cao, Zhang et al. 2025).

Given the narrow interdependence of the ZP and PS with the amount of dispersed NOF molecules and that the induced pH evolves during ozonation, adsorption is also expected to evolve in time and significantly influence the global process kinetics. Hence, the mere presence of clay catalysts was found to accelerate the ozonation process at least due to the adsorption contribution even before complete dissolution of ozone in the aqueous medium (Benghaffour, Foka-Wembe et al. 2022).

The potential occurrence of ternary $[\text{M}^{n+}:\text{Mt}:\text{NOF}]$ interactions is supported by composing binary ones. The theoretical modeling of such interactions is based on metal cation capture/release by the following: 1. ion-exchange by the permanent charges of the octahedral Al layer, partially substituted by Mg^{2+} and/or Fe^{2+} ions; 2. the pH-dependent charges of the silanol groups; 3. the Lewis acid–base chelation of excess metal cation by non-binding electron pairs belonging the lattice oxygen atoms; 4. pH-dependent charges of NOF carboxyl groups; 5. the Lewis acid–base chelation of excess metal cations by the non-binding electron pairs of the N-atoms of NOF; 6. NOF adsorption on the clay surface by cation exchange through protonated NOF amino groups at a low pH; 7. repulsive interaction between deprotonated clay silanols and NOF carboxyls at a high pH; 8. H-bridges of clay silanols and aluminols, mainly with NOF carboxyls; 9. hydrophobic interactions between NOF molecules and -Si-O-Si-rich islands. In the presence of ozone, quaternary $[\text{M}^{n+}:\text{Mt}:\text{NOF}:\text{O}_3]$ interactions are also possible if one assumes, at least, that the mere presence of a clay surface favors ozone adsorption, mainly via ozone molecule polarization and electrostatic interactions (Shahidi, Roy and Azzouz 2014, Shahidi, Moheb et al. 2015, Shahidi, Roy and Azzouz 2015, Larouk, Ouargli et al. 2017).

4.3.9 Clay dispersion effect on toxicity

The pH evolution in time should be a key-factor in governing the surface charge and clay dispersion, which, in turn, control their very catalytic activity, ozonation process advancement, product distribution and consequently the toxicity of the ozonized NOF mixture. In this regard, Zeta potential and particle size measurements may provide valuable information on the toxicity of heavy metals and organic pollutants (Alfarsi, Weir et al. , Lage, Bondoso and Catita 2012, Yusefi-Tanha, Fallah et al. 2020). This narrow interdependence between all these factors can be illustrated by an increasing tendency in the FN/Control ratio with increasing Zeta potential (**Figure 4.7a**).

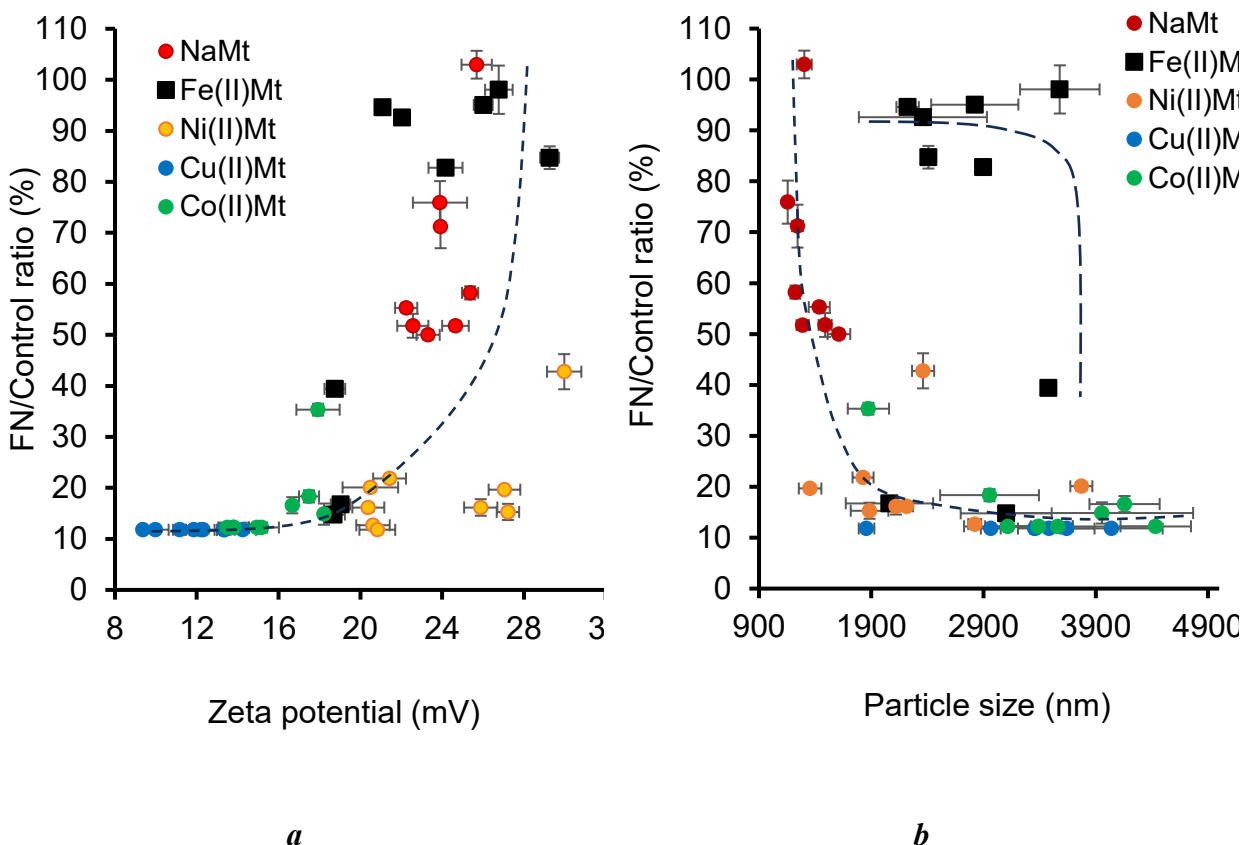


Figure 4.7. Effect of the Zeta potential absolute value (a) and particle size (b) on the frond number of *Lemna minor*. The frond number (FN)/Control ratio was defined as being the (measured FN/Control FN ratio) x 100%. The control consisted of an average fresh weight of 98 mg of plant with and 67 fronds in normal growth conditions in SIS medium, non-contacted with ozonized NOF mixtures.

Given that the FN is a primary indicator used to assess the phytotoxic effects of antibiotics and heavy metals (Alfarsi, Weir et al. , Lage, Bondoso and Catita 2012), a first plausible explanation should consist in that NaMt and Fe(II)Mt contain less toxic and even more nutritive cations towards *L. minor* as compared to the other clay catalysts. Nonetheless, these beneficial features of NaMt and Fe(II)Mt must be combined with that of their lower instant particle sizes with increasing instant ZP (**Figure 4.5b**). As expected, such a data combination gave rise to a decreasing tendency of the FN/Control ratio with increasing particle size (**Figure 4.7b**).

A similar but weaker detrimental effect of the large particle size was noticed for Fe(II)Mt at high FN/Control ratio due to its lower toxicity and higher nutritive properties as compared to the other exchangeable cations. Thus, both Na^+ and Fe^{2+} promote clay dispersion at a specific pH and via different mechanisms, leading to increased clay activity, high NOF conversion, and reduced toxicity (Djidja, Dewez and Azzouz 2025). The basicity of Na^+ enhances silanol deprotonation, repulsive forces, and clay particle dispersion. Fe^{2+} hydration induces Brønsted acidity via hydrolysis into free protons and additional negative charges on the $[\text{Fe}^{2+}(\text{H}_2\text{O})_{(n-1)}\text{OH}]^-$ cation on the clay surface and reduces its sandwiching effect between clay lamellae (Zhu, Marchuk and Bennett 2020).

4.3.10 Cation intrinsic toxicity on frond number

Here, the toxicity towards *L. minor* should unavoidably include that of the metal cations, which was reflected by increasing frond number (FN) inhibition rate and the production of oxygen reactive species (ROS) with increasing cation concentration, expressed in terms of $\text{Log}_{10} C$ (**Figure 4.8**). This phenomenon was a common behavior for all metal cations but increasingly more pronounced for Co^{2+} , Ni^{2+} and Cu^{2+} with the increasing concentration.

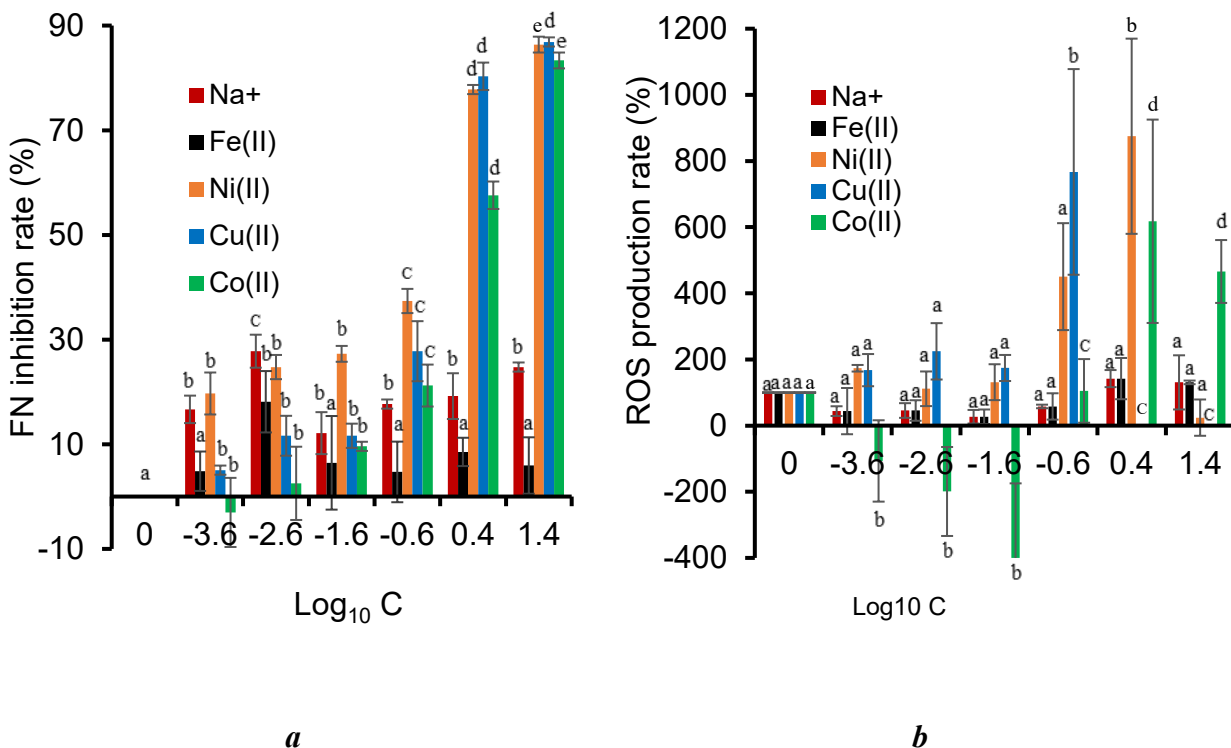


Figure 4.8. Concentration effect of cations alone dissolved in water on toxicity on *L. minor* as expressed in terms of inhibition of frond number growth (a) and ROS relative amount (b) as reported to the blank sample (0 mg/L). These toxicity parameters were measured after 7 days of exposure in SIS medium containing cations solutions at concentrations ranging from 0.00025 to 25 mg/L (the solutions were prepared by dilution of stock solution of 25.10 mg/L $\pm$ 0.1). The results are expressed as percentages relative to the control sample (SIS medium). Triplicates measurements were achieved for all concentrations considered including the metals-free control sample). The control consisted of plants grown in SIS medium, an average fresh weight of 98 mg and 67 fronds. Certain negative values indicate an increase in growth (a) and decrease in ROS level (b) compared to control group (0 mg/L). The different symbols used (a, b and c) account for different data significances at $p < 0.05$. Data with similar significance are marked with similar symbols in the same group.

It is worth mentioning that Co^{2+} displayed the lowest FN inhibition rate and ROS production for low concentration around 25×10^{-4} ppm. At this concentration, Co^{2+} is known to act as a micronutrient that stimulates the plant growth. Co^{2+} at concentration higher than 0.059 ppm was already found to inhibit *L. minor* growth by inhibiting iron transport from the roots to shoots without affecting iron uptake (Sree, Keresztes et al. 2015). Other metal cations such as Cu^{2+} can exhibit toxicity towards *Lemna minor* even at low concentrations by favoring the activity of the

antioxidant enzymes catalase and guaiacol peroxidase (Razinger, Dermastia et al. 2007). Plants are known to rise the activity of their antioxidant defense systems in response to oxidative stress that reduces the ROS levels.

ROS formation is one of the primary drivers of toxicity when dealing with genotoxicity (Peng, Chai et al. 2024, Stannard, Doherty et al. 2024) or phytotoxicity assessment (Zhao, Li et al. 2022). At high cation concentration, the ROS disappearance is often attributed to severe cellular damage and dysfunction/destruction of ROS producing organelles, such as chloroplasts, mitochondria and peroxisomes (Das and Roychoudhury 2014). Other living species such as microalga *Raphidocelis subcapitata* showed increased ROS production after exposure to Co^{2+} (0.5 mg/L) as well as a decay in the fluorescence of chlorophyll *a* (0.10 mg/L). The same species rather showed decreased ROS production after Ni^{2+} exposure due to an activation of their antioxidant mechanisms (Dos Reis, de Abreu et al. 2024).

The observed toxic responses to Na^+ concentrations involved only growth inhibition. Salt excesses above certain thresholds can lead to physiological disruption in *Lemna minor* and *Pseudokirchneriella subcapitata*, and no ROS involvement and no reduction in chlorophyll was noticed (Simmons 2012). Indeed, Na^+ toxicity appears to only arise from osmotic stress caused by salt excess (de Moraes, Barbosa-Neto et al. 2019). Depending on the dose and type of metals, redox active (Co, Cr, Fe and Cu) and non-redox active (Ni and Zn) are expected to act via different toxicity mechanisms.

This intrinsic toxicity of metal cations that inhibits *Lemna minor* growth more particularly at relatively high concentrations beyond admissible levels was somehow expected. Certain metal cations can compete with other essential micronutrients at physiologically important chelating sites leading to functional deficiencies that affect living species metabolisms (Begović, Mlinarić et al. 2016). Comparison with cation-exchanged clay materials revealed a higher intrinsic toxicity of free dissolved cations for *L. minor* as compared to their clay-supported counterparts (Djidja, Dewez and Azzouz 2025). This is mainly due to a lower availability in the dispersed form in the bulk aqueous media. Negatively charged soil surfaces including cationic clay-based materials such as smectites are well known to control pollutions by metals through both cation-exchange and

chelating processes by atoms acting as Lewis bases and exhibiting available non-binding electron pairs.

4.3.11 Effect on photosynthetic efficiency

The exposure of *L. minor* to cations affected the photosynthetic efficiency and electron transfer in PSII. Cation concentrations of 0.25–25 µg/L showed no marked effect on the pigment production (**Figure S9**). The pH set in the SIS medium or induced by the addition of reaction mixtures resulting from cation- and clay-catalyzed NOF ozonation and clay's intrinsic effect on FN are separate key-factors in ecotoxicity changes (**Figures S10–S12**).

Deeper insights into fluorescence kinetics revealed a decay in the relative fluorescence intensity from ca. 3.5 down to approximately 2.5 with the increasing cation concentration up to 25 mg/L (**Figure S13**), indicating that metal cations interfere with the photosynthetic electron transport and energy transfer processes in the thylakoid membrane. In addition, the photosynthetic parameters calculated herein, i.e., the inhibition rate of the Performance Index, PI_{ABS} (**Figure 4.9**), the maximum PSII quantum yield, F_v/F_m (**Figure S14a**), and the variable fluorescence relative to quinone A reduction, (V_j) (**Figure S14b**), showed noticeable changes, particularly for high concentrations of all the cations. These results, obtained on the photosynthetic response to the metal concentration, confirm the PSII multi-site toxicity pattern across various transitional metal cations.

Among these parameters, PI_{ABS} is particularly interesting, reflecting possible structural and functional variations associated with the PSII complex and offering a holistic assessment of plant vitality (Rodríguez, Vilas et al. 2023). A 55% decrease of PI_{ABS} was observed for copper, sodium, and nickel for the lowest concentration tested of 0.00025 mg/L ($\log_{10} C = -3.6$). This indicates that transitional metal cations are highly toxic to the photosynthetic performance, even at low concentrations. Under similar conditions, the minimum effects of Fe^{2+} and Co^{2+} suggest the existence of specific physiological mechanisms that allow plants to tolerate or assimilate these cations more effectively. Interestingly, exposure to Fe^{2+} and Co^{2+} had only negative effects on the PI_{ABS} values compared to the control sample at high tested concentrations, suggesting differential toxicity mechanisms for transitional metal cations. The decrease of PI_{ABS} confirmed the inhibitory impact of cations such as Pd^{2+} and Cd^{2+} on PSII photochemistry and electron transport in the

thylakoid membrane (Perreault, Samadani and Dewez 2014, Samadani, Perreault et al. 2018, Jmii and Dewez 2021)

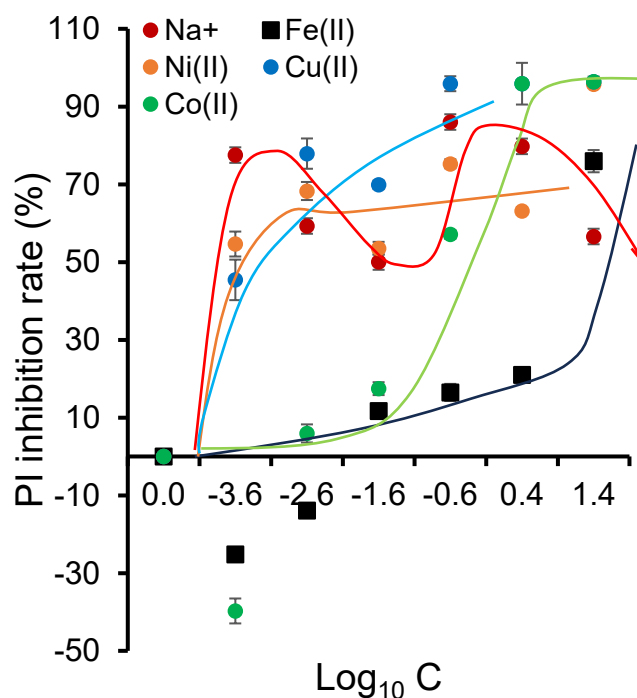


Figure 4.9. Change in the Inhibition rate of the Performance Index PI_{ABS} (% of the control). *L. minor* were exposed for 7 days to different cation concentrations (mg/L). Triplicates measurements were achieved including the metals-free control sample. The control sample consisted of plants grown in SIS medium, an average fresh weight of 96 mg and 66 fronds non-contacted with ozonized mixtures.

Reduced F_v/F_m values were already reported for *L. minor* plants exposed to different concentrations of metal cations such as Co, Cs, Mn, Ni and Zn (Van Dyck, Vanhoudt et al. 2023). In addition, the release of Cu^{2+} cations from CuO nanoparticles was also found to inactivate the PSII reaction center (RC) in *Lemna gibba* primarily through photoinhibitory processes (Samadani, Perreault et al. 2018). Cd^{2+} cations accumulation on green algae *Chlamydomonas reinhardtii* caused the inactivation of PSII RC but through cation interference with the electron flow required

for the light reactions of photosynthesis (Perreault, Samadani and Dewez 2014). Metal cations exhibit also a catalytic activity in the formation of reactive free radicals contributing to additional oxidative stress in plant cells (Frankart, Eullaffroy and Vernet 2002). In this study, the changes in the photosynthetic efficiency observed in the presence of cations and clay catalysts was an accurate indicator of how the [clay–metal–organic substrate] interactions affect the toxicity toward the *L. minor* plants.

In normal cellular metabolism, the electron transfer through electron transport chains occurs in the chloroplasts (Photosystem I and II), mitochondria (Complexes I and III), and peroxisomes (Chauhan, Yadav et al. 2021). Random electron leaks first generate ROS and superoxide anions is the first ROS formed by reacting with molecular oxygen (O_2) (Andrés, Pérez de la Lastra et al. 2023). Other simultaneous processes also take place (Bienert, Schjoerring and Jahn 2006, Sharma, Jha et al. 2012, Bienert and Chaumont 2014, Anjum, Gill et al. 2022, Alp, Arikan et al. 2023). Transition metal cations are known to convert H_2O_2 into highly toxic hydroxyl radicals ($OH\cdot$) that cannot be neutralized by plant enzymes (Kehrer 2000, Richards, Wilkins et al. 2015). This induces a negative impact on PSII and, mainly, on chlorophyll *a* degradation that affects carbon dioxide fixation and cellular structures and metabolisms. (Gao, Chang et al. 2020, Hu, Xu et al. 2025, Tanaka and Ito 2025). Besides, metal cations also enhance the activity of chlorophyllase enzyme and can replace Mg^{2+} in the porphyrin ring of the chlorophyll molecules (Riyazuddin, Nisha et al. 2021).

4.3.12 Toxicity of cation-catalyzed ozonation

A quick overview of frond number measurements during cation-catalyzed ozonation revealed that the nature of the cations has specific effects (**Figure 4.10a**). After ozonation time of up to 10 min, Na^+ -containing reaction mixtures showed the highest FN inhibition rate (ca. 46-58 %), while Fe^{2+} cation induced the lowest levels of approximately 13-17 %. Ni^{2+} , Cu^{2+} and to a lesser extent Co^{2+} cation showed a marked toxicity increase after 10 min ozonation. In agreement with previous statements, this was only accompanied by a weak ROS production by both cations. This ROS production thoroughly depleted below the natural level on the control sample after 1 min of Na^+ catalyzed ozonation (**Figure 4.10b**).

In the meantime, decreases in chl *a/b* ratio but barely detectable for Fe²⁺ and Co²⁺ were noticed once ozonation triggered (**Figure S11**). After an ozonation time of 10 min in the presence of Co²⁺ and Fe²⁺, the NOF conversion yield increased up to 94.9 % and 94.5 %, respectively, without inducing significant ROS production. Fe²⁺ cation also allowed affording total mineralization of fluoroquinolone and reduced ecotoxicity (Bobu, Yediler et al. 2013). The fact that organic substrates such as ciprofloxacin (CIP) can chelate metals cations through their Lewis base sites is a plausible explanation of the toxicity mitigation, as already reported for *E. aeruginosa* (Cuprys, Pulicharla et al. 2018).

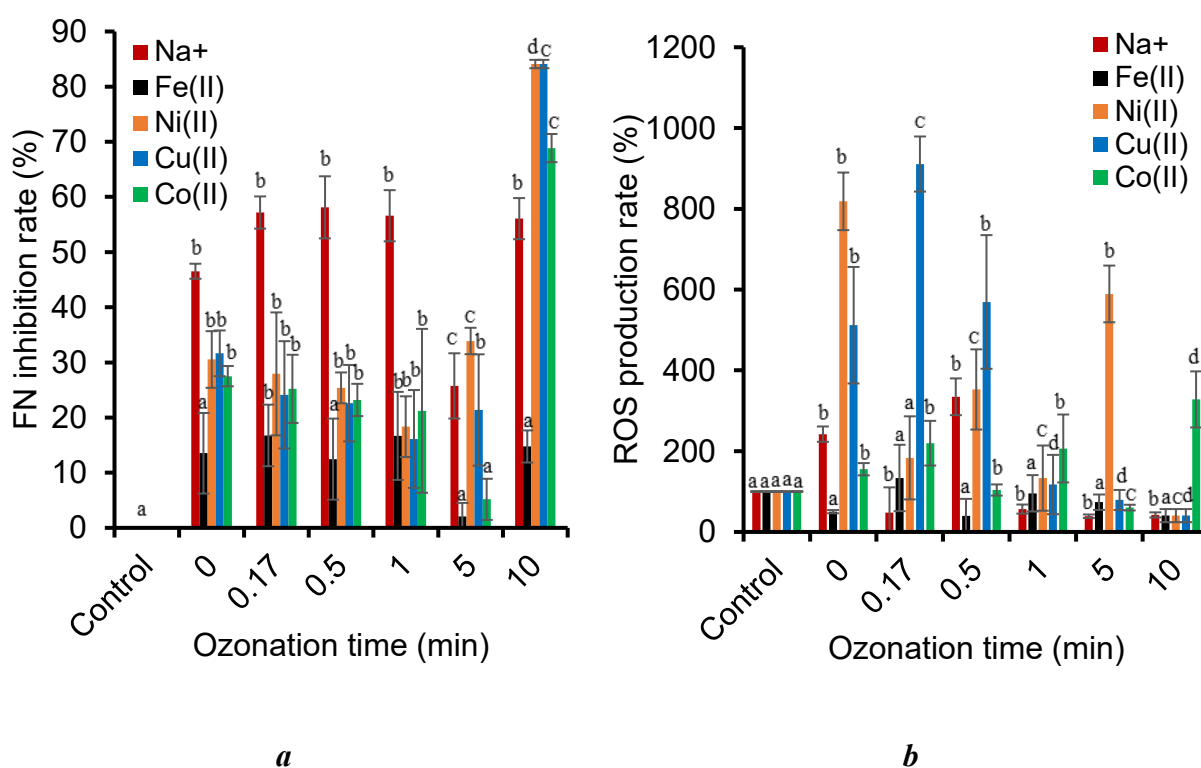


Figure 4.10. Toxicity of ozonized NOF solution in presence of metal cation on frond number inhibition rate (a) and ROS production (b) of *Lemna minor* after 7 days of exposure. Triplicates measurements were achieved, including the metals-free control sample. The control sample consisted of plants grown in SIS medium, an average fresh weight of 96 mg and 66 fronds non-contacted with ozonized mixtures. The different symbols used (a, b c, and d) account for different data significances at $p < 0.05$. Data with similar significance are marked with similar symbols in the same group.

Notwithstanding that longer contact times with metals are known to enhance toxicity, the mere presence of NOF molecules should reduce the cation mobility/release in the bulk solution. In addition, the acidic conditions induced by cation-catalyzed ozonation unavoidably favor cation exchange with negatively charged NOF molecules (Shahidi, Roy and Azzouz 2014). NOF can also bind to divalent metal ions via chelation between the cation and the 4-oxo and the carboxyl group adjacent of the organic substrates which may result in changes in their toxicity with possible adverse effects on fauna and flora induced by FQs-metal complexes (Serafin and Stańczak 2009). There, both cation adsorption via ion-exchange and chelation along with ozonation are expected to mitigate not only the toxicity of the cation but also those of the residual and progressively depleting NOF molecules and oxidized derivatives that should also bear additional oxygen atoms and non-binding electron pairs. This is a plausible explanation of the decay in ROS production as the ozonation process progresses.

4.4 Conclusion

This research provides another contribution for a better understanding of the role of clay-containing media in nature self-regeneration, which allows the tailoring of nature-inspired and clay-based water treatments. The results obtained allow us to conclude that a judicious compromise between acidic media and a small clay particle size is favorable for the degradation of norfloxacin. Excessively acidic aquatic media in contact with decaying vegetation and soluble humus derivatives are expected to mitigate the contact surface and catalytic activity of the clay suspension, leading to exchangeable cation loss, the low degradation of organic pollutants, and increased ecotoxicity. Conversely, weakly acidic-to-neutral and even alkaline media are also detrimental to pollutant adsorption and oxidative degradation, and thus to biodiversity. Different metal cations appear to strongly influence this compromise and the toxicity towards the vegetal species. Metal cation interactions with organic pollutants reciprocally modify their respective intrinsic toxicities. Cation capture by norfloxacin mainly involves ion-exchange and chelation and appears to contribute, with ozonation, to attenuating the global toxicity of the ozonized aqueous media. The presence of Na^+ and Fe^{2+} cations improves the clay dispersion and reduces the toxicity in oxidized, antibiotic-contaminated clay media. The presence of a clay surface modifies the cation behavior in aqueous media, and vice versa. In addition, the photosynthetic organism *L. minor* acted as a reliable and sensitive indicator of the toxicity impact of [clay–metal–organic substrate] interactions,

depending on the concentration and type of metal cations. The combined toxicity of metal cations and organic pollutants on the photosynthetic efficiency of vegetal living species still remains to be elucidated. Therefore, these results provide valuable information on the impact of organic pollutant oxidation on oxidative water treatments and on aquatic media.

CHAPITRE 5
MOLECULAR INTERACTIONS OF NORFLOXACIN IN METAL-LOADED CLAY
SUSPENSIONS-EFFECTS ON DEGRADATION AND INDUCED TOXICITY

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Roumaissa Djidja

Development of the methodology and execution of the experimental work, including adsorption, ozonation and catalytic ozonation studies as well as ecotoxicological assays. Processing and analysis of experimental data, interpretation of results. Investigation and preparation of the original manuscript draft.

David Dewez

Revision and optimization of experimental and ecotoxicological protocols. Review and validation of ecotoxicological data, contribution to data interpretation and correction of the final manuscript.

Abdelkrim Azzouz

Conceptualization and design of the research project on the catalytic ozonation of organic pollutants. Supervision of experimental work, validation and interpretation of experimental results, supervision of the research work, and participation in manuscript writing, reviewing and editing.

Abstract

The role of the metal valence state on the surface properties of metal-loaded clay minerals in the adsorption/oxidative degradation of an antibiotic was investigated. Transitional metal cations and their zero-valent counterparts such as Fe^0 , Ni^0 , Co^0 and Cu^0 supported on montmorillonite were comparatively investigated for their interactions during adsorption and toxicity tests of antibiotic norfloxacin (NOF). UV-Vis spectrophotometric and Fourier transform infrared (FTIR) spectroscopic analyses confirmed the involvement of the hydroxyl and carboxyl groups and/or piperazinyl nitrogen of NOF in the complexation with metal cations. Ecotoxicological assessment using aquatic plants *Lemna minor* showed that the metal cations reduce the bioavailability of the organic pollutant and that the zero-valent metals display higher toxicity due to their specific interaction with NOF and clay mineral surface. This evaluation will provide insights into potential environmental impacts of the co-occurrence of antibiotics and metals and will certainly contribute to correlating the safety of the water treatment by assessing the residual toxicity and its fluctuations.

Keywords: zero valent metals; norfloxacin; Montmorillonite; adsorption; *Lemna minor*; toxicity

5.1 Introduction

Antibiotics have become a significant environmental issue due to the persistent impact of the parent compound and/or its derivatives in various ecosystems (Haciosmanoğlu, Mejías et al. 2022). For instance, antibiotics can induce toxic effects on growth and photosynthesis of plants (Kovalakova, Cizmas et al. 2020) and depletion of microbial communities responsible for essential ecological functions (Saxena and Gauba 2025). This disruption can also impact pathogenic bacteria such as enterotoxigenic *Escherichia coli* (ETEC), potentially altering host-microbiome interactions and exacerbating infections (Tchoumi Neree, Noori et al. 2024) in spite of the well-known plant strategy to combat the oxidative stress (Hernandez-Viezcas, Castillo-Michel et al. 2011, Miazek, Iwanek et al. 2015). Due to their extensive use and slow biodegradability, fluoroquinolones (FQs) and more particularly norfloxacin (NOF) are interesting to be investigated in this regard (Zhang, Deng et al. 2021, Mangla, Sharma and Ikram 2022, Chen, Song et al. 2024). The similarity of FQs with quinones confers norfloxacin (NOF) a similar action as a quinone site inhibitor in photosystem II (PSII) (Zhao, Huang et al. 2024).

This impact should be governed by specific behavior and interactions of the organic substrate in clay-containing media and in even more in the presence of metal (Morel, Spadini et al. 2014, Weng, Cai et al. 2017). Here, these interactions are expected to unavoidably lead to the formation of complexes that can modify the reactivity towards oxidizing/reducing agents but the induced residual ecotoxicity. In this regard, research on FQs behavior combined with metals present in soils and aqueous media and its effect remains limited (Chen, Song et al. 2024). The co-occurrence of FQs and heavy metals in the environment may induce changes in their slow degradation and their specific toxicities. For instance, NOF and copper salts have been detected in livestock and poultry wastewaters and soils with concentrations reaching up to 225.45 ppm and 160 ppm, respectively (Qian, Wu et al. 2016, Wu, Fan and Song 2025). The different natures of pollutants imply different behaviors in ecosystems (López, Fabiani et al. 2022) and possibly synergistic ecotoxicity on the environment and human health (Shahzad, Tanveer et al. 2018, Li, Chen et al. 2021).

In natural media, antibiotics can undergo diverse degradation pathways, such as photolysis and photo-oxidation (Ding, Jiang et al. 2020, Xue, Li et al. 2021, Rivera-Vera, Muñoz-Lira et al. 2023), aerobic/anaerobic bacteria-driven processes and ozonation on the outermost surfaces under severe or prolonged exposure to air and sun radiation. Ozonation already turned out to be quite effective in quinolone removal (Mangla, Sharma and Ikram 2022). The pH (Cao, Zhang et al. 2025), light exposure (Tong, Qiu et al. 2023) and presence of reactive minerals (Wang, Li et al. 2010) were found to play key-roles in this process. Among many minerals, clays dispersed in soils and waters are assumed to have a high capacity to retain and interact with organic molecules and metals regardless of their oxidation state with beneficial and/or detrimental effects on the polluted media. For example, bentonite-supported zero-valent iron showed effectiveness in the simultaneous removal of Cr(VI) and phenol (Diao, Xu et al. 2016). Besides, clay mineral cation exchange is a conventional strategy for water remediation (Ciğeroğlu, El Messaoudi et al. 2024).

Due to their variable oxidation states, chelating ability, Lewis acid behavior, and redox properties transition metals can form stable complexes with organic drugs (Baral, Bhattarai and Chaudhary 2024). FQs can interact with the exchangeable cations of clay minerals through both ion exchange or complexation mechanisms. Ciprofloxacin (CIP) was found to easily adsorb on pillared bentonite in alkaline media (Roca Jalil, Toschi et al. 2018) mainly through van der Waals interactions in spite the repulsive force of negatively charge CIP and clay mineral surface (Roca Jalil, Baschini and

Sapag 2017). The ammonium and carboxyl groups of Norfloxacin can promote cation exchange and chelating processes that should strongly influence its mobility in soils and groundwater according to the pH and competitive interactions of dissolved metal cations (Morel, Spadini et al. 2014). Metal cations were already found to strongly interact with norfloxacin, influencing both its adsorption and oxidative degradation pathways (Djidja, Dewez and Azzouz 2025, Djidja, Dewez and Azzouz 2025). Thus, the metal nature and valence are expected to modulate the strength and nature of [Metal:NOF] interactions and the evolution of their toxicities.

In anaerobic environments, zero-valent metals (ZVM) may promote the reduction of organic substrates and even their already oxidized derivatives. This was supported by the contribution of transitional metal particles in nitroaromatic reduction (Ghosh, Mandal et al. 2004, Bouazizi, El achari et al. 2018, Bouazizi, El achari et al. 2018, Bouazizi, Vieillard et al. 2018, Bouazizi, El-Achari et al. 2019, Bouazizi, Morshed et al. 2019, Bouazizi, Vieillard et al. 2019), dechlorination of chlorinated hydrocarbons (Wang, Zhu et al. 2013), and even of trichloroethylene (TCE) when supported on bentonite (Baldermann, Kaufhold et al. 2021). This was explained in terms of electron transfer improvement by high amount of surface hydroxyl groups and a synergistic effect of $\text{Fe}^{2+}/\text{Fe}^{3+}$ and $\text{Cu}^0/\text{Cu}^{2+}$ redox cycles (Zhang, Li et al. 2023). Here, the surface oxidation of ZVM generates metal oxides that promote ozone decomposition (Wang and Bai 2017) but also Fe(II) cation release with additional potential toxicity (El-Temsah and Joner 2013, Ševců, El-Temsah et al. 2017). This can be elucidated through deeper investigations. The beneficial role of surface hydroxyl groups was also reported for MnOx/SBA-15 -catalyzed ozonation of NOF (Chen, Li et al. 2017). Such catalysts displayed fairly extended specific surface areas of $405\text{--}671\text{ m}^2\cdot\text{g}^{-1}$, affording however moderate but higher mineralization rate as compared to the non-catalytic ozonation. Furthermore, the presence of chelating sites such as hydroxyl groups is an essential requirement not only for preventing particle agglomeration and loss in catalytic surface (Ezzatahmadi, Ayoko et al. 2017), but also for promoting additional adsorption contribution for complete remediation (Mangla, Sharma and Ikram 2022).

Given the high number of factors that influence the heterogeneous catalytic process, adsorption resulting toxicity, the present research was completed by factorial experiment designs, a judicious approach for assessing the simultaneous individual effects and interactions (Demirtas, Açıkel and Aşçı 2025) around supposedly optimum values of much lower number of parameters (Zahmatkesh

Anbarani, Najafpoor et al. 2023). Similar approaches were already tackled for maximizing the adsorption capacity of ciprofloxacin (Gulen and Demircivi 2020), tetracycline (Zahmatkesh Anbarani, Najafpoor et al. 2023) and norfloxacin on various materials (Wan, Liu et al. 2018). In the present work, the pH, contact time, and clay catalyst concentration were considered as key factors in the retention, degradation and toxicity of NOF in metal-containing aqueous clay suspensions. This is intended not only to understand their complex interactions in natural clay-containing media such as soils and clay suspensions in ponds, lakes and rivers but also to explain toxicity evolution using *Lemna minor* as an aquatic plant bioindicator.

5.2 Methods and Materials

5.2.1 Chemicals and Stock Solutions

Norfloxacin (Lot No LRAD2292) and Sodium tetrahydroborate (NaBH_4) were purchased from Sigma Aldrich. Metallic salts $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 7\text{H}_2\text{O}$ were supplied by Fisher Scientific, Pittsburgh, PA, USA (purity of 99%) and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ supplied by Sigma-Aldrich, Burlington, MA, USA (purity of 99.995%). Stock solutions of NOF (10 mg/L) were prepared in nanopure water and stored at 4 °C in the dark. Metals stock solutions (100 mg/L) were also prepared in nanopure water and stored under the same conditions.

5.2.2 Preparation of Montmorillonite-Based Adsorbent/Catalysts

Ion-exchanged montmorillonites Fe(II)Mt , Ni(II)Mt , Co(II)Mt , and Cu(II)Mt were prepared by impregnating NaMt with aqueous solutions of the corresponding metal salts following a previously described procedure (Djidja, Dewez and Azzouz 2025, Djidja, Dewez and Azzouz 2025). Zero-valent metal modified montmorillonites (Fe^0Mt , Ni^0Mt , Co^0Mt , and Cu^0Mt) were prepared by dispersing 0.2 g of NaMt in 15 mL of 0.12 M aqueous solutions of the corresponding metal salts and stirred vigorously for 2 h. Then, 10 mL of NaBH_4 (0.2 M) was added dropwise as a reducing agent under a nitrogen atmosphere, and the mixture was stirred for an additional 15 min. The color of the mixture turned dark, indicating the formation of ZVM. The resulting M^0/Mt were then filtered, washed and dried for 3 h at 80 °C under N_2 atmosphere and then stored in a sealed enclosure with dry oxygen-free nitrogen (Bouazizi, Barrimo et al. 2017, Noori, Neree et al. 2021). M^0/Mt were characterized by Attenuated total reflectance-Fourier transform infrared spectroscopy, ATR-FTIR (Thermo Scientific, Nicolet 6700 instrument, Madison, WI, USA).

5.2.3 Metal-NOF Interaction Study

Prior to NOF adsorption and ozonation tests, the potential interaction of norfloxacin (NOF) in aqueous solution (3.31×10^{-6} M) with aqueous media containing various zero-valent metal and cation was investigated. The mixtures were stirred continuously at room temperature and was monitored by UV–Vis spectrophotometry using an Agilent-Cary 60 instrument and a 1 cm quartz cell and analyzed through ATR-FTIR. A few drops of the aqueous complex sample were previously deposited directly onto the ATR crystal then air-dried at room temperature until complete evaporation.

5.2.4 Adsorption Tests

Adsorption experiments were carried out by contacting 50 mg of metal-exchanged montmorillonites (M^{2+} -Mt) and zero-valent metal-modified montmorillonites (M^0 /Mt) with 25 mL of NOF solution at intrinsic pH. The suspensions were vigorously stirred at room temperature. The samples were analyzed immediately after filtration through a 0.45 mm membrane. The residual concentration of NOF in the supernatant was measured by UV–Vis spectrophotometry at 275 nm and High-Performance Liquid Chromatography coupled to a Diode Array Detector detector (HPLC-DAD, Agilent Technologies model 1290 equipment) under specific conditions (Table S1). The adsorption yield was defined as $(1-(A/A_0)) \times 100\%$, where A is the instant HPLC-DAD peak area and A_0 the initial HPLC-DAD peak area.

Zeta potential (ZP) and particle size variation was measured during adsorption through a Malvern Zetasizer device Ultra Red Label (Malvern Panalytical, UK). The concentration of metal cations released from the clay minerals into the solution was determined after each adsorption or ozonation test using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Agilent 5100, Santa Clara, CA, USA), with an axial plasma configuration and a concentric quartz nebulizer under optimized instrumental conditions (Table S2).

5.2.5 Ozonation Tests

Ozonation experiments were conducted in a series of glass tubes (25×200 mm) containing 25 mL samples of NOF solution (3.31×10^{-6} M) and 50 mg of dry clay catalyst in a similar procedure as previously. Ozone was generated by an A₂Z generator (A₂Z Ozone Inc., Louisville, KY, USA) and

continuously bubbled into the solution through a porous glass diffuser at a 600 mg/h throughput. Prior to UV–Vis analysis, the resulting ozonized mixtures were centrifuged and then measured using UV-Vis spectrophotometry (Agilent-Cary 60 instrument, Santa Clara, CA, USA) and 1 cm quartz cell) over the range 190–800 nm.

To complete the role of the different parameters studied previously (Djidja, Dewez and Azzouz 2025, Djidja, Dewez and Azzouz 2025), deeper insights in their combined effects (Interactions) on the ozonation process were achieved through a 3³-factorial design of experiments in agreement with previous works (Bodo, Ahmanache et al. 2004, Assaad, Azzouz et al. 2007, Shahidi, Roy and Azzouz 2014). For this purpose, 27 ozonation tests were carried out for each catalyst, by varying the initial pH of the solution (X₁), catalyst amount (X₂) and ozonation time (X₃) (Table S3). The individual effects and interactions of these parameters were assessed based on measurements of the relative absorbance of three UV-Vis key bands of NOF (Y₁, Y₂, Y₃), the resulting toxicity evaluated by fresh weight, frond number, number of specimens, Chl *a/b* and ROS (Y₄, Y₅, Y₆, Y₇, Y₈) and the final pH obtained after ozonation (Y₉).

The instant pH was measured periodically during ozonation using an Accumet[®] model 15 pH-meter (Fisher Scientific, Pittsburgh, PA, USA), with an accuracy of ±0.01 units. The concentration of metal cations released from the clay minerals after each ozonation test was determined using ICP-OES under the same instrumental conditions.

5.2.6 Toxicity Tests on *Lemna Minor*

Aquatic plant *Lemna minor* was used as bioindicator for toxicity assessment, after cultivation under laboratory conditions in a nutrient medium previously prepared according to the Swedish Institute for Standards (SIS) (Table S4). A photoperiod of 16 h light and 8 h dark was maintained with light intensity maintained of 100 ± 10 μmol of photons m⁻²s⁻¹ at 24 °C ± 2 °C and a relative humidity of 60 ± 5%. The cultivation medium was renewed every 7 days to ensure stable nutrient conditions and pH. The plants were exposed to *i.* NOF ozonation or adsorption mixtures in the presence of modified clay minerals and *ii.* NOF in the presence of various metal species exhibiting different oxidation states in the absence of such materials.

The number of fronds, fresh weight, the ROS production and Chlorophyll *a* fluorescence measurements were assessed through procedures fully described elsewhere (Djidja, Dewez and Azzouz 2025, Djidja, Dewez and Azzouz 2025). Shortly, the plant growth inhibition was evaluated on the basis of the frond number and fresh weight. The production of reactive oxygen species (ROS) was quantified using CellROX Green, while the chlorophyll contents was determined by UV–Vis spectrophotometry after ethanol extraction.

Chlorophyll *a* fluorescence measurements were performed with Handy-PEA fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd., King's Lynn, UK). This is commonly used in plant ecotoxicology to evaluate photosystem II efficiency through OJIP transient analysis and to assess the performance index (PI_{ABS}) as detailed elsewhere (Jmii and Dewez 2021, Djidja, Dewez and Azzouz 2025). The latter was determined as $PI_{ABS} = RC/ABS \times \phi Po/(1 - \phi Po) \times \psi Eo/(1 - \psi Eo)$, where: 1. RC/ABS indicates the density of active reaction centers (RC) per energy dissipation from chlorophyll antenna; 2. ϕPo , estimated as the quantum yield of primary photochemistry, which reflects the efficiency of light energy conversion into redox energy, $\phi Po = 1 - F_o/F_m$. The F_o accounts for the fluorescence intensity at 20 μs , while F_m is the maximum fluorescence intensity; 3. ψEo is the efficiency of electron transport beyond the quinone A (Q_A) in the PSII, derived from $\psi Eo = 1 - [(F_j - F_o)/(F_m - F_o)]$. The F_j represents the fluorescence intensity at 2 ms (Strasser, Tsimilli-Michael and Srivastava 2004, Stirbet 2011).

The metals concentration was determined in the testing medium and plant biomass by using the ICP-OES. Before the analysis, the digestion was first carried out in 2 mL of HNO_3 (Fisher Scientific, purity 70%). The tubes were placed on a sand bath at 90 °C for 2 h. After digestion, each sample was collected and acidified with HNO_3 to obtain a final concentration of 5% HNO_3 .

5.3 Results and Discussion

5.3.1 Metal Cation: NOF Interaction

NOF interaction with metal cations was reflected by noticeable changes in the UV-Vis spectrum of their mixture (**Figure 5.1**). The marked decrease in absorbance intensity at 275 nm and to a lesser extent at 315 nm indicates a depletion of free NOF molecules, which appears to evolve in time (**Figure S1**). This must be likely due to cation exchange on deprotonated carboxylic groups

and complexation with free electron pairs of the carbonyl, ammonium and piperazine groups of NOF (**Schéma 5.1**). Here, the piperazine ring is assumed to be much less involved in the cation complexation, as already reported for fluoroquinolones with transition metals (Ahmad, Ziaullah et al. 2024).

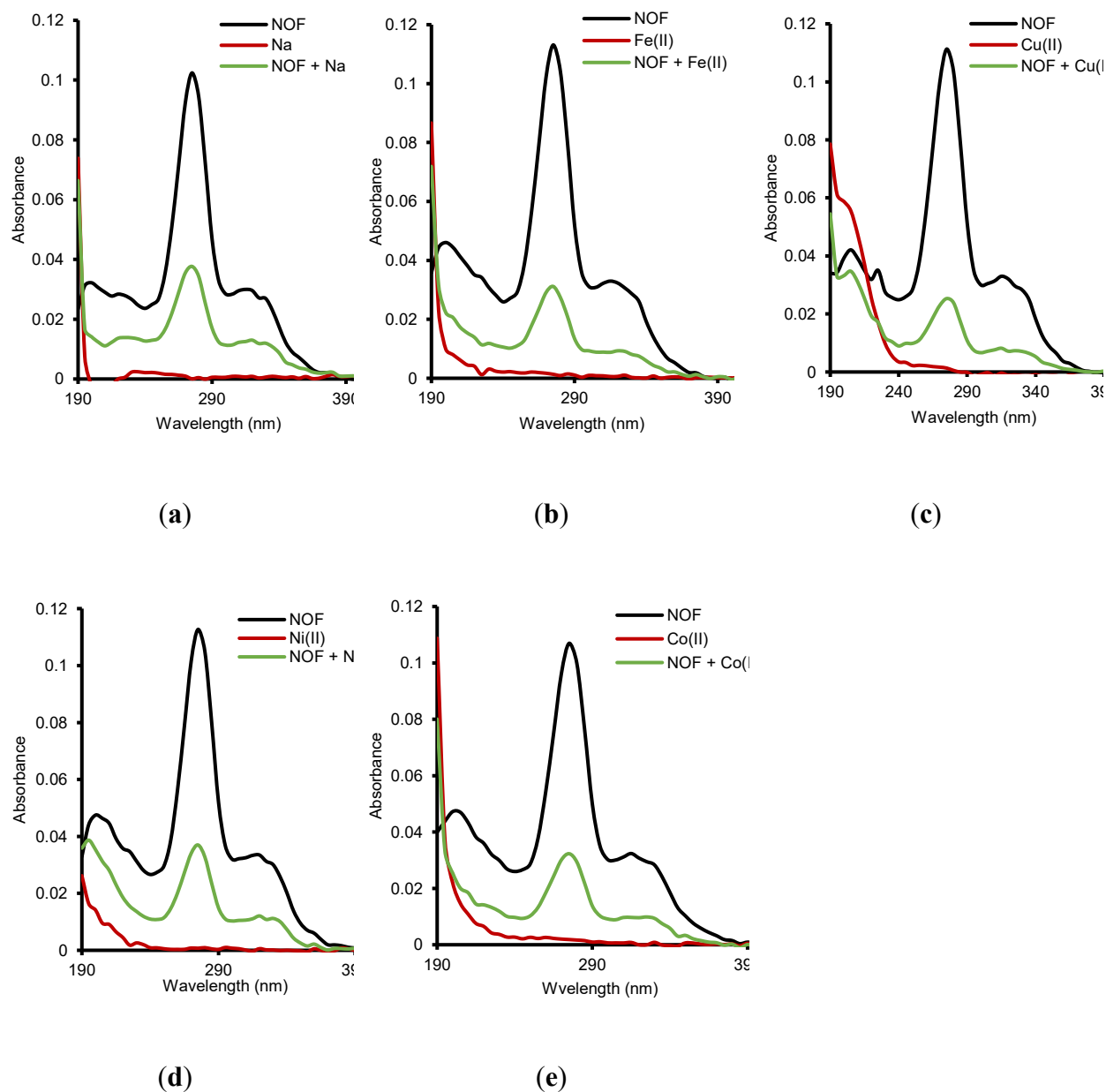


Figure 5.1. UV-Vis absorption spectra for Norfloxacin in the presence of Na^+ (a), Fe^{2+} (b), Cu^{2+} (c), Ni^{2+} and Co^{2+} (e). NOF concentration: 3.31×10^{-6} M. Metal cation concentration: 10 ppm.

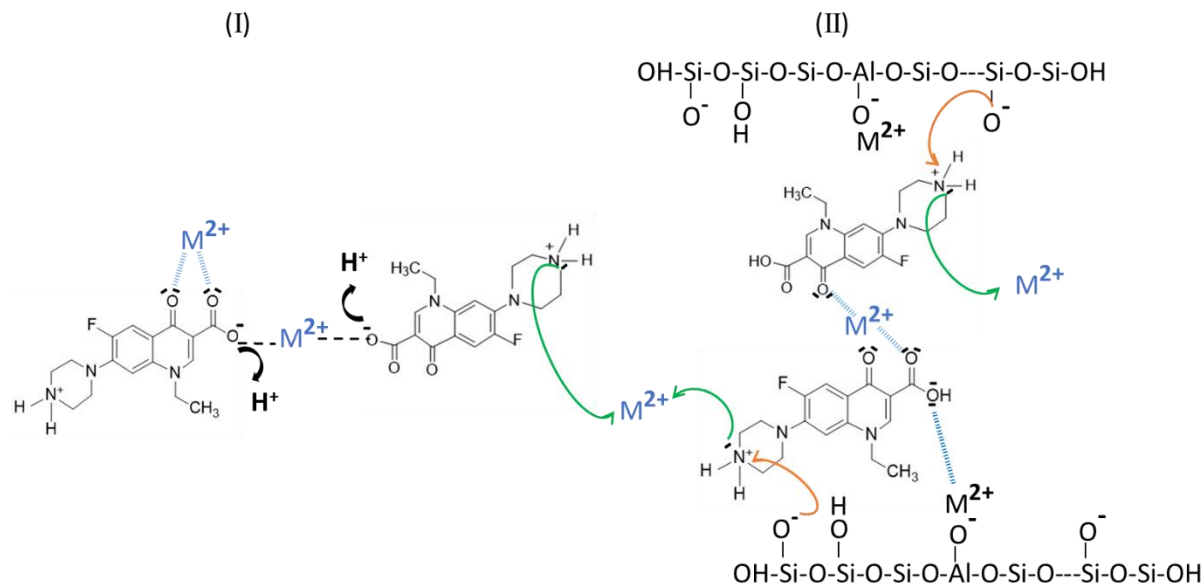


Schéma 5.1. Potential NOF interactions with metal cation (I) and with metal cation and clay mineral surface (II). Orange: NOF ion exchange on deprotonated silanols; Green: NOF capture by adsorbed and free cation; Blue: Cation capture by chelation on NOF; Black: Cation capture by NOF though ion exchange. Part I describes i. the formation of NOF:NOF bridges via bivalent cation exchange on two deprotonated carboxyls of two free NOF molecules and ii. Lewis acid-base interaction between the metal cation and non-binding electron pairs of oxygen atoms (and potentially of N atoms also). Part II illustrates additional interactions involving NOF adsorption through exchange of i. ammonium cation on the clay mineral surface and ii. the formation of NOF-Clay bridges between NOF carboxyls and exchangeable sites sharing a bivalent metal cation.

Such [Metal:NOF] interactions may contribute to the chemical stability of the antibiotic molecule, as supported by an increased photostability of $[Cu^{2+}\text{-ciprofloxacin}]$ system (Zhang, Cai et al. 2012). The pronounced decrease in intensity during the first 5–10 min followed by a bump between 5 and 30 min is a precise indicator of the occurrence of successive interactions involving slow ion-exchange and/or chelation processes (**Figure 5.2**).

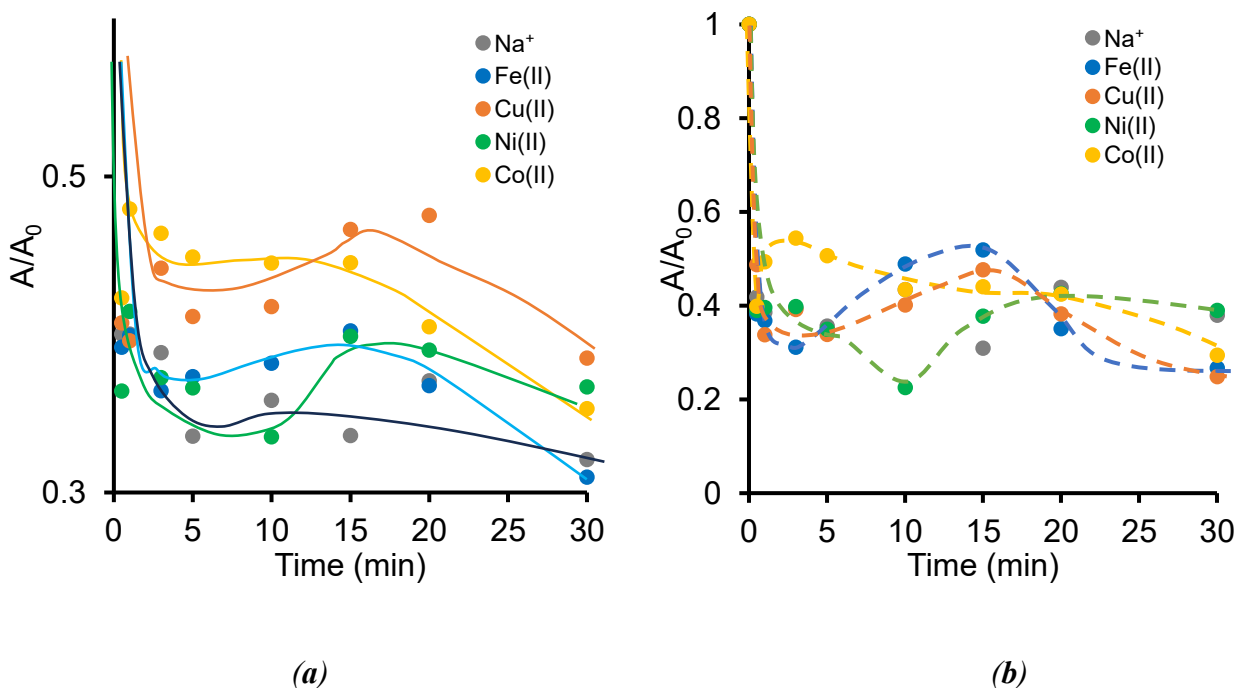


Figure 5.2. Evolution of the relative absorbance at 275 nm (a) 315 nm (b) during metal:NOF complex formation. The relative absorbance corresponds to A/A_0 , where A is the absorbance of NOF in the presence of metal ions and A_0 is the absorbance of the initial NOF solution without metal with a standard deviation of ± 0.04 .

Deeper insights through FTIR analysis allowed explain i. the involvement of the carboxyl and carbonyl groups in the cation capture, ii. the cation interaction with the N-H group of the imino-moiety of piperazinyl groups and/or the O-H of the carboxylic groups and iii. those occurring between the zero-valent metal and surface hydroxyl groups. The total disappearance of the C=O bond stretching around 1740 cm^{-1} confirmed that the carboxyl and carbonyl groups are involved in the cation capture (**Figure S2**). Cation addition to NOF also induced a significant intensity decrease and a slight shift of the broad band around 3260 cm^{-1} towards higher wavenumber of up to 3270 cm^{-1} for $[\text{NOF}:\text{Na}^+]$, 3370 cm^{-1} for $[\text{NOF}:\text{Fe}^{2+}]$, 3380 cm^{-1} for $[\text{NOF}:\text{Cu}^{2+}]$, 3280 for $[\text{NOF}:\text{Ni}^{2+}]$ and 3320 cm^{-1} for $\text{NOF}:\text{Co}^{2+}$ suspected interactions (**Table S5**). This can be attributed to cation interaction with the N-H group of the imino-moiety of piperazinyl groups and/or the O-H of the carboxylic group, in agreement with the previous statement. These FTIR band shifts were assigned to the NH- and OH bond stretching and appear to be stronger for Fe^{2+} , Cu^{2+} and Co^{2+} as

compared to Ni^{2+} and to a lesser extent Na^+ . This indicates the occurrence of different interaction strengths of the corresponding groups according to the metal cation, which remains to be confirmed by X-ray photo-electron spectroscopy which provides an accurate assessment of the slight variations of the binding energy of the electrons belonging to the chelating groups (Noori, Megoura et al. 2022, Tchoumi Neree, Noori et al. 2024). In the meantime, new signals were observed at $1620\text{--}1670\text{ cm}^{-1}$ attributed to N-H bending vibration of the quinolone group (Sahoo, Chakraborti et al. 2012, Ahmad, Ziaullah et al. 2024, Cao, Zhang et al. 2025).

These interactions are expected not only to drive metal cation transfers between the bulk solution, NOF molecules and clay mineral surface. These wavelength shifts should, in turn, govern NOF adsorption in the forms of free molecules, metal cation/NOF salts and [NOF:Cation] complexes on the clay mineral surface but also the dispersion of the resulting NOF-loaded clay mineral lamellae.

5.3.2 Effect of [NOF:Metal] Interaction on Clay Mineral Dispersion During Adsorption

In the presence of Na^+ - montmorillonite (NaMt) and its and bivalent metal cation exchanged counterparts (Fe(II)Mt, Co(II)Mt, Ni(II)Mt and Cu(II)Mt), it appears that both adsorption and ozonation induce change in the chemical composition of the aqueous media as observed through periodical ICP-OES analysis in time.

Increasing contact time during adsorption induced a pronounced Na^+ release in the liquid media (**Figure 5.3a**). This is presumably due to a relatively strong cation exchange imposed by the low initial pH (5.2) of the starting NOF solution with respect to the basic character of Na^+ (Djidja, Dewez and Azzouz 2025). This low pH should not produce noticeable release of transition metal cations in the bulk solution, as reflected through their barely detected amounts by ICP-OES. In contrast, the ozonation progress in time led to a continuous increase of the concentration of dissolved transition metal cation, except for Na^+ which rapidly reached almost the same level as during adsorption. The latter was slightly mitigated after only 1–2 min contact time probably due to interaction with NOF and rise of acidic NOF derivatives (**Figure 5.3b**).

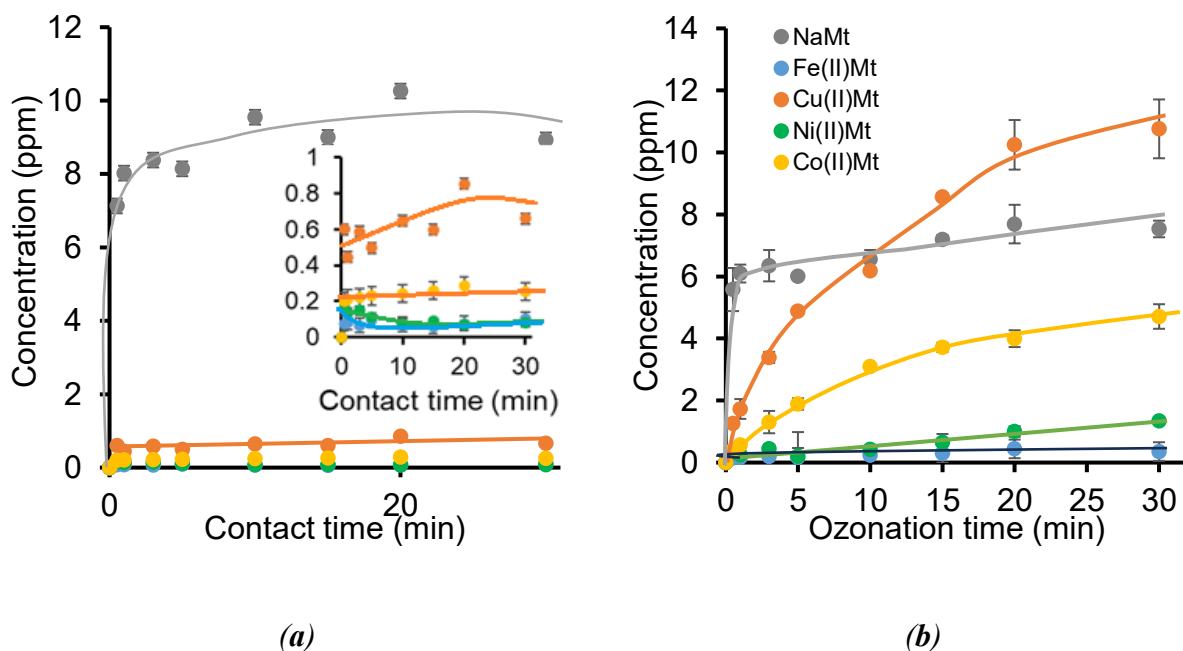


Figure 5.3. Evolution of the metal cation concentration in the NOF solution during adsorption (a) and clay-catalyzed ozonation (b). $T = 25\text{ }^{\circ}\text{C}$. $\text{pH} = \text{intrinsic level}$. Clay mineral amount: 50 mg. Sample volume: 25 mL. NOF initial concentration = $3.31 \times 10^{-6}\text{ M}$.

The most plausible explanation resides in a progressive cation release through ion-exchange promoted by the graduation formation of acidic species and pH decrease. This cation release was found to decrease in the following sequence $\text{Cu(II)} > \text{Co(II)} > \text{Ni(II)} > \text{Fe(II)}$. Attempts to correlate the effect of this cation release in the bulk solution with the clay mineral dispersion gave only a global decrease in the particle size with increasing amount of cation detected (**Figure 5.4a**). The finest particle sizes were observed around 1200–1700 nm for in the presence of dissolved Cu^{2+} and Na^{+} , while the bulkiest ones were noticed for Ni^{2+} , Co^{2+} and Fe^{2+} cations.

This cannot be explained only by the decreasing polarizing power and Lewis acidity of the transitional cations in the sequence $\text{Ni(II)} > \text{Cu(II)} > \text{Co(II)} > \text{Fe(II)}$. Here, the unavoidable effect of the presence of NOF molecules and competitive interactions with both forms of dissolved and adsorbed cations must also be involved, in agreement with Scheme 1. The particle size decrease contrasts with the decrease in the Zeta potential (ZP) and increase in the amount of free metal cation, which should rather trigger progressive surface protonation and clay lamella aggregation

and compaction (**Figure 5.4b**). This suggests that increased amount of released cation should globally produce an acidity improvement that promotes the adsorption of positively charged NOF species through their protonated ammonium groups, without necessarily leading to clay mineral aggregation.

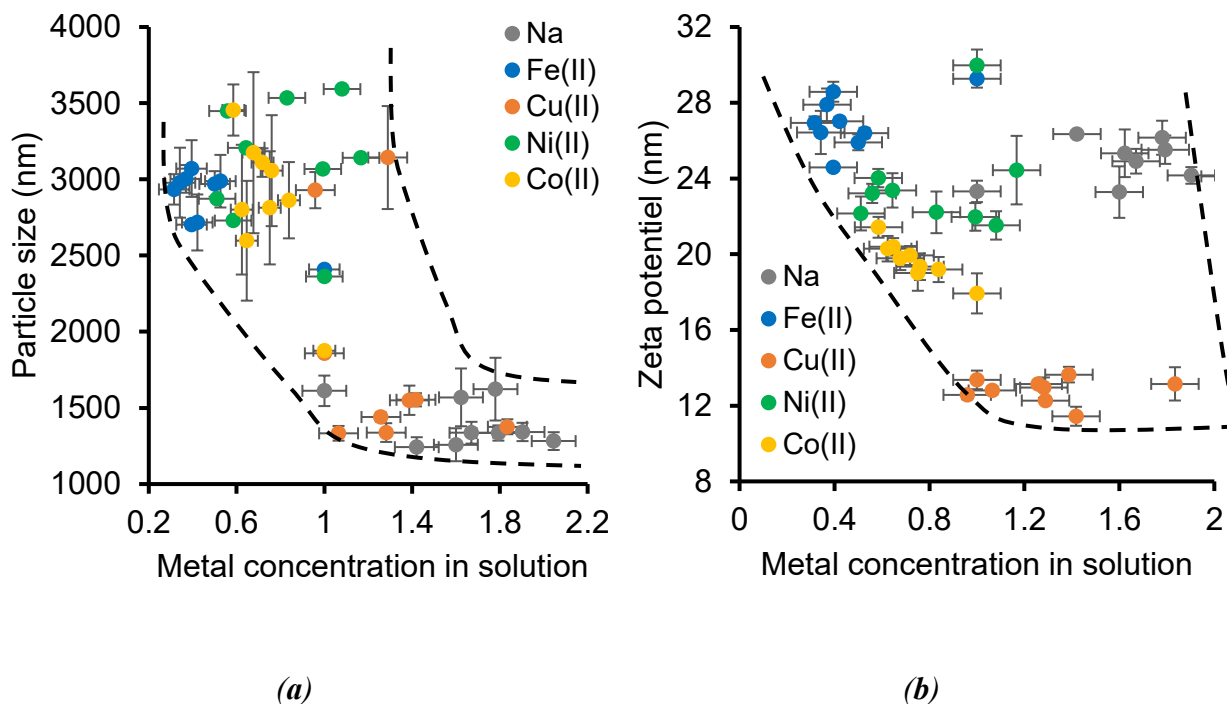


Figure 5.4. Particle size (a) and zeta potential (b) as function of the fraction of free metal ions detected in NOF solution during adsorption. T = 25 °C. NOF solution: initial pH = 5.2. Clay mineral amount: 50 mg. Sample volume: 25 mL. NOF initial concentration = 3.31×10^{-6} M.

At this pH level, NOF occurs predominately as a protonated species that adsorbs on the clay mineral surface via electrostatic interactions and simultaneously chelates both free and adsorbed metal cations. The most common coordination sites include the carboxylic and ketone groups. In addition, nitrogen atom of the piperazine ring and, in some cases hydroxyl atoms can also participate in coordination (Refat 2007, Zhang, Cai et al. 2012, Cao, Zhang et al. 2025). The involvement of these sites depends on factors such as pH, metal cation properties, and ligand-to-metal ratio (Cuprys, Pulicharla et al. 2018). Possible interactions between NOF molecules and the clay mineral surface may involve mainly hydrogen bonding and electrostatic forces. The metal ion

can coordinate with NOF either in a 1:1 (I) or 1:2 (II) (Metal:Ligand) ratio depending on the metal type, pH, and experimental conditions. Some studies, based on the Lineweaver–Burk equation, indicates that Cu(II) can form 1:1 complexes with enrofloxacin (Xu, Yu et al. 2025). That is why pH variation during ozonation plays a crucial role in controlling all binary [NOF:Cation], [NOF:NOF], [Clay:Clay] and [Clay:Cation] and ternary [NOF:Clay:Cation] and [Clay:NOF:Cation] interactions through protonation/deprotonation of the functional groups involved (Djidja, Dewez and Azzouz 2025).

Montmorillonite shows adsorption capacity that vary according to the acid-base character of the exchangeable cation and the charges of NOF molecules. NOF retention involves pH-dependent interactions that strongly influence the clay mineral aggregation/dispersion and the extent of the adsorption surface. At a intrinsic pH of the 1 mg/L NOF solution, Fe(II)Mt and Ni(II)Mt gave a 100% NOF removal rate (0.5 mg/g), versus 99–98% with Cu(II)Mt (0.47 mg/g) and barely 55–60% with NaMt (0.275–0.30 mg/g) (Djidja, Dewez and Azzouz 2025). These values vary according the pH and initial NOF concentration, which should be close to those of most NOF-contaminated wastewaters, and are in the same magnitude order as those provided by the literature under nearly similar conditions (Zelaya Soule, Flores et al. 2020, Sun, Wang et al. 2024). These different values appear to strongly influence the degradation of NOF and induced toxicity towards *L. minor*, which is the main objective of the present research. Nevertheless, the use of montmorillonite as adsorbent for NOF removal purposes often require improvements of the hydrophobic interaction by incorporating organic moiety as reported by ample literature.

5.3.3 Clay Mineral Amount Effect on Ozonation and Toxicity

Previous works already demonstrated the key role of clay minerals on the ozonation of organic substrates (Shahidi, Roy and Azzouz 2014, Larouk, Ouargli et al. 2017, Benghaffour, Foka-Wembe et al. 2022, Zekkari, Ouargli-Saker et al. 2022, Djidja, Dewez and Azzouz 2025, Djidja, Dewez and Azzouz 2025), but only few were devoted to such interaction, more particularly in correlation with the role of the catalyst amount on adsorption (Shahidi, Roy and Azzouz 2014, Mirilă, Boudissa et al. 2020, Benghaffour, Dewez and Azzouz 2023). In this regard, correlating the effects of pH and ozonation time to the catalyst amount could be a judicious approach for assessing the optimum parameter effects for maximum removal of NOF and minimum toxicity. For this purpose, an given

the high number of parameters and metal cations, a comparative study of ozonation through two 3^3 factorial experiment designs was restricted to only NaMt and Fe(II)Mt as the catalysts. In this regard, 27 ozonation attempts were performed for each clay catalyst within judiciously established variation ranges (**Table S6**) and model coefficient calculations (**Table S7**) revealed a favorable individual effect of pH in presence of NaMt for characteristic UV bands of NOF (Y_1 , Y_2 and Y_3). The sign of the coefficient describes the effect nature while the absolute value of the coefficient expresses the effect intensity (Dumitriu, Lutic et al. 1999, Azzouz, Rotar et al. 2002, Bodo, Ahmanache et al. 2004, Assaad, Azzouz et al. 2007, Shahidi, Roy and Azzouz 2014). The mere occurrence of optimum values of the pH, clay mineral concentration and ozonation time with optimum productions of NOF intermediates absorbing at the three wavelengths and of optimum values of the toxicity biomarkers considered (**Table S8**). This provides clear evidence of the occurrence of optimum favorable interaction, more particularly of the initial pH and clay mineral amount, for maximum NOF degradation in presence of NaMt and Fe(II)Mt and minimum induced toxicity (**Figure 5.5**).

As a general feature, increase in clay amount influences NOF ozonation (Y_2 and Y_3), which, in turn, determine the optimum pH for maximum clay mineral dispersion and contact surface according to the Critical Micellar Concentration of montmorillonite (Assaad, Azzouz et al. 2007). This also impacts the ratio chl a/b (Y_7). NOF conversion increase in time in presence of Fe(II)Mt ($a_3 = 14.786$) more than NaMt ($a_3 = 4.965$). As the ozonation process progresses, the production of reactive oxygen species (ROS) increases (Y_8) especially with Fe(II)Mt. The most significant interaction was registered for NOF toxicity ($a_{13} = 233.395$) through the initial pH and ozonation time. This indicates a synergy of these two parameters on ROS level (Y_8) with Fe(II)Mt. Strong interactions were also noticed between the pH and clay catalyst (a_{12}) for NOF conversion in presence of NaMt (Y_1 , Y_2 , Y_3). pH fluctuations are expected to impact the catalyst activity, and conversely, as already reported for the ozonation of oxalic acid with different catalyst (Shahidi, Roy and Azzouz 2014) and Orange-G with hematite/SBA-16 (Larouk, Ouargli et al. 2017).

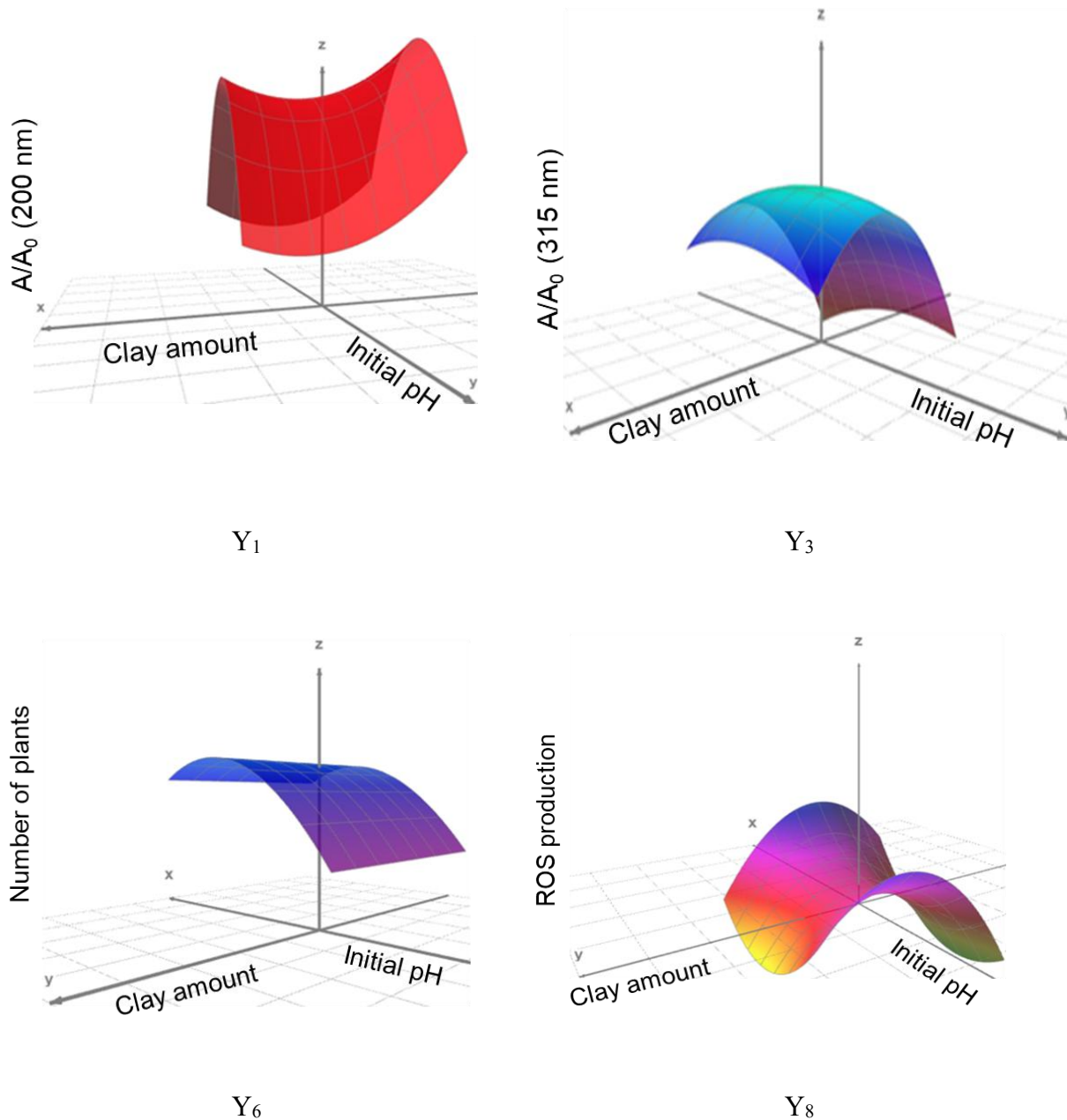


Figure 5.5. Response-surface of NOF ozonation progress as expressed in terms of A/A_0 at 200 nm (Y_1) and 315 nm (Y_3) for Fe(II)Mt and central reaction time ($X_3 = 0$) and toxicity as expressed by the number of plant specimens (Y_6) and ROS production (Y_8).

A favorable interaction for the three parameters with NaMt for the formation of hydroxylated and acidic intermediates was noticed for the relative absorbance (A/A_0) at 200 nm (Y_1) and ROS

production (Y_8). This indicates the formation of intermediates with high toxicity towards *L. minor*, which contrasts with Fe(II)Mt where a positive a_{123} coefficient was obtained for fresh weight and fronds number (Y_4 et Y_5). This result is of great importance, because it demonstrates that an increase in initial pH, clay mineral amount and ozonation time gave rise to less toxic end products.

5.3.4 Ozonation Effect on Photosynthetic System

Clay-catalyzed ozonation of NOF induced changes in the frond number, fresh weight, chlorophyll content and ROS production by *Lemna minor* indicating alteration in the pigment composition of the photosynthetic apparatus (Djidja, Dewez and Azzouz 2025) but its influence on photosynthetic activity remains unexplored. In photosynthesis, photosystem II defined as an enzyme complex located in the thylakoid membranes of chloroplasts (Sharma, Siedlewicz and Pazdro 2021). Deeper analysis of photosynthetic efficiency was carried out focusing on PSII performance. The altered OJIP transients based on a fluorescence induction curve plotted on a logarithmic time scale to describe the PSII electron transport activity (**Figure S3**) revealed a progressive decline in the photosynthetic performance following exposure *Lemna minor* to ozonated NOF solutions. A correlation between PI_{ABS} and production of ROS showed a general tendency (with few exceptions) consisting in decrease in photosynthetic performance is accompanied by an increased oxidative stress (**Figure 5.6**).

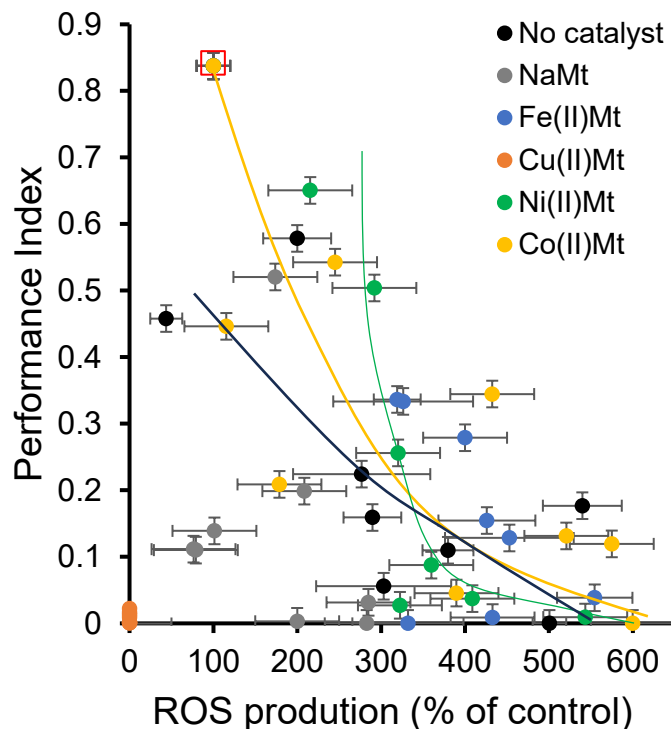


Figure 5.6. Correlation between the performance index of the photosynthetic system (PI_{ABS}) and ROS production of *Lemna minor* exposed to clay-catalyzed ozonation of norfloxacin. The red square accounts for the control sample used for ROS normalization. ROS are expressed as a percentage with respect to this control, which showed the highest performance index. The orange points represent the values assessed for the copper-containing samples. All these values are close to zero due to an almost total plant mortality.

FQs such as CIP disrupt the energy transfer between antenna chlorophyll molecules and the reaction center of photosystem II. This disruption leads to an accumulation of non-functional excited chlorophylls, a slower photoreduction of the primary quinone acceptor Q_A and consequently a reduction in the overall photochemical efficiency of PSII (Aristilde, Melis and Sposito 2010). FQs such as NOF were already found to exhibit a similar effect, but this toxicity appears to be enhanced by the presence of metal cations (Djidja, Dewez and Azzouz 2025). Ozonation was found to trigger a progressive release of metal ions revealed by ICP-OES analysis in the liquid medium from the clay mineral (**Figure 5.3b**). This arises from the progressive pH decrease due to the rise of acidic derivatives during ozonation, inducing cation exchange and release in the aqueous media by a proton excess. This explains, at least partly, the ozonation

mitigation time, imposing periodical catalyst regeneration by reverse cation exchange (Shahidi, Roy and Azzouz 2014). The consecutive accumulation of metal ions in the SIS medium (**Figure S4a–e**) is expected to result in higher contents in plant tissues. Cu(II) and Co(II) showed marked increases in their concentrations during the ozonation process (**Figure S4c,e**), confirming their high bioavailability and potential involvement in metal–pollutant complexes taken up by the plants. Once accumulated in plant tissues, metals cations can promote oxidative stress by generating reactive oxygen species (ROS). Moreover, metal ions can interact directly with chlorophyll molecules or photosynthetic enzymes, altering their structure and function. Based on these results, the toxic impact of clay-catalyzed ozonation of norfloxacin was stronger on the PSII activity of *L. minor*, due to a higher intracellular accumulation of metals and/or norfloxacin and its transformation products. Simultaneous exposure *Lemna minor* to NOF and metal ions reduced the growth parameters such as frond number and fresh weight (**Figure S5**). This combination also affected the chlorophyll content and ROS accumulation (**Figure 5.7**).

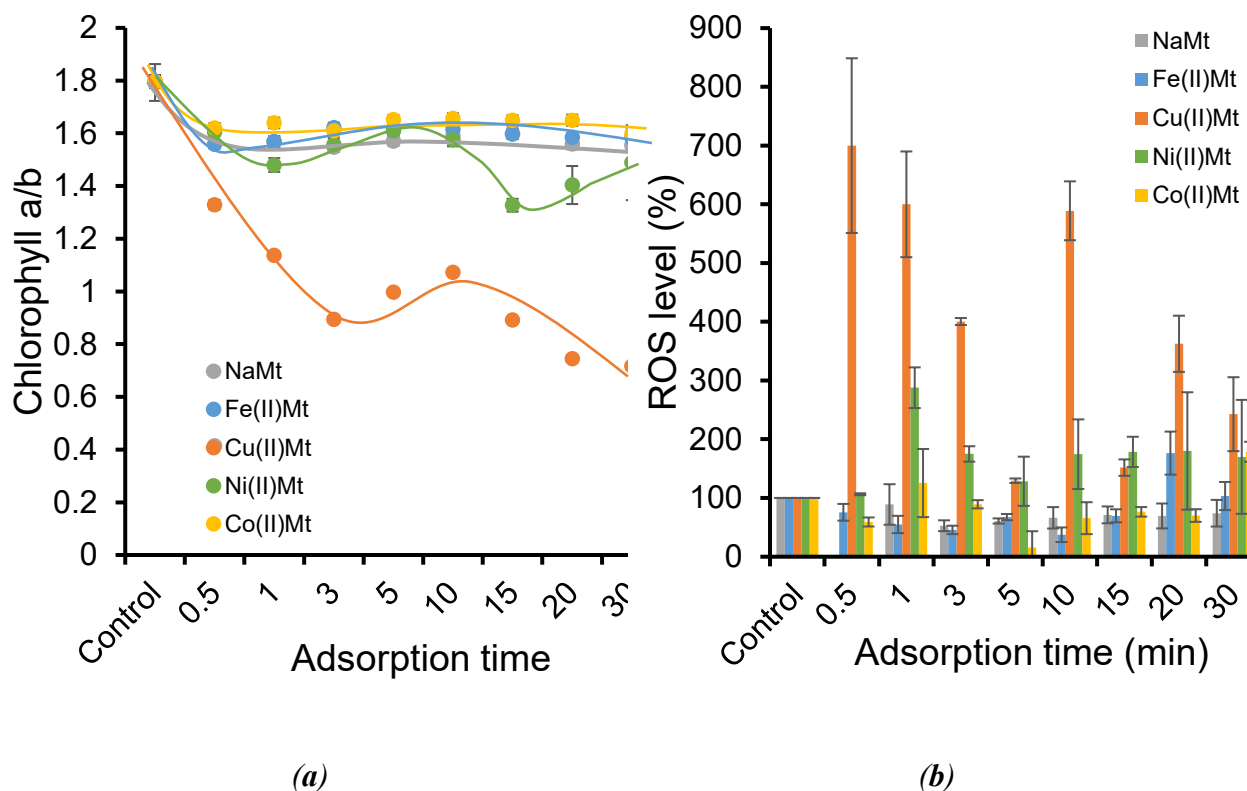


Figure 5.7. Effect of combined NOF and metal ion on chlorophyll *a/b* ratio (a) and ROS level (b) after 7 days of exposure to *Lemna minor*. Triplicates measurements were achieved including the control sample. The control sample consisted of plants grown in SIS medium.

5.3.5 NOF Interaction with Clay-Supported Zero-Valent Metal

The incorporation of ZVMs into montmorillonite induced a red shift of the broad FTIR band around 3500 cm^{-1} (Figure S6). This band is assigned to O-H stretching vibrations (Khan, Sohni et al. 2023). This shift toward lower wavenumbers indicates interactions between ZVMs and surface hydroxyl groups of the clay mineral likely through coordination or hydrogen bonding. For Cu^0Mt , the new band at 1412 cm^{-1} confirmed the immobilization or reduction of Cu^0 (Mekewi, Darwish et al. 2016).

M^0 incorporation in clay mineral particles is expected to contribute to surface interactions and possible complexation with both free and adsorbed NOF molecules. The intensity of the absorbance of the characteristic UV-Vis band of NOF at 275 nm (**Figure 5.8**) rapidly decreased up to total disappearance upon direct contact with M^0/Mt , suggesting strong NOF interaction with the surfaces of the metal-loaded clay minerals and advanced depletion of free NOF molecules.

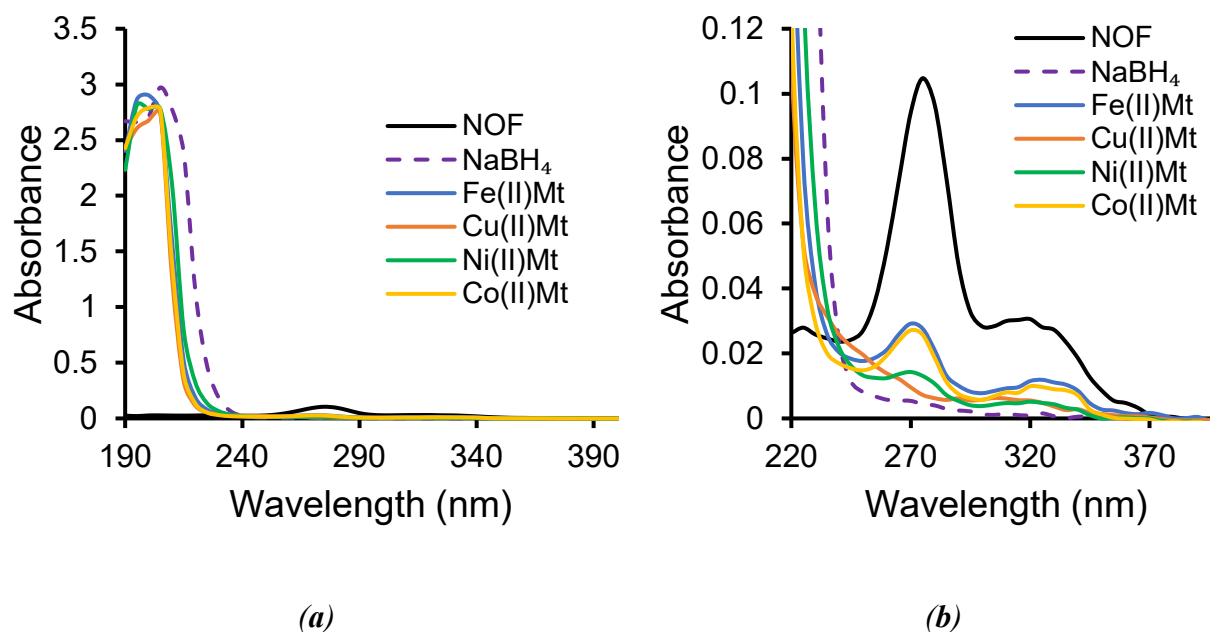


Figure 5.8. UV-Vis Spectra of NOF-Metal exchanged montmorillonite mixtures upon NaBH₄ reduction under nitrogen atmosphere (a) and close-up for the region 220–370 nm (b) as compared to NOF alone, NOF + NaBH₄ mixture and NaBH₄ alone. Clay mineral amount: 50 mg. Sample volume: 25 mL. NOF initial concentration = 3.31×10^{-6} M. 10 mL NaBH₄ (0.1 M).

This was confirmed by HPLC analysis, which showed a ca. 60–70% reduction in the NOF peak area upon NaBH₄ addition, slightly higher in presence of Ni⁰Mt and slightly lower for Co⁰Mt (**Figure 5.9a**). This provides clear evidence of the reduction of transitional cation on the montmorillonite surface. The disappearance of the cationic species which commonly induce acidic pH agrees with the visible pH increase during the metal reduction process (**Figure 5.9b**).

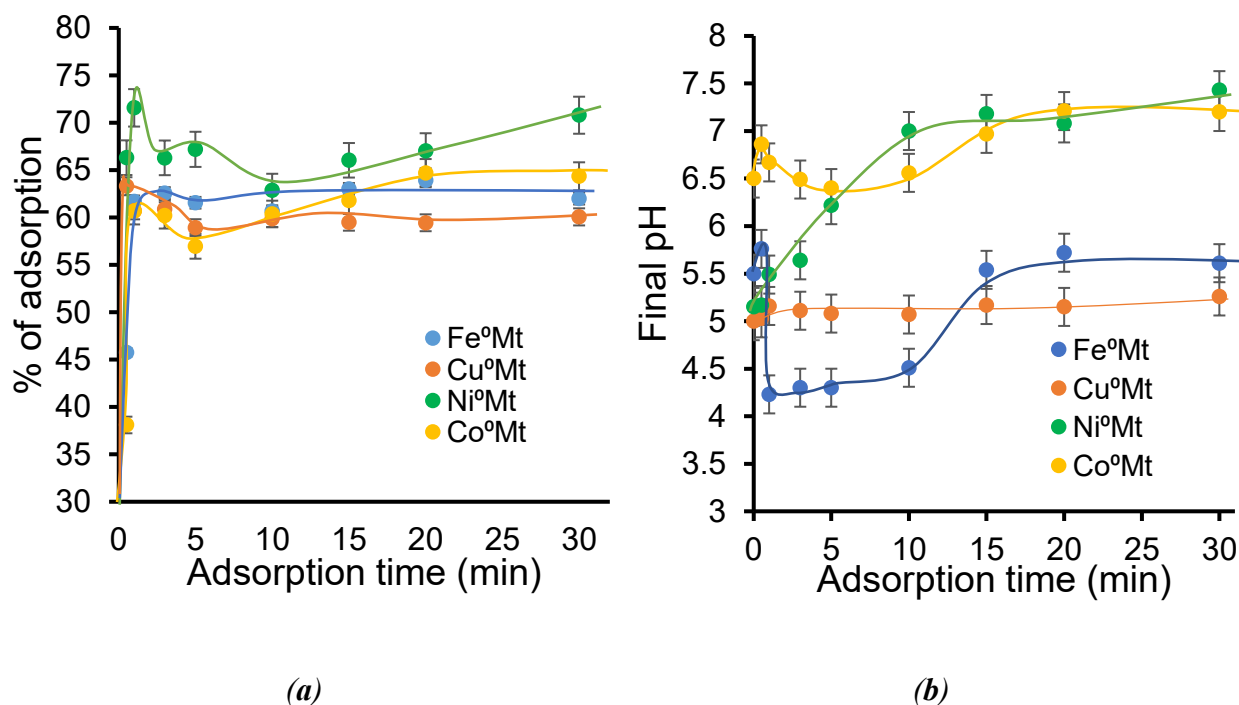


Figure 5.9. Evolution in time of adsorption yield as determined by HPLC-DAD (a) and evolution of instant pH (b). Clay mineral amount: 50 mg. Sample volume: 25 mL. NOF initial concentration = 3.31×10^{-6} M.

Cu⁰Mt displays almost similar adsorptive and catalytic properties with almost similar effects as Fe⁰Mt on the biomarkers that allow assessing NOF toxicity towards *L. minor*. Nevertheless, copper is known to be a more toxic metal in both cationic and zero-valent forms as compared to iron, which can rather act as nutrient for plants in suitable concentrations.

5.3.6 Toxicity of NOF Adsorbed on ZVM-Loaded Montmorillonite

As compared to the starting exchanged montmorillonite M(II)Mt, ZVM-loaded montmorillonite (M⁰Mt) exhibited higher toxicity towards *L. minor* (**Figure 5.7**). After 30 min of exposure, the Chl *a/b* ratio decreased from 0.4 for Cu⁰Mt compared to 0.7 for Cu(II)Mt, from 1.5 for Ni(II)Mt and Co(II) to 0.45 for Ni⁰/Mt and 0.7 for Co⁰/Mt, and from 1.6 for Fe(II)Mt to 1.50 for Fe⁰Mt (**Figures 7a and 10a**). The most pronounced reduction was observed for nickel, where the Chl *a/b* ratio dropped by approximately 70%, followed by copper with 43%, cobalt with a 53% decrease. Iron showed only a slight reduction of 6%. M⁰/Mt induced a decrease in the chlorophyll *a/b* ratio

(Figure 10a), while Fe^0/Mt and Co^0/Mt gave higher ROS production as compared to the control sample (Figure 5.10b). In the meantime, a strong plant growth inhibition was observed, as reflected by a decrease in both frond number and fresh weight (Figure S7).

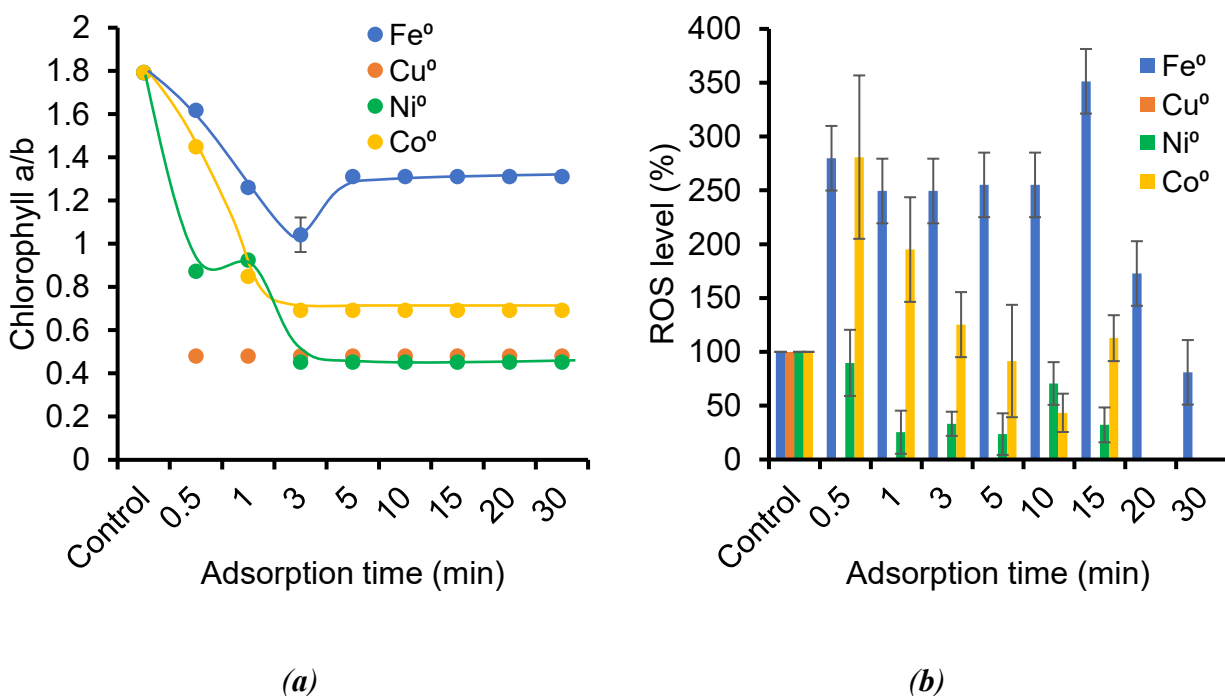


Figure 5.10. Change in Chlorophyll ratio (a) and ROS level (b) of *Lemna minor* in NOF adsorption in clay-supported ZVM after 7 days of exposure. Triplicates measurements were achieved including the control sample. The control sample consisted of plants grown in pure SIS medium.

This difference is explained by the distinct mechanisms involved in each system. In the case of M(II)Mt , the toxicity involves ionic interactions through ions that can bind to cellular components but are partially regulated by physiological defense mechanisms into lower concentration (Djidja, Dewez and Azzouz 2025). Conversely, M^0Mt undergoes gradual surface oxidation with the production of reactive oxygen species more particularly with water and dissolved (Deng, He et al. 2025), which amplifies the overall toxic response. ZVM were already used for the aerobic removal of dyes contaminants methyl orange and congo red at neutral pH, involving Cu^0 reduction into Cu(I) and formation of hydroxyl radicals (Dong, Ai and Zhang 2014, de Sousa, de Oliveira et al. 2019). Reduction appears as being less efficient than oxidation, given that ciprofloxacin removal,

for instance, was about 100% in presence of oxygen and 35% in anaerobic conditions after 2 h of reaction (Sousa, Oliveira et al. 2019). Here, Cu^0 is assumed to oxidize into Cu(I) and Cu(II) releasing electrons that reduce oxygen and generate ROS, which are responsible for the CIP degradation.

However, under oxygen-free conditions and the presence of a reducing agent, zero-valent iron (Fe^0) is assumed to react with protons and/or dissolved oxidizing agent in water, releasing electrons and consuming protons to generate highly reducing active hydrogen species (Wei, He et al. 2024). These active hydrogen atoms subsequently donate electrons to organic cations, with potential cleavage of $-\text{C}=\text{N}$ and $-\text{C}=\text{C}$ bonds. ZMV were recognized as being highly effective for the aerobic degradation of azo dyes, but usually show lower efficiency in non-azo organic pollutants such as rhodamine B, methylene blue, and sodium pentachlorophenate (Dong, Ai and Zhang 2014). The presence or absence of oxygen determines whether pollutant removal is dominated by oxidative/coagulation processes or by reductive transformations.

5.4 Conclusions

This study highlights the critical role of metal speciation and valence state in determining both the reactivity of norfloxacin in metal-loaded suspensions in water and ecotoxicological impact. NOF binds to metals through carboxyl, carbonyl, and nitrogen donor groups. Dissolved metals play a central role in the clay mineral dispersion in the aqueous media and determine its interactions with organic molecules and catalytic activity. Zero valent metal-loaded montmorillonite exhibited markedly higher toxicity, because they amplify the ecotoxicological risks through combined effects of metal release, ROS generation, and pollutant-metal complexation. Such findings provide evidence that coupling degradation studies with ecotoxicological assessments is an essential requirement when evaluating the performance of advanced clay-based treatment in the presence of metals and environmental safety.

CONCLUSION GÉNÉRALE

Cette thèse s'inscrit dans un contexte actuel lié à la contamination croissante des milieux aquatiques par les antibiotiques, et plus particulièrement par les fluoroquinolones. L'objectif principal était d'élucider le rôle des cations métallique dans la dégradation oxydative de la Norfloxacin avec une approche inspirée des processus naturels dans les écosystèmes aquatiques. Cette approche combinant les propriétés intrinsèques des matériaux argileux naturels à l'action oxydative de l'ozone a permis d'atteindre l'objectif principal de la thèse, qui consistait à démontrer que les polluants organiques se dégradent dans les sols et les suspensions argileuses des milieux aquatiques par des processus similaires. Une évaluation écotoxicologique approfondie a permis de comprendre que la dégradation oxydative produit inévitablement une acidification du milieu néfaste aux espèces qui y vivent et génère une grande variété d'intermédiaires dont l'évolution de la distribution fait fluctuer dans le temps l'écotoxicité en fonction du milieu. Les résultats obtenus ont permis d'aboutir aux conclusions propres à chaque partie de ce travail.

Première partie

Les résultats obtenus dans cette partie ont tout d'abord permis de démontrer que les matériaux argileux à base de montmorillonite en raison de leur large présence dans les sols et les milieux aquatiques, constituent des matrices catalytiques pertinentes pour l'ozonation de la norfloxacin en milieu aqueux. L'ajout de montmorillonite améliore la dégradation de la NOF, mais conduit simultanément à la formation de produits pouvant être toxiques pour *Lemna minor*, d'où les principales conclusions suivantes :

1. La NOF présente une toxicité intrinsèque qui exerce un stress physiologique et oxydatif direct sur les macrophytes aquatiques, affectant à la fois leur croissance et leur fonctionnement photosynthétique.
2. La disparition partielle de la NOF ne s'accompagne pas systématiquement d'une diminution de la toxicité car son oxydation progressive par l'ozone entraîne une évolution non linéaire de la toxicité au cours du temps.

3. Le processus d'oxydation gouverne la distribution, la nature et la prédominance des dérivés issus de la molécule-mère.
4. Une ozonation non catalytique assez prononcée semble produire une dégradation de la structure même des groupements phényle et pyridinyle conduisant à des clivages de cycles, sans forcément atteindre une minéralisation de la molécule-mère.
5. L'amélioration de l'efficacité de l'ozone dans la dégradation de NOF repose sur une combinaison optimale de plusieurs facteurs tels que l'adsorption sélective de la molécule et/ou de ses intermédiaires sur les sites actifs de la surface argileuse, les interactions de complexation avec le cation métallique et autres. Cette sélectivité gouverne l'orientation de molécule de NOF lors de son adsorption, et détermine les voies réactionnelles et la formation ou la suppression de certains intermédiaires.

Dans ce contexte, les systèmes Fe(II)Mt et Cu(II)Mt se distinguent par une génération plus limitée d'intermédiaires suggérant des voies d'oxydation plus efficaces. Sur le plan écotoxicologique, une toxicité globalement plus faible est observée en présence de NaMt et Fe(II)Mt comparativement aux autres catalyseurs argileux, indiquant que ces matériaux favorisent la formation de produits de transformation moins toxiques pour *Lemna minor*.

Deuxième partie

Cette partie a permis d'analyser l'évolution des interactions moléculaires impliquant les cations métalliques, les catalyseurs argileux et la norfloxacin au cours de l'ozonation en milieu aqueux. Les résultats obtenus montrent que l'évolution du pH pendant le processus joue un rôle central, en influençant simultanément l'état de dispersion du catalyseur argileux, son efficacité dans la décomposition oxydative de la NOF et la toxicité finale du milieu traité. Ce qui a permis d'aboutir aux plus importantes conclusions suivantes :

1. La variation du pH modifie les propriétés de surface des argiles, notamment la charge de surface et le potentiel Zêta, l'état de dispersion colloïdale ainsi que l'accessibilité aux sites, influençant directement les interactions avec la NOF et l'efficacité du processus d'ozonation catalytique.
2. Un milieu acide favorise la dégradation de NOF, mais une acidité excessive réduit la surface de contact et l'activité catalytique des suspensions argileuse par agrégation des feuillets d'argile.
3. À pH plus élevé, le matériau argileux présente un état de dispersion plus favorable, résultant d'une forte augmentation de la répulsion par interaction électrostatique entre les feuillets dû à une déprotonation accentuée des groupements silanols. Cependant une alcalinité excessive affecte l'adsorption de la NOF qui acquiert des charges négatives répulsives envers la surface déjà chargée négativement.
4. La réduction de la taille des particules argileuses et l'amélioration de leur dispersion augmentent la surface spécifique accessible et favorisent le contact entre la NOF, l'ozone et les sites catalytiques.
5. Le processus global implique une synergie entre les différents types d'interaction dont la chélation Cation:NOF, NOF:Montmorillonite, Cation :NOF:Montmorillonite et NOF:Cation: Montmorillonite, ce qui favorise l'adsorption et ultimement l'ozonation.
6. Les interactions entre le métal et la NOF modifient réciproquement leurs toxicités intrinsèques, en altérant la biodisponibilité de la NOF et en orientant la nature des produits de transformation générés au cours de l'ozonation.
7. La présence de surfaces argileuses modifie le comportement des cations en influençant leur pouvoir polarisant, ce qui affecte leurs interactions avec les espèces chimiques présentes en solution.

8. Les cations Na^+ et Fe^{2+} se distinguent par leur capacité à améliorer la dispersion des particules argileuses et à limiter l'agrégation, favorisant ainsi une oxydation plus prononcée conduisant à la formation de moins d'intermédiaires et une toxicité plus faible.
9. Certains cations métalliques induisent des perturbations plus marquées des paramètres photosynthétiques, soulignant l'impact direct de la nature du cation sur les réponses physiologiques de *Lemna minor* et confirmant le rôle central des interactions [Métal:Montmorillonite:NOF] dans la modulation de la toxicité finale.

Troisième partie

Cette étude a mis en évidence le rôle central des interactions moléculaires impliquant un polluant organique, des cations métalliques et des suspensions argileuses contaminées par des métaux, tant au cours des étapes d'adsorption que lors de l'ozonation en milieu aqueux, et leur impact direct sur un *L. minor*. Les résultats obtenus montrent que la toxicité observée ne résulte pas d'un facteur isolé, mais de l'équilibre entre les comportements physicochimiques des différentes espèces dispersées dans le milieu réactionnel. Parmi les conclusions les plus saillantes, figurent les suivantes:

1. La molécule de NOF se lie aux métaux par l'intermédiaire de ses groupements à comportement de base de Lewis. Ces groupements sont donneurs de paires d'électrons non liants tels le carboxyle, carbonyle et les fonctions azotées, Ceci conduit à la formation de complexes dont la stabilité et la réactivité dépendent étroitement de la nature et de la spéciation du métal.
2. La libération partielle de cations métalliques à partir de la surface de l'argile au cours du processus d'oxydation altère l'activité catalytique des matériaux argileux, en modifiant à la fois la réactivité chimique du système, la distribution des espèces oxydantes et la toxicité du milieu.
3. L'analyse conjointe des performances de dégradation et des réponses écotoxicologiques a permis d'établir une corrélation entre l'existence d'interactions binaires et ternaires

optimales impliquant la NOF, les cations métalliques et la matrice argileuse et une toxicité minimale vis-à-vis de *L. minor*.

4. La spéciation du métal, influence à la fois la réactivité de l'ozone et la toxicité envers l'organisme exposé.
5. La présence de métaux zéro-valents favorise la génération accrue d'espèces réactives de l'oxygène, intensifiant le stress oxydatif chez *Lemna minor*.

Perspectives

1. Les résultats obtenus dans cette thèse ouvrent de nombreuses perspectives scientifiques et applicatives pour approfondir la compréhension des mécanismes impliquées dans l'adsorption et l'ozonation de la norfloxacin, ainsi que le développement de procédés de traitement des eaux et des sols plus efficaces, sélectifs et respectueux des écosystèmes.
2. Une première perspective consiste à étendre l'approche développée à d'autres familles de polluants organiques persistants. L'étude de composés présentant des propriétés physicochimiques différentes de celles de la norfloxacin permettrait notamment de déterminer dans quelle mesure les mécanismes observés dans cette thèse peuvent être généralisés. Une telle approche contribuerait également à mieux comprendre l'influence de la structure moléculaire des contaminants sur leur adsorption à la surface des matériaux, leur réactivité vis-à-vis de l'ozone ainsi que leur transformation au cours du traitement.
3. L'étude pourrait également être élargie à d'autres matrices environnementales, telle que les eaux salines, afin d'évaluer l'influence de la salinité et de la composition ionique sur les processus d'adsorption, d'ozonation et sur l'évolution de la toxicité. En particulier, l'étude des interactions avec la matière organique naturelle, notamment les acides humiques et fulviques. Ces composés peuvent entrer en compétition avec les contaminants pour les sites d'adsorption et consommer une partie de l'ozone et des espèces radicalaires formées au cours du traitement. Leur présence pourrait donc modifier l'efficacité de dégradation, la biodisponibilité des contaminants ainsi que la nature et la toxicité des produits de transformation.

4. Une autre perspective réside dans l'étude de systèmes multi-contaminants plus représentatifs des conditions environnementales réelles. La présence simultanée de plusieurs contaminants organiques ou inorganiques peut modifier les phénomènes d'adsorption, de transformation et de biodisponibilité. L'évaluation conjointe de la NOF avec d'autres polluants organiques ou inorganiques, tels que les microplastiques, pourrait permettre d'analyser les phénomènes de co-adsorption.
5. Sur le plan écotoxicologique, l'élargissement des bio-indicateurs à d'autres espèces et à différents niveaux trophiques, incluant des microalgues, des invertébrés aquatiques et des microorganismes du sol, permettrait d'obtenir une vision plus intégrée et plus réaliste des impacts environnementaux des procédés étudiés. Une approche multi-espèces permettrait également de vérifier si les effets observés chez *Lemna minor* sont représentatifs d'autres organismes et de mieux caractériser les risques associés aux contaminants résiduels, aux produits de transformation ainsi qu'aux éventuelles espèces métalliques présentes dans le milieu.

Ainsi, les travaux futurs pourraient permettre de passer progressivement de systèmes expérimentaux contrôlés à des conditions environnementales plus complexes. Cette démarche contribuerait à mieux définir le potentiel et les limites de l'utilisation des montmorillonites modifiées combinées à l'ozonation pour le traitement de contaminants émergents, tout en intégrant simultanément les performances de dégradation, les mécanismes physicochimiques et les impacts écotoxicologiques.

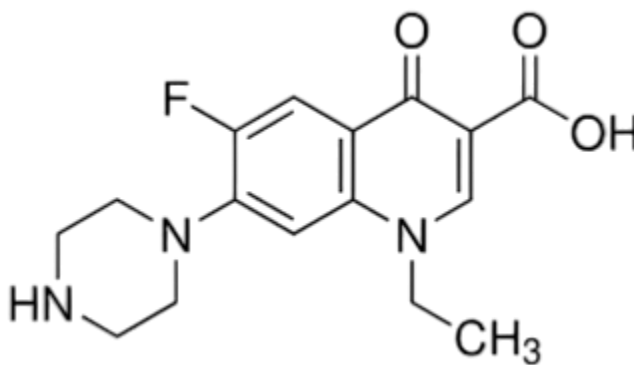
ANNEXE A
SUPPORTING INFORMATION DE L'ARTICLE 1

Clay-catalyzed ozonation of Norfloxacin - Effects of metal cation and degradation rate on aqueous media toxicity towards *Lemna minor*

Roumaissa Djidja^a, David Dewez^{a,*} and Abdelkrim Azzouz^{a,b,*}

Supporting information

1. Norfloxacin - molecular structure and features



Scheme S1. Molecular structure of Norfloxacin (NOF).

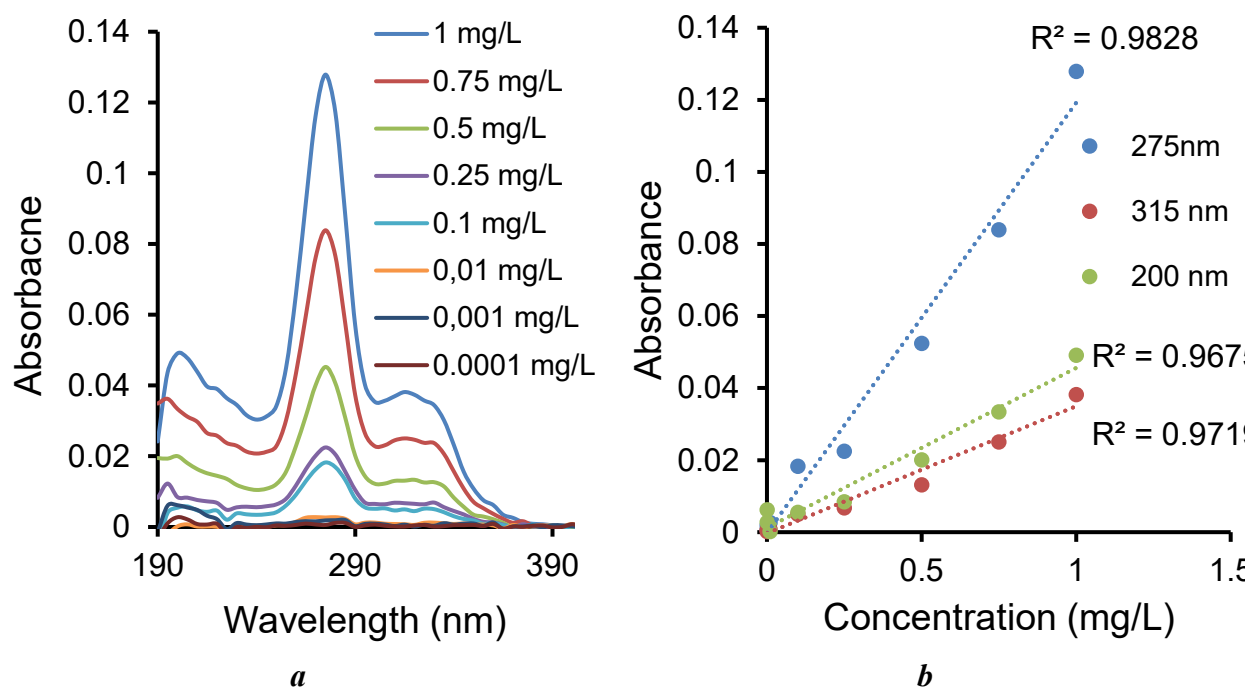
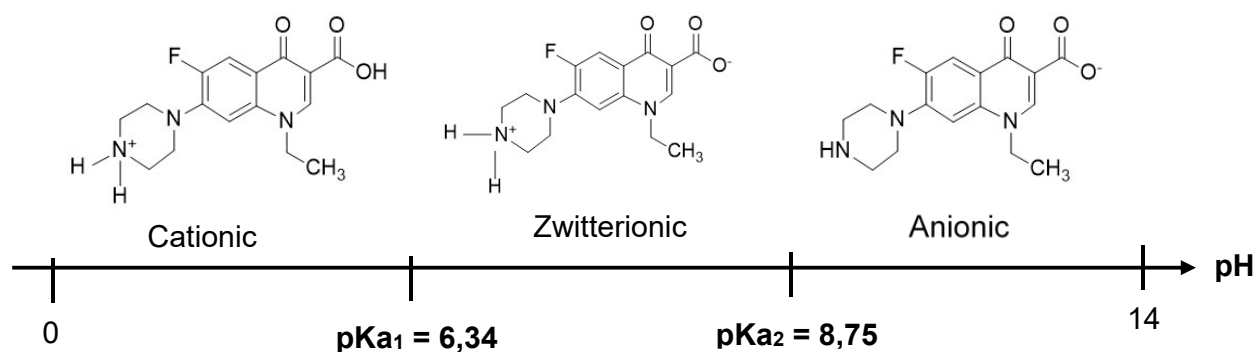


Fig. S1. UV-Vis spectrum of NOF aqueous solutions at different concentrations (a) and calibration plots for the different absorption bands (b). The spectra display characteristic absorption bands corresponding to different electronic transitions in the NOF molecule, showing a linear relationship with the concentrations of NOF.

Table S1. Some features of Norfloxacin

Compound	Norfloxacin (NOF)
Supplier	Sigma aldrich. Product No. LRAD2292
IUPAC denotation	1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid
Chemical formula	C ₁₆ H ₁₈ FN ₃ O ₃
Classification family	Fluoroquinolone
Molecular weight	319.33 g/mol
Functional groups	Quinolone: two-ring nitrogen containing with a ketone Piperazinyl ring: six-membered ring containing two nitrogen atoms at opposite positions.
Values of pKa	pKa1 = 6.34 and pKa2 = 8.75 (Wang, Cheng et al. 2020)
Main property	Antibiotic activity
Solubility	450 mg/L at 25 °C at pH 7.5 (it pH dependent) (Loh, Tan and Peh 2016) 1.01g/L (Zhao, Li et al. 2022)
Lamda max	274, 325, 336 nm
Solution concentration mol/L	3.31x10 ⁻⁶
Intrinsic pH	5.28 in nanopure water



Scheme S2. Ionic forms of NOF as a function of pH.

2. Clay catalyst interaction with aqueous NOF solution

Table S2. Initial physico-chemical features of different clay suspensions in water and NOF solution before ozonation.

Clay catalyst	Aqueous media	pH	Particule size (nm)	Zeta potentiel (mv)
Bentonite	Water	9.07	5897	-25.06
NaMt		9.03	2270	-22.86
Fe(II)Mt		3.83	2829	-30.91
Cu(II)Mt		5.45	2327	-9.77
Bentonite	Norfloxacin solution (3.31×10^{-6} M)	9.10	5759	-25.60
NaMt		9.00	2614	-21.14
Fe(II)Mt		3.85	2409	-29.26
Cu(II)Mt		5.48	2133	-10,06

3. UV-Vis spectrum changes upon non-catalytic ozonation

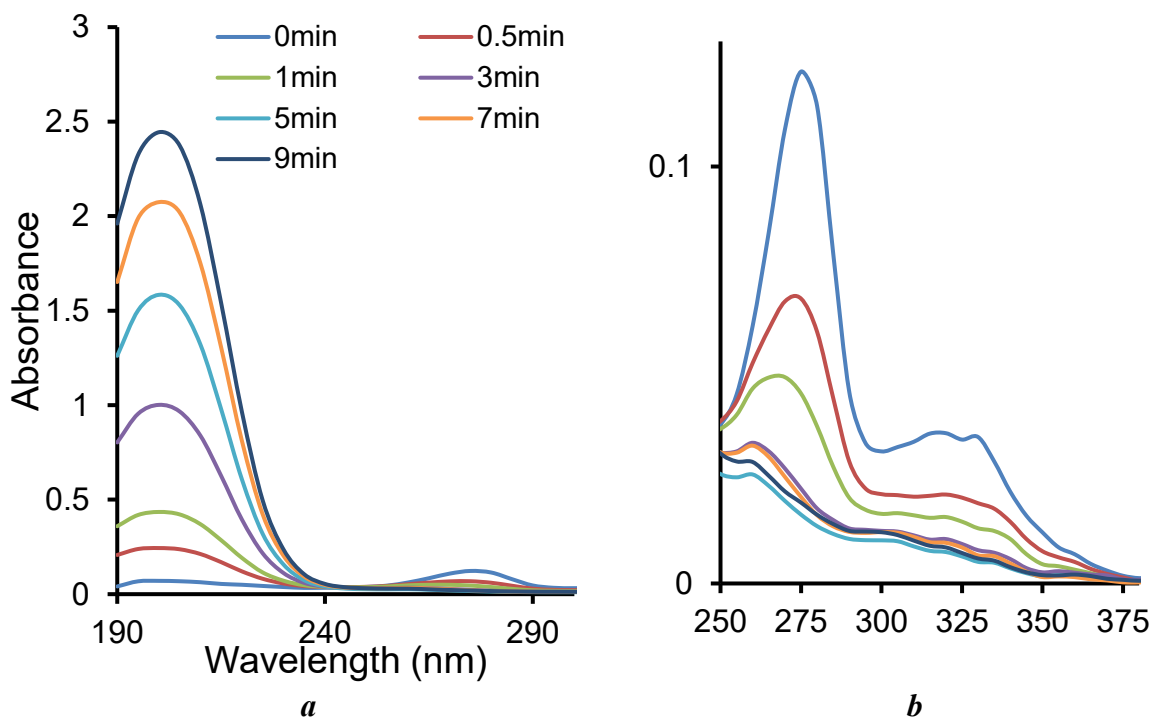


Fig. S2. Evolution of the UV-Vis spectrum of norfloxacin during non-catalytic ozonation (a) and close-up on the 250-375 nm region (b). T = 25 °C. pH = 5.28. Quartz cell = 1 cm. O₃ throughput: 600 mg h⁻¹. Sample volume = 25 mL. NOF initial concentration: 3.31×10⁻⁶ M.

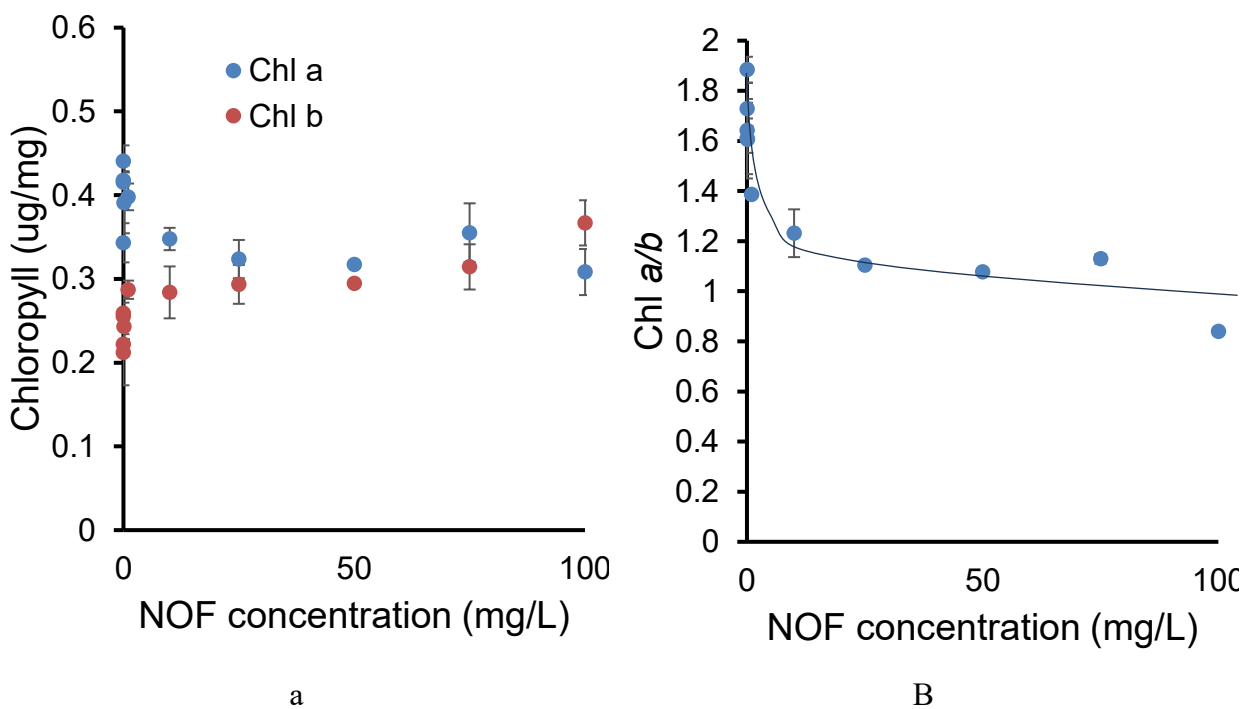


Fig. S3. Effect of varying NOF concentrations (ranging from 0.001 to 100 mg/L) on the content of *L. minor* chlorophyll *a* and *b* ($\mu\text{g}/\text{mg}$ fresh weight) (a) and chlorophyll *a/b* ratio (b). The average data and standard deviation presented in these figures resulted from triplicate measurements under similar operating conditions as in Fig. 1. The control sample (Blank) consisted of *L. minor* grown in antibiotic-free SIS medium for 7 days. Triplicates measurements were achieved for all concentrations considered including the same antibiotic-free control sample.

4. UV-Vis spectrum changes upon upon catalytic ozonation

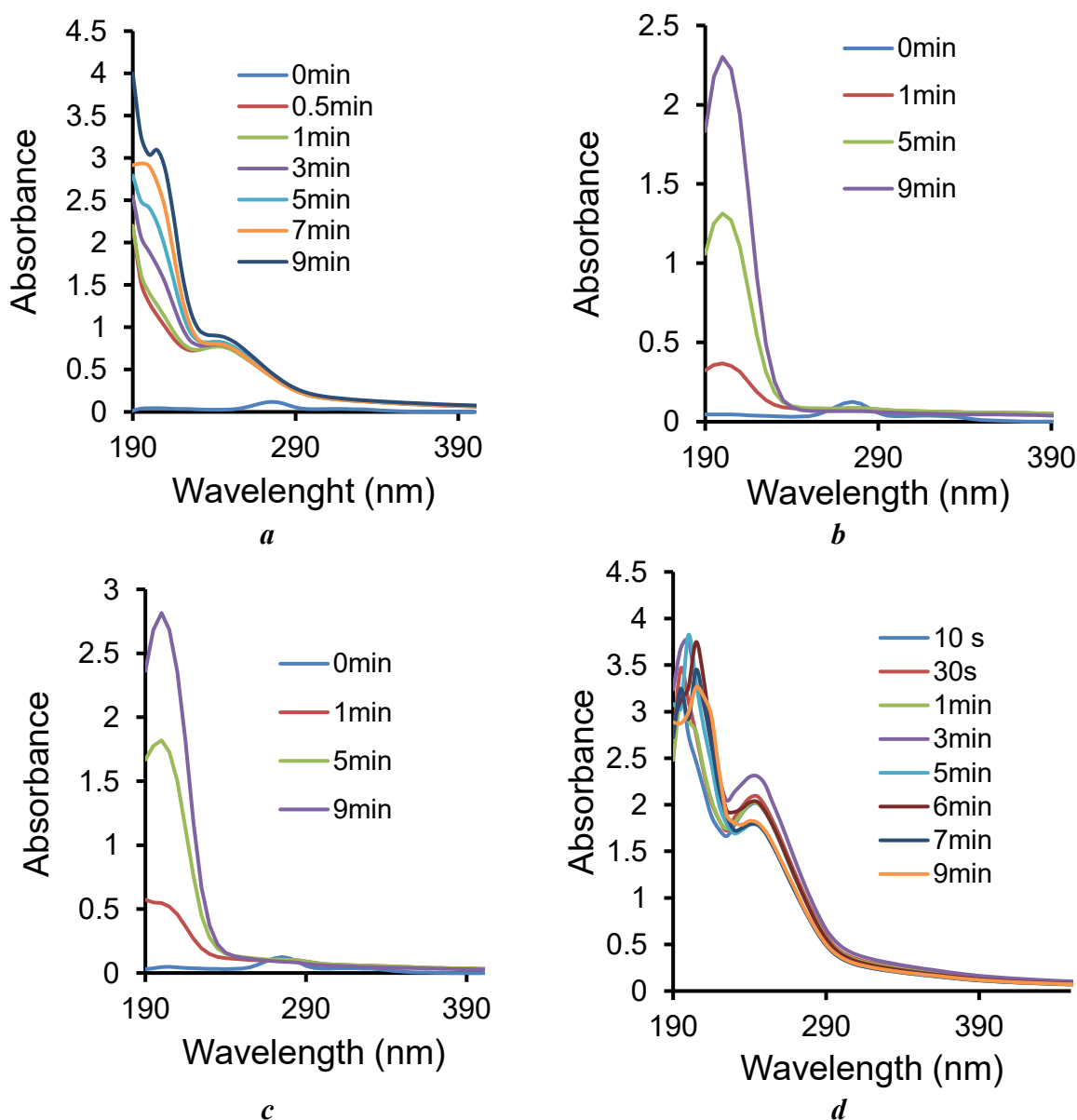


Fig. S4. Evolution of NOF UV-Vis spectra during ozonation in the presence of 2 g/L NaMt (a), Fe(II)Mt (b), Cu(II)Mt (c) and Bentonite (d). Sample volume = 25 mL. Triplicate

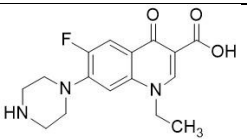
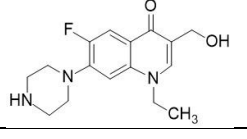
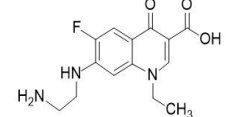
measurements were achieved including the non-ozonized NOF solution taken as the starting control sample (1 mg/L).

5. LC-MS identification of commercial NOF impurities

Table S3. Operating conditions of LC-MS analysis of the ozonized reaction mixture.

Module	Parameter	Specification
LC	HPLC	Agilent Technologies 1260 series
	Column	Hypersil GOLD PFP, 100 x 2.1 mm, 3µm, Thermo Scientific
	Mobile phases	A: water with 0.1% Formic Acid B: methanol
	Gradient	0-0.5 min, 5% B; 0.5-2.0 min, 5-10% B; 2.0-8.0 min, 10-25% B; 8.0-10.0 min, 25-90% B; 10.0-11.0 min, 90% B; 11.0-11.2 min, 90-5% B; 11.2-18.0 min, 5%B.
	Flow rate	0.4 mL /min
	Column temperature	40°C
	Sample volume	5 µl
MS	MS	G6224A TOF, Agilent Technologies
	Ion source	Dual ESI (Dual Electrospray Ionization)
	Capillary voltage	3500 V
	Capillary temp.	350°C
	Data acquisition	Full scan, m/z 100 - 1500

Table S4. Products identified by LC-MS in the starting and non-ozonized aqueous solution of NOF (Millipore-Sigma) prepared in Nanopure water.

m/z (g/mole)	Molecular structure	Molecular formula	Retention time (min)	I/I ₀	Proportion (mol. %)
320		C ₁₆ H ₁₈ F N ₃ O ₃	7.83	1	97.86
306		C ₁₅ H ₂₀ F N ₃ O ₂	6.8	0.017286	1.69
294		C ₁₄ H ₁₆ F N ₃ O ₃	6.56	0.004628	0.45

6. SIS media preparation

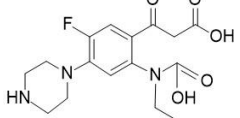
Table S5. Preparation and composition according to Swedish Standard for *Lemna* growth medium (SIS).

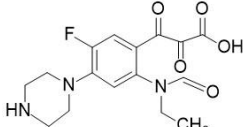
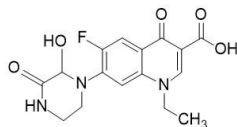
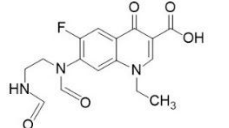
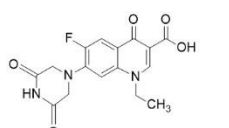
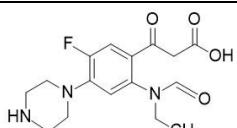
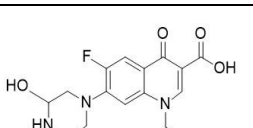
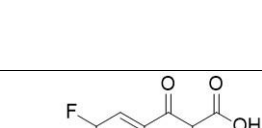
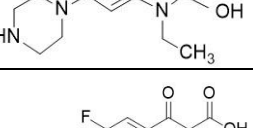
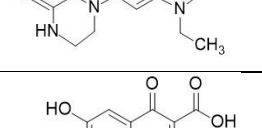
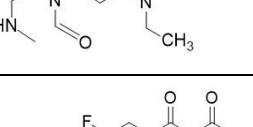
Stock solution	Chemicals	Concentration in		Manufacturer
		Stock solution (g/L)	SIS medium* (mg/L)	
I	Na NO ₃	8.500	85.000	Anachemia, ACS grade Anachemia
	KH ₂ PO ₄	1.340	13.400	
II	MgSO ₄ .7H ₂ O	15.000	75.000	Anachemia
III	CaCl ₂ .2H ₂ O	7.200	36.000	Anachemia, ACS grade
IV	Na ₂ CO ₃	4.000	20.000	Fisher Scientific
V	ZnSO ₄ .7H ₂ O	0.050	0.050	Anachemia, ACS grade
	H ₃ BO ₃	1.000	1.000	Anachemia, ACS grade
	MnCl ₂ .4H ₂ O	0.200	0.200	Anachemia, ACS grade
	Na ₂ MoO ₄ .2H ₂ O	0.010	0.010	Fisher Chemicals
	CuSO ₄ .5H ₂ O	0.005	0.005	Anachemia, ACS grade
	Co(NO ₃).6H ₂ O	0.010	0.010	Anachemia, ACS grade
VI	FeCl ₃ .6H ₂ O	0.170	0.840	Anachemia
	Na ₂ -EDTA.2H ₂ O	0.280	1.400	
VII	MOPS (buffer)	490.000	490.000	Anachemia

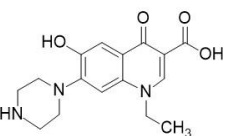
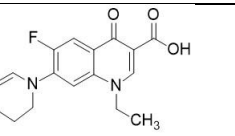
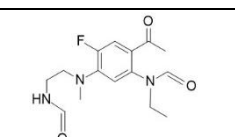
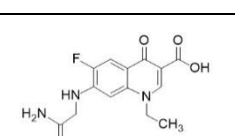
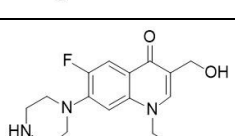
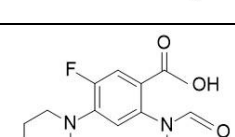
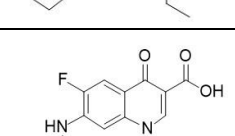
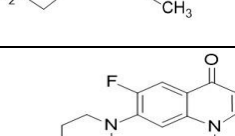
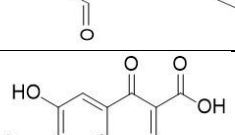
* The SIS medium was prepared through the dissolution of 10 mL of solution I, 5 mL of solution II, 5 mL of solution III, 5 mL of solution IV, 1 mL of solution V, 5 mL of solution VI, 1 mL of solution VII in a 1 L flask containing ca 900 mL of ultrapure water and dissolution of the following stock solutions. The pH was adjusted to 6.5 ± 0.2 with either 0.1 M HCl or 0.1 M NaOH. The volume was finally adjusted to 1 litre.

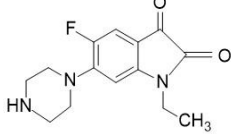
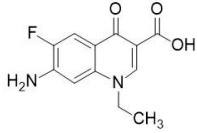
7. LC-MS identification of NOF derivatices after ozonation

Table S6. Main intermediates detected by LC-MS in NOF ozonized mixtures in agreement with the literature including diverse oxidative processes

m/z	Molecular structure	Molecular Formula	No catalyst	NaMt	Fe(II)Mt	Cu(II)Mt	Bentonite	Process referred to	Ref
362		C ₁₈ H ₂₀ F N ₃ O ₄	OK	OK					
354		C ₁₆ H ₂₀ F N ₃ O ₅	OK					Cathodic micro-arc plasma electrolysis	(Wang, Gao et al. 2023)

352		C ₁₆ H ₁₈ F N ₃ O ₅		OK			OK	Photocatalysis-self-Fenton system	(Wang, Zhao et al. 2023)
350		C ₁₆ H ₁₆ F N ₃ O ₅					OK	Catalytic ozonation (Metal-organic frameworks (MOFs))	(Chen and Wang 2021)
							OK	MnO ₂ /GO nano-hybrids coupled With peroxymonosulfate	(Wu, Li et al. 2020)
348		C ₁₆ H ₁₈ F N ₃ O ₅	OK	OK			OK	Electrochemical	(Carneiro, Aquino et al. 2020)
338		C ₁₆ H ₂₀ F N ₃ O ₄		OK		OK	OK	Photocatalysis-self-Fenton system	(Wang, Zhao et al. 2023)
336		C ₁₆ H ₁₈ F N ₃ O ₄	OK	OK			OK	Photocatalysis-self-Fenton system	(Wang, Zhao et al. 2023)
			OK					Pxidation under permanganate treatment	(Hubicka, Żmudzki et al. 2013)
334		C ₁₆ H ₁₆ F N ₃ O ₄	OK	OK			OK	E ⁺ -ozonation	(Wang, Cheng et al. 2020)
								Photocatalytic	(Chen and Chu 2015)
322		C ₁₅ H ₁₆ F N ₃ O ₄	OK	OK		OK	OK	Fenton-like catalysts	(Liu, Wang et al. 2024)

320		$C_{16}H_{18}FN_3O_3$	OK	OK	OK	OK	OK		NOF
318		$C_{16}H_{16}N_3O_4$ Instable					OK	E+-ozonation	(Wang, Cheng et al. 2020)
		$C_{16}H_{14}FN_3O_3$					OK	Photocatalysis-self-Fenton system	(Wang, Zhao et al. 2023)
310		$C_{15}H_{20}FN_3O_3$	OK	OK			OK	Fenton-like catalysts	(Liu, Wang et al. 2024)
308		$C_{14}H_{14}FN_3O_4$		OK		OK	OK	UV/chlorine	(Lu, Li et al. 2021)
306		$C_{15}H_{20}FN_3O_2$	OK	OK		OK	OK	Adsorption	(Zhao, Sun et al. 2023)
296		$C_{14}H_{18}FN_3O_3$	OK	OK	OK	OK	OK	Adsorption	(Zhao, Sun et al. 2023)
294		$C_{14}H_{16}FN_3O_3$	OK	OK		OK	OK	MnO ₂ /GO nano-hybrids coupled with peroxymonosulfate	(Wu, Li et al. 2020)
								Fenton-like catalysts	(Liu, Wang et al. 2024)
292		$C_{15}H_{18}FN_3O_2$	OK					Fenton-like catalysts	(Liu, Wang et al. 2024)
		$C_{14}H_{16}FN_3O_4$	OK					Photocatalytic	(Chen and Chu 2015)

278		C ₁₄ H ₁₆ F N ₃ O ₂	OK					Photo-Fenton	(Zhao, Wang and Wang 2022)
251		C ₁₂ H ₁₁ F N ₂ O ₃	OK	OK		OK	OK	MnO ₂ /GO nano-hybrids coupled with peroxymonosulfate	(Wu, Li et al. 2020)
								Photocatalytic	(Chen and Chu 2015)

8. Correlation between the fluctuations in derivative distribution and toxicity

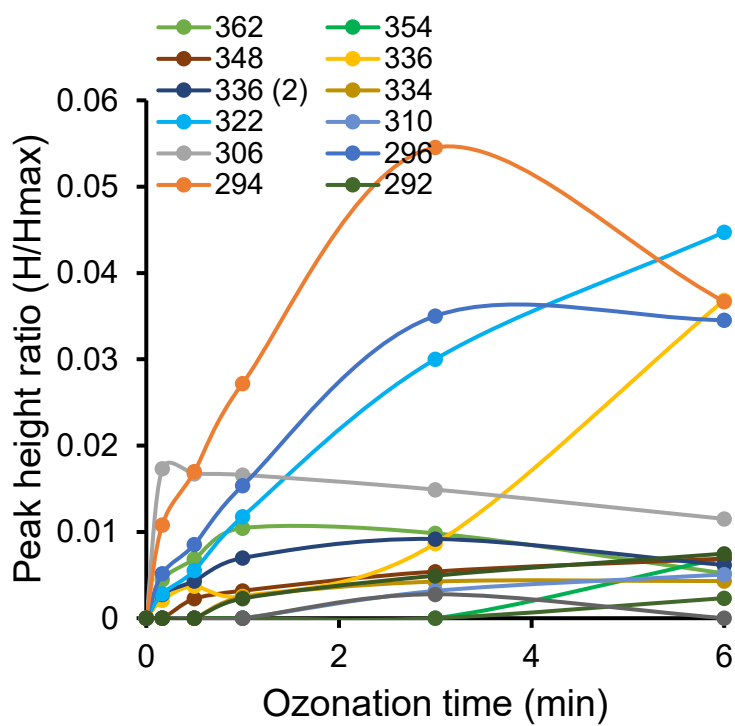


Fig. S5. Evolution in time of the relative peak height ratio for NOF derivatives identified by LC-MS ($H/H_{\max}$) for NOF ozonized mixture in the absence of catalysts.

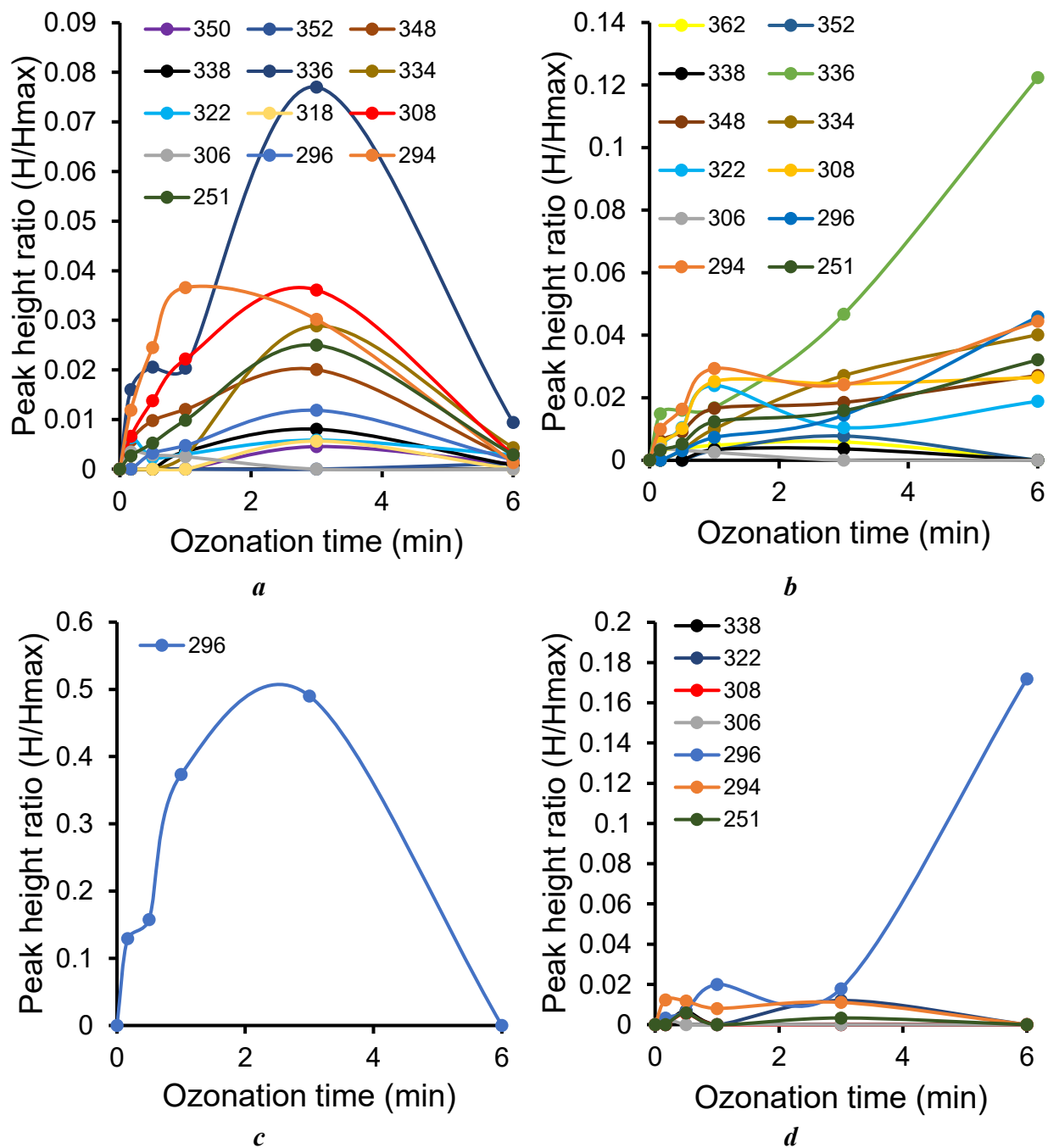
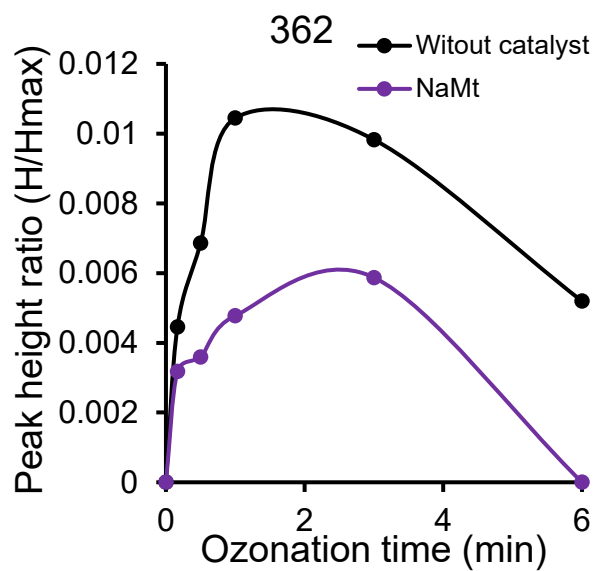
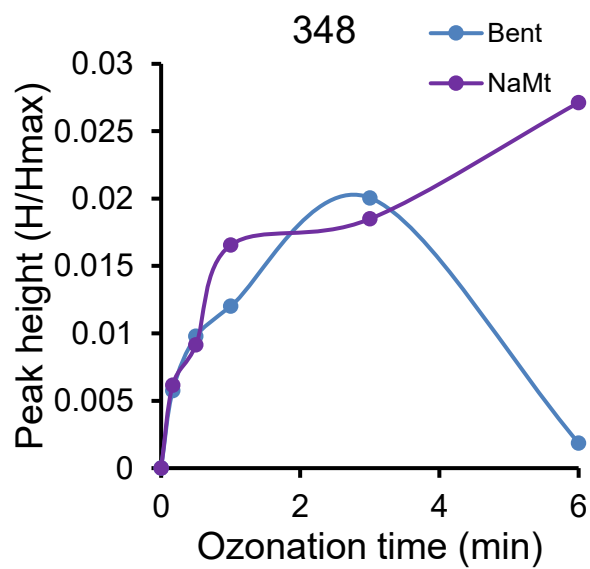


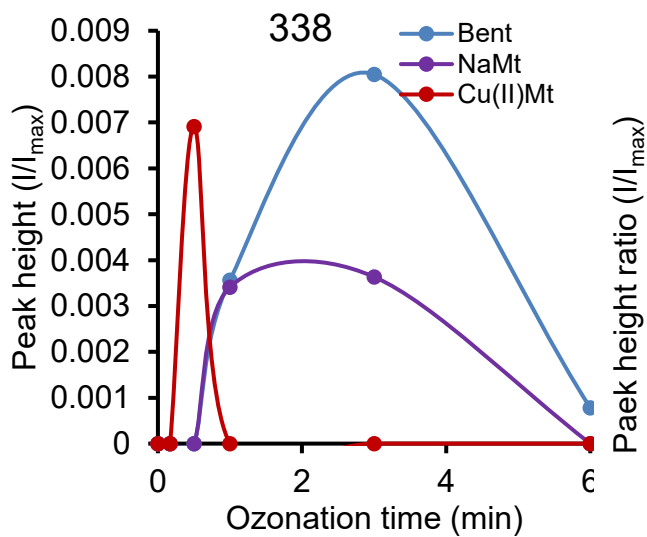
Fig. S6. Evolution in time of the relative peak height ratio for NOF derivatives identified by LC-MS (H/H_{max}) in presence of different catalyst bentonite (a), NaMt (b), Fe(II)Mt (c) and Cu(II)Mt (d).



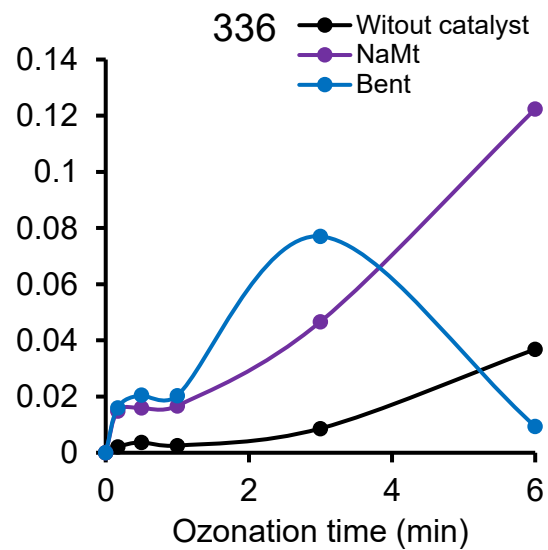
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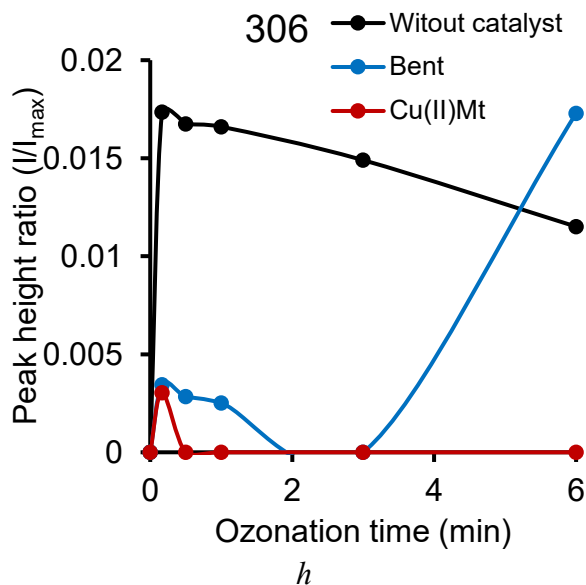
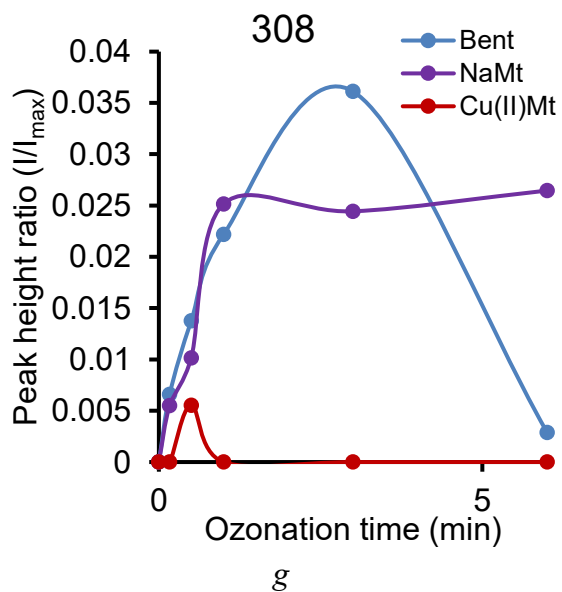
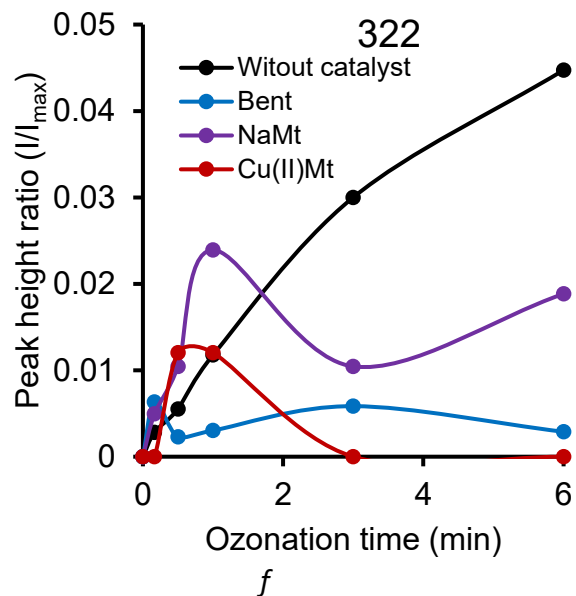
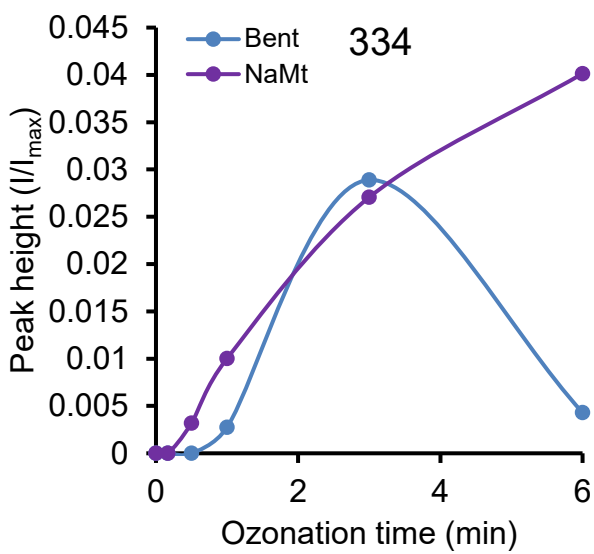
b



c



d



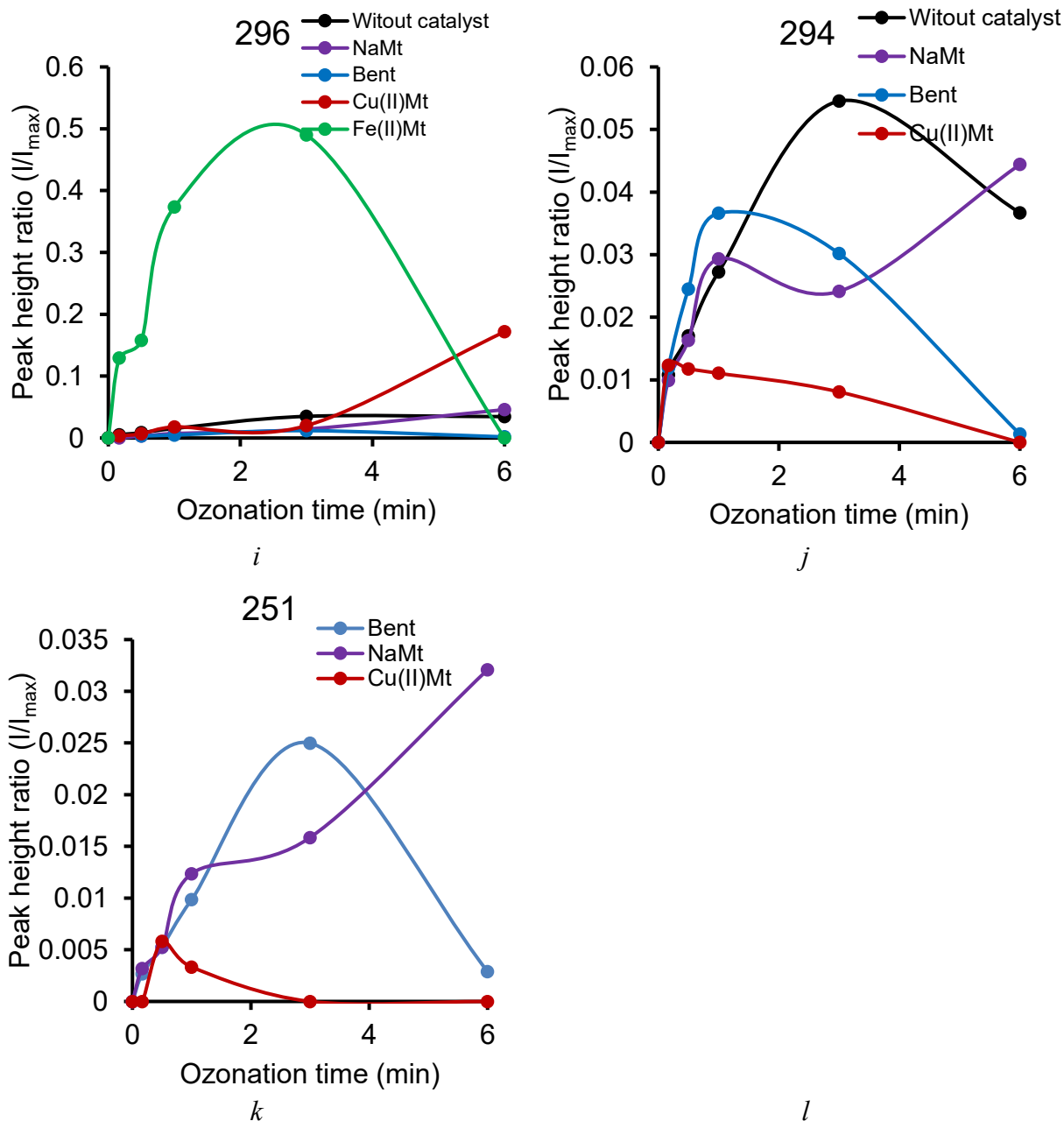
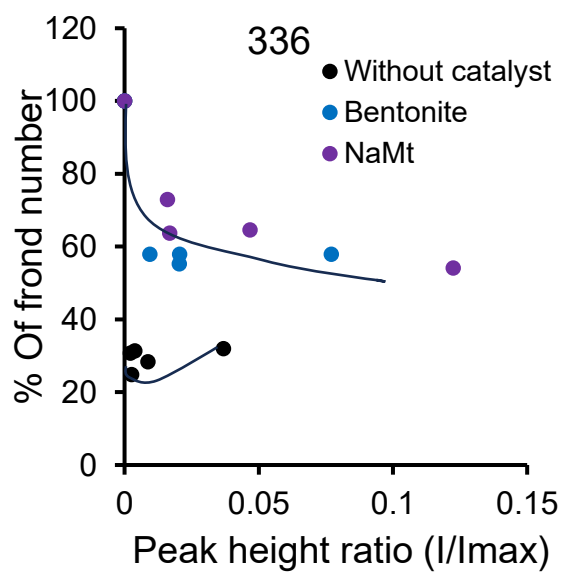
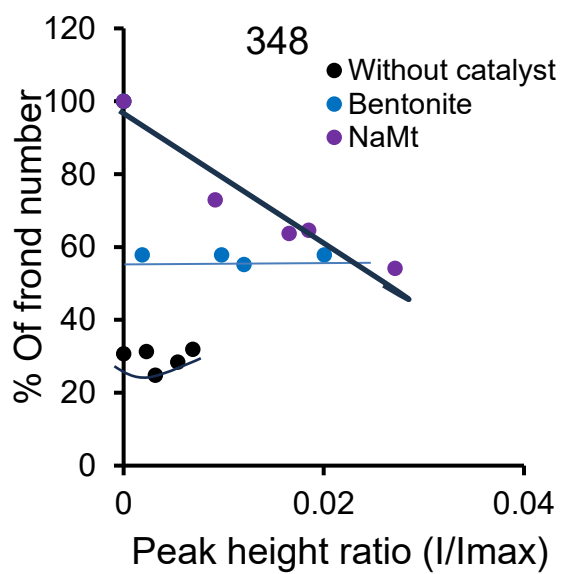
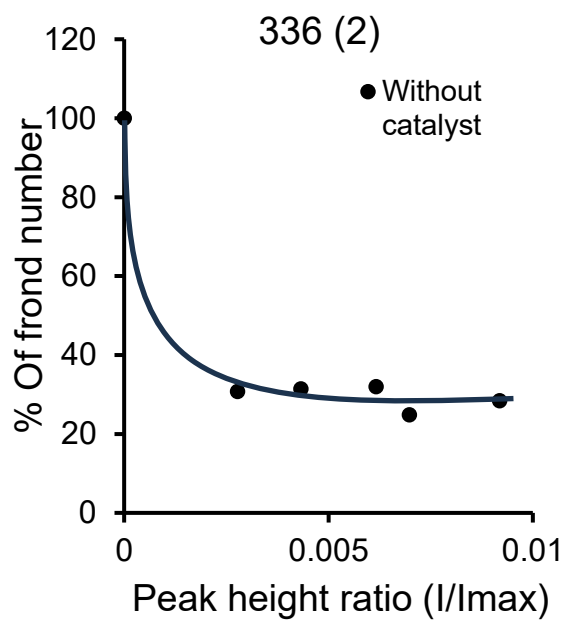
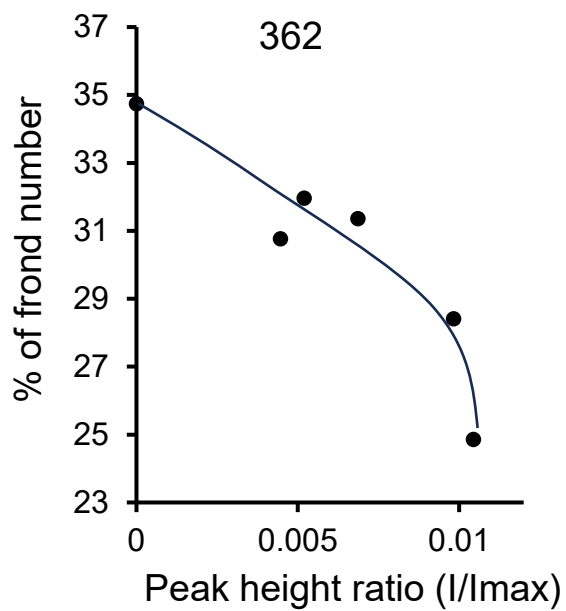
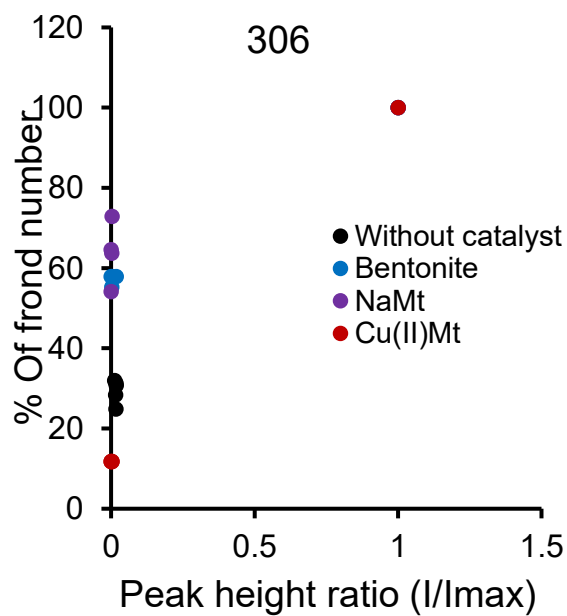
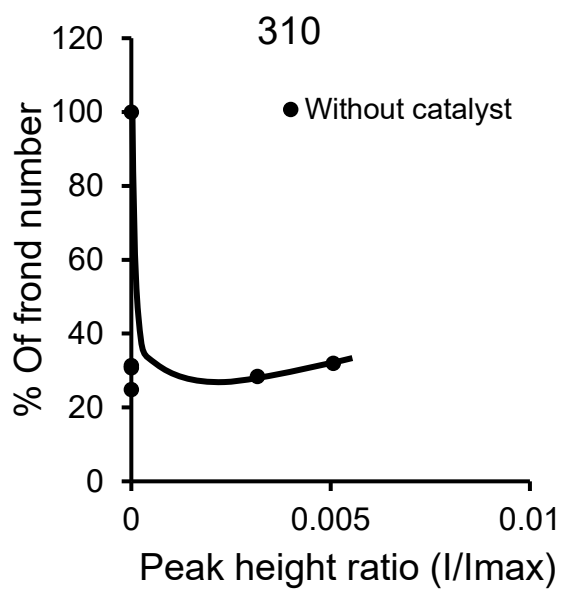
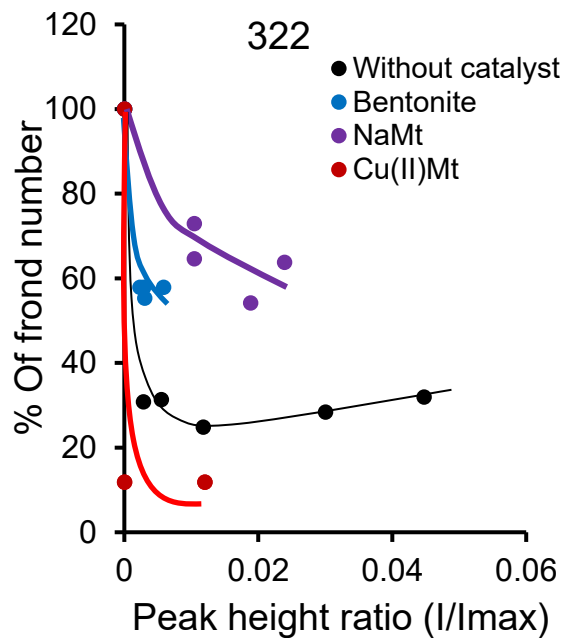
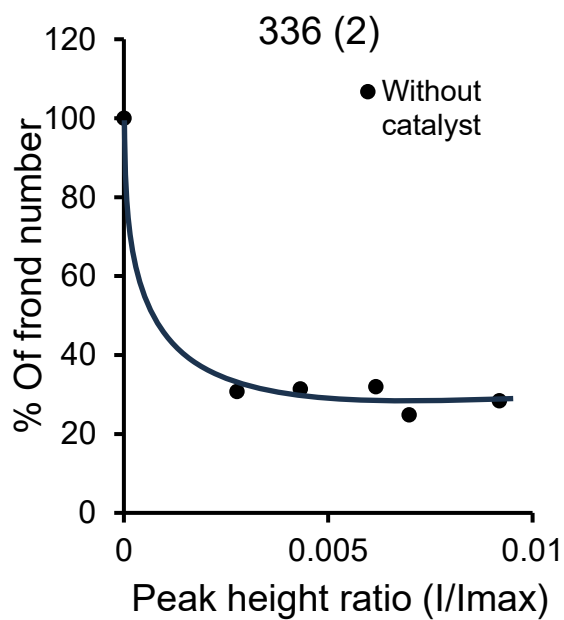


Fig. S7. Evolution in time of NOF derivatives distribution during clay-catalyzed ozonation. The distribution was assessed by LC-MS and expressed in terms of instant/initial peak height ratio for each intermediate separately ($I/I_{\max}$). in presence of different catalyst bentonite (a), NaMt (b), Fe(II)Mt (c) and Cu(II)Mt (d).





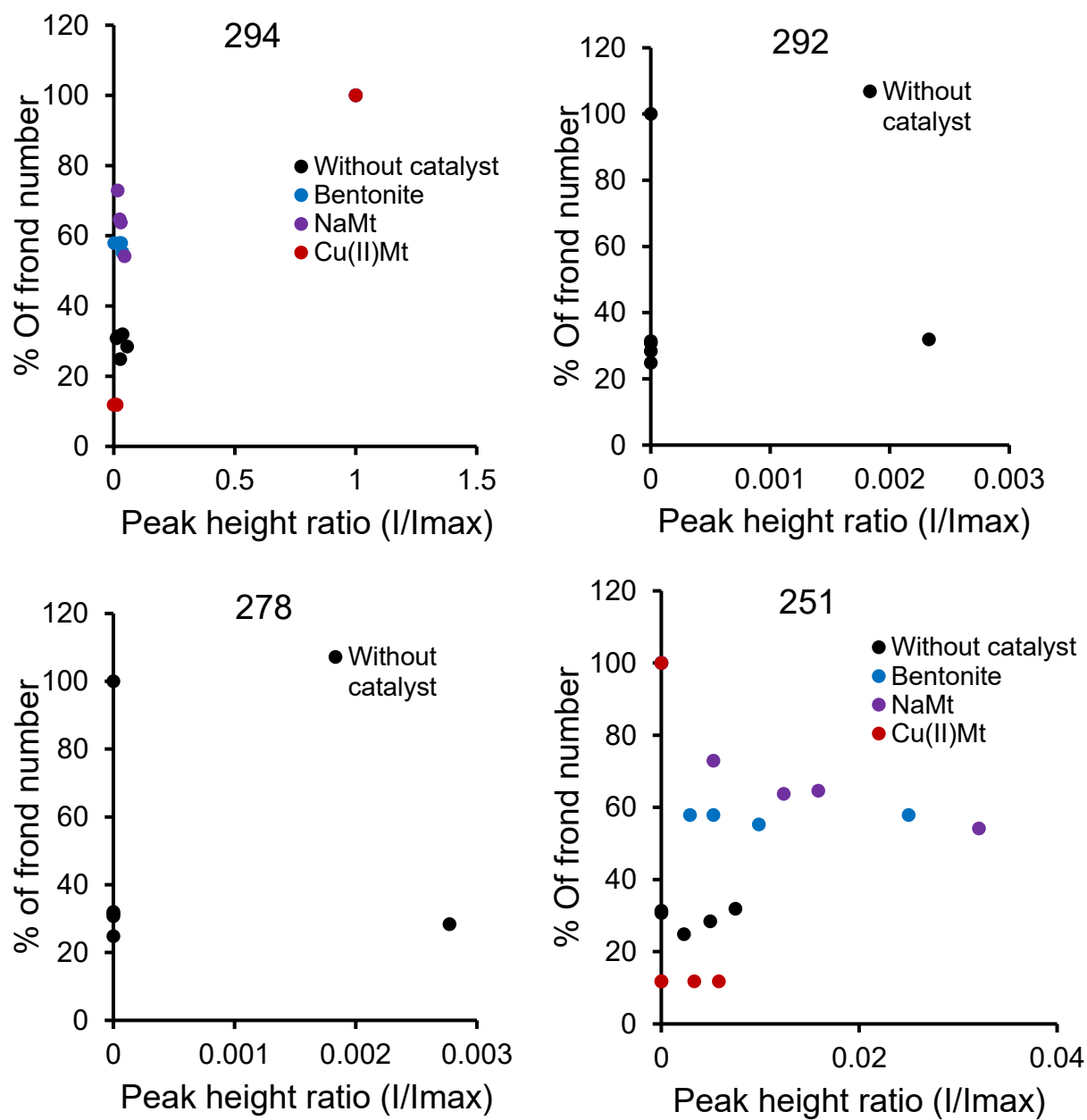


Fig. S8. Frond number (%) in function of peak height ratio for NOF derivatives distribution during clay-catalyzed ozonation

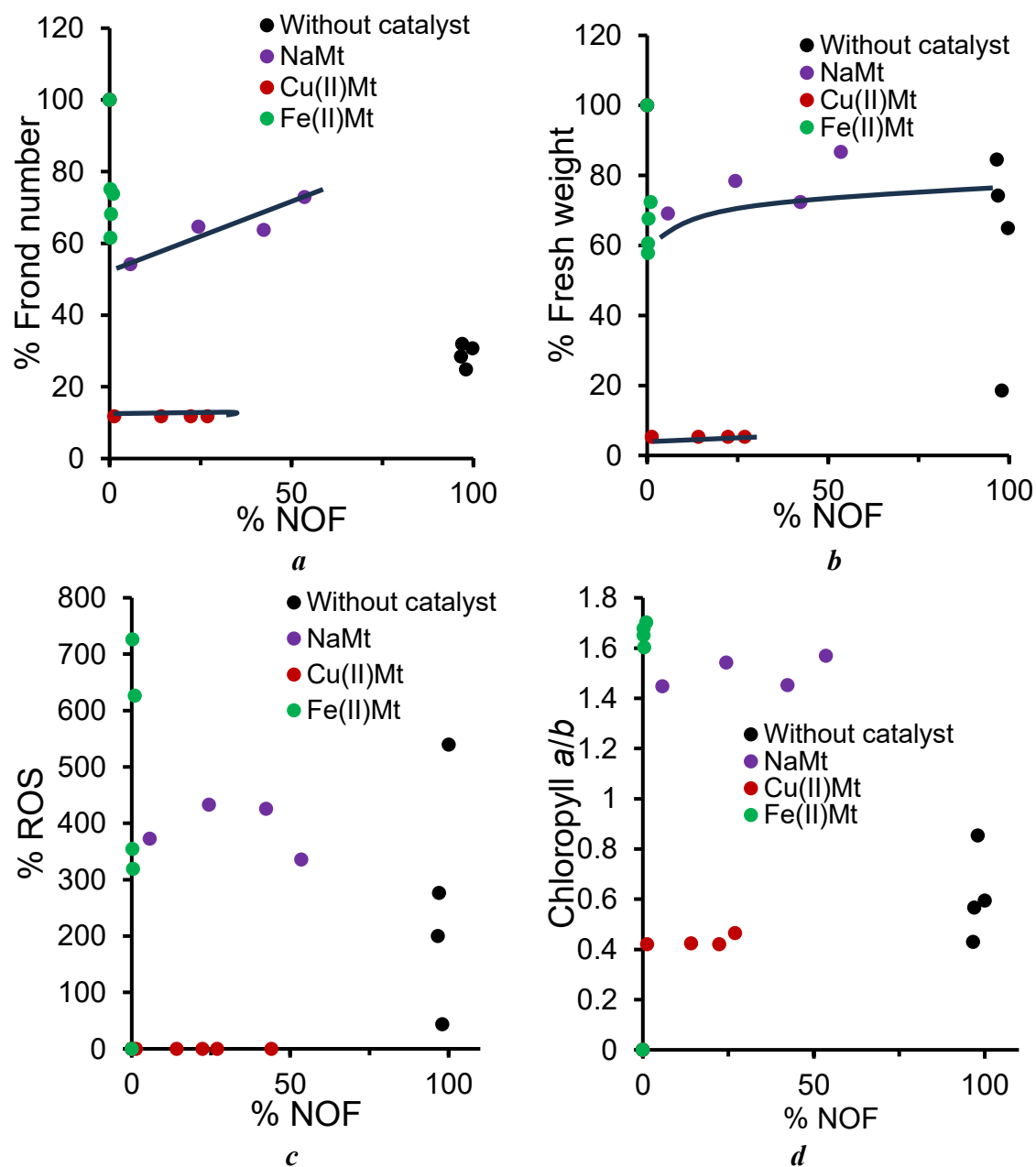


Fig. S9. Effect of NOF (%) yield on the toxicity towards *Lemna minor* plants exposed for 7 days to the ozonised mixtures of NOF. Frond number (a) fresh weight (b) ROS amount (c) and Chlorophyll *a/b* ratio (c).

9. References

7. 1. Wang, H., et al., *pH-dependent norfloxacin degradation by E+-ozonation: Radical reactivities and intermediates identification*. Journal of cleaner production, 2020. **265**: p. 121722.
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9. 3. Zhao, X.-L., et al., *Phytotoxicity of environmental norfloxacin concentrations on the aquatic plant Spirodela polyrrhiza: Evaluation of growth parameters, photosynthetic toxicity and biochemical traits*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 2022. **258**: p. 109365.
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17. 11. Liu, Y., et al., *Nitrogen-doped carbon-coated CuO activates molecular oxygen for norfloxacin degradation over a wide pH range*. Journal of Colloid and Interface Science, 2024. **665**: p. 945-957.
18. 12. Lu, J., et al., *Kinetics, structural effects and transformation pathways for norfloxacin oxidation using the UV/chlorine process*. Journal of Water Process Engineering, 2021. **44**: p. 102324.
19. 13. Zhao, Y., et al., *Construction of Fe/N/C nano-clusters anchored on porous diatomite for efficient removal of norfloxacin via the adsorption-PMS activation*. Separation and Purification Technology, 2023. **310**: p. 123127.

20. 14. Zhao, Q., C.-C. Wang, and P. Wang, *Effective norfloxacin elimination via photo-Fenton process over the MIL-101 (Fe)-NH₂ immobilized on α -Al₂O₃ sheet*. Chinese Chemical Letters, 2022. **33**(11): p. 4828-4833.

ANNEXE B

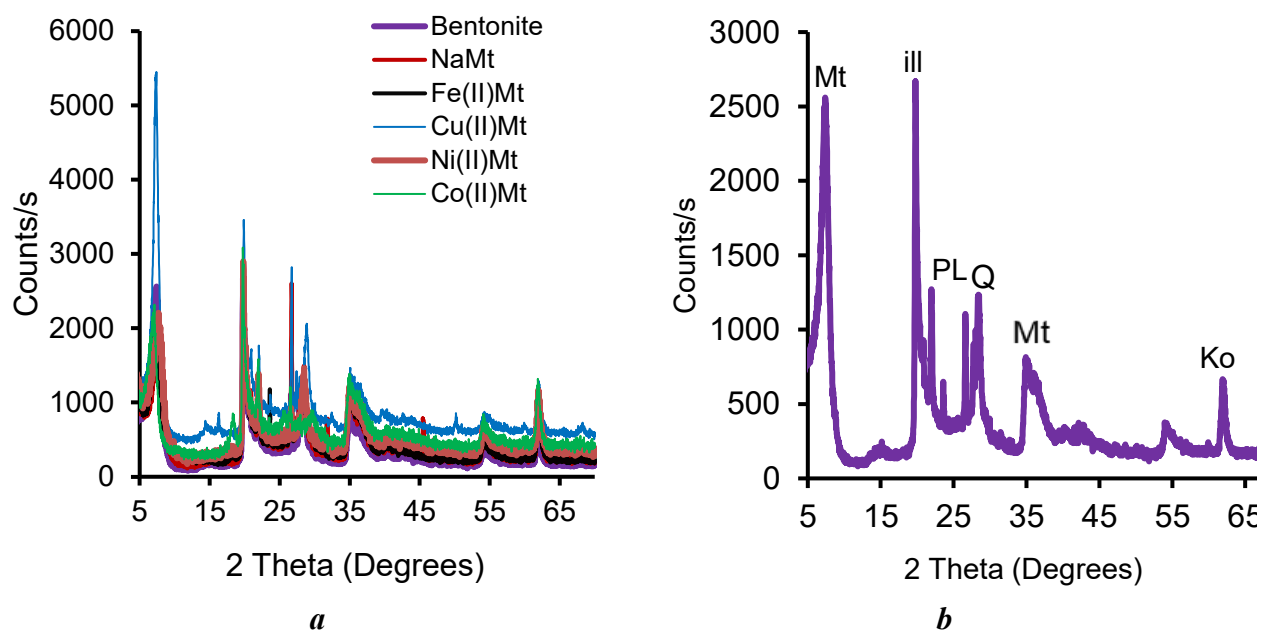
SUPPORTING INFORMATION DE L'ARTICLE 2

Reciprocal effects of acidity evolution on metal cations and clay catalyst dispersion in Norfloxacin oxidative degradation and toxicity in aqueous media

Roumaissa Djidja et al.

Supporting information

1. XRD patterns of bentonites and exchanged montmorillonites



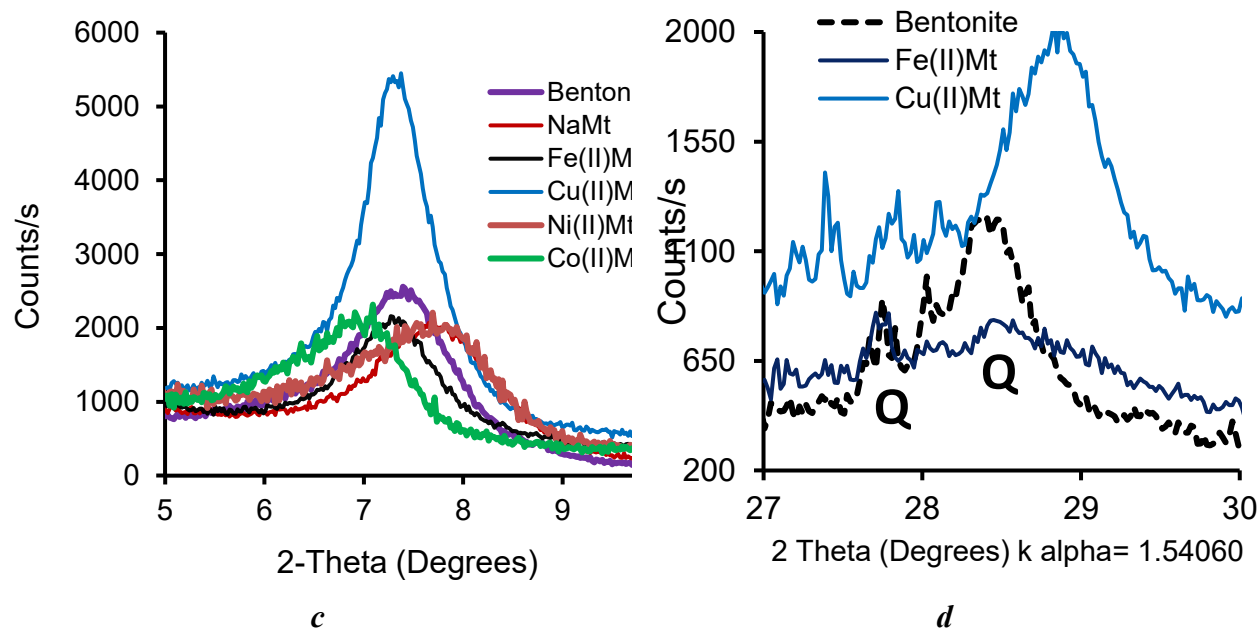


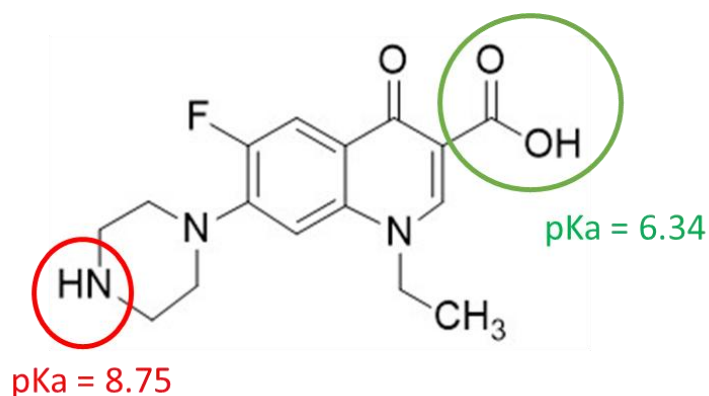
Fig. S1. X-ray diffraction patterns of the clay materials investigated (a), XRD reflexion of main bentonite impurities (b) and close-ups on changes in the d_{001} basal spacing in the 2-Theta ranges 5-10 degrees (c) and 25-30 degrees for quartz removal detection after clay purification (d).

2. Concentration of dissolved ozone

An ozone generator A₂Z (A₂Z Ozone Inc., Louisville, KY, USA) with throughput 600 mg/h was used for all ozonation experiments. The dissolved ozone concentration was then determined using the same iodometric method, applied to 20 mL samples of distilled water at different ozonation times (1, 2, 5, 10, 15, 20, 30, 40, and 60 minutes). For this purpose, ten 250 mL erlenmeyer flasks were prepared containing an acidified potassium iodide solution. The solution was prepared by dissolving 0.5 g of KI and 0.05 g of KIO₃ in 100 mL of distilled water. After complete dissolution, the volume was adjusted to 200 mL with additional distilled water. The solution was then acidified with 10 mL of 1 M HCl. Following this, 7.8 mL of 0.1 M sodium thiosulfate (Na₂S₂O₃) was added, resulting in a dark yellow color. Each flask was ozonated for 5 minutes at different ozone concentrations (10% to 100%). The samples were analyzed by UV-Vis spectrophotometry. The iodine (I₂) generated from the oxidation of iodide by ozone was subsequently titrated with a 0.1 M of Na₂S₂O₃ in the presence of 1 mL of starch as indicator until the disappearance of the blue color and the appearance of a pale-yellow endpoint. The number of moles of ozone was calculated based on the volume of thiosulfate consumed, using the following equation: $n(\text{O}_3) = [\text{S}_2\text{O}_3^{2-}] \times V(\text{S}_2\text{O}_3^{2-}) / 2$. The corresponding mass flow of ozone was then calculated as: $D_{\text{mass}} = n(\text{O}_3) \times M(\text{O}_3) / \Delta t$ where $M(\text{O}_3) = 48 \text{ g/mol}$ and Δt is the ozonation time. The volume of thiosulfate used is directly proportional to the amount of dissolved ozone (Chasanah, Yulianto et al. 2019). The concentration of dissolved ozone increases almost linearly up to its maximum level of ca. 0.35-0.40 mmol/L after 10-15 min gas bubbling depending on ozone concentration in the carrier gas (Benghaffour, Foka-Wembe et al. 2022) and operating conditions (Zekkari, Ouargli-Saker et al. 2022). This value is much lower than the reported 20.8 mmol/L in water at 0 °C (Battino, Rettich and Tominaga 1983) or 11.9 mmol/L in water at 20 °C (Roth and Sullivan 1981, Egorova, Voblikova et al. 2015,

Shahidi, Roy and Azzouz 2015). The resulting O₃/NOF molar ratio of ca. 121 ensures a sufficient excess of ozone after 10 min ozonation.

3. *pKa of NOF molecule and UV-Vis spectrum change during non-catalytic ozonation*



Scheme. S1. Chemical structures and related pKa constants for Norfloxacin

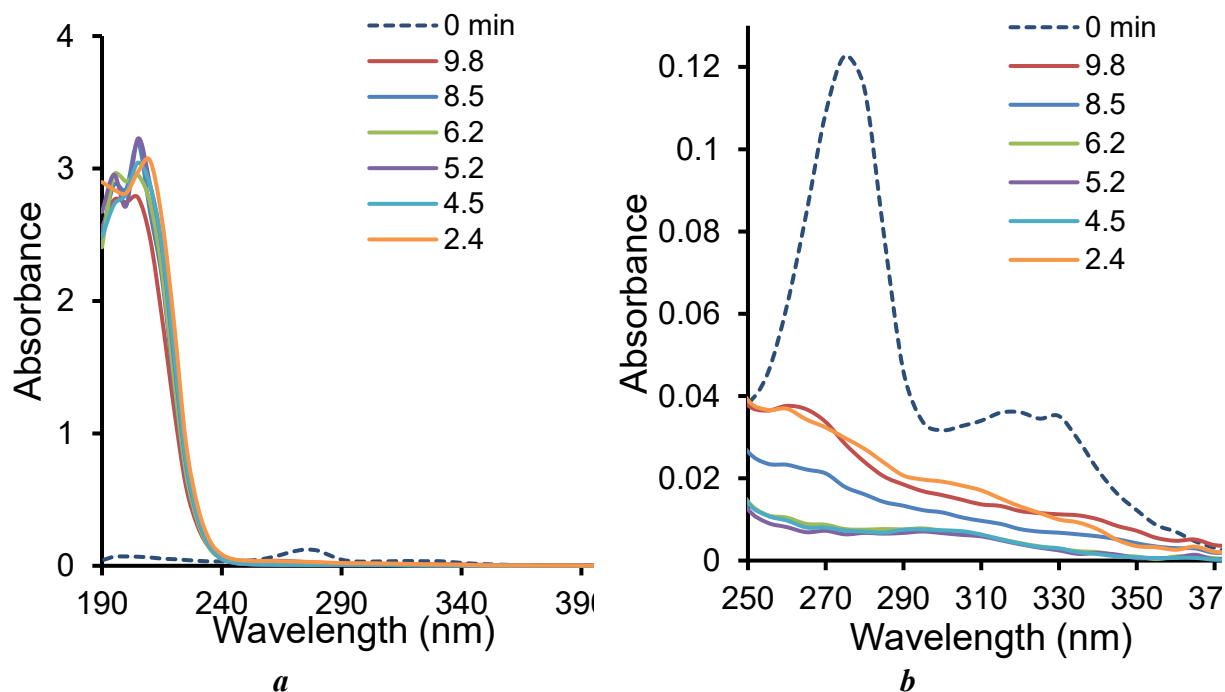


Fig. S2. pH effect on UV-Vis spectra of NOF (a) and close up on the 250-370 region (b) after 10 min of non-catalytic ozonation as compared to the non-ozonized NOF solution (Dashed line).

4. *Effect of cations and exchangeable Mt samples on pH decrease during ozonation*

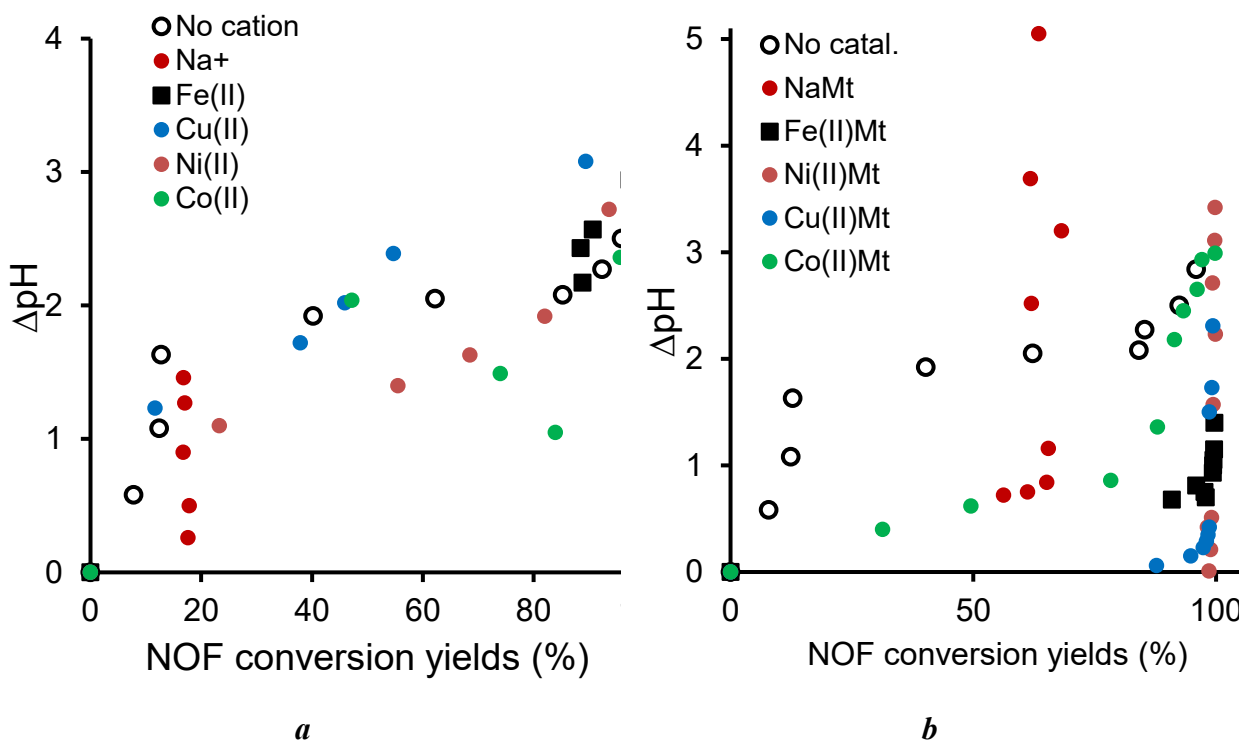


Fig. S3. pH variation (difference between initial pH and instant pH) as function of NOF conversion yield in the presence of dissolved (a) and clay-supported metal cations (b). NOF conversion was determined up to 10 min of cation-catalyzed ozonation and up to 30 min of clay-catalyzed ozonation.

NOF conversion was assessed up to 30 min of non-catalytic ozonation.

5. UV-Vis spectra during Na^+ -catalyzed NOF ozonation during ozonation

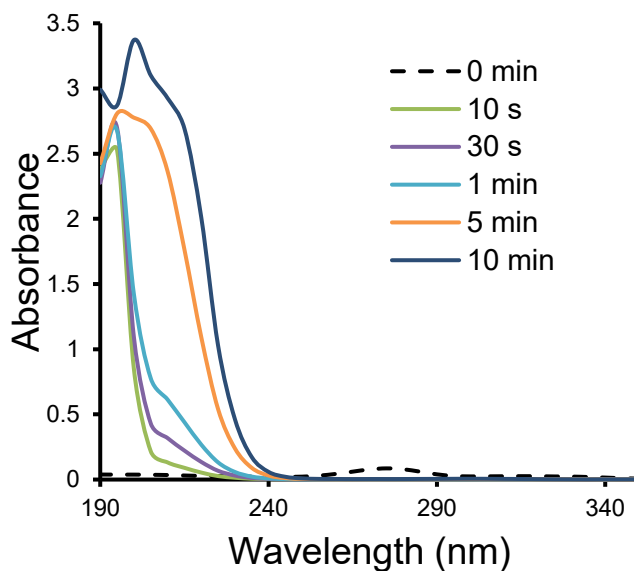


Fig. S4. Evolution of UV-Vis spectra during Na^+ -catalyzed NOF ozonation

6. pH Evolution during ozonation

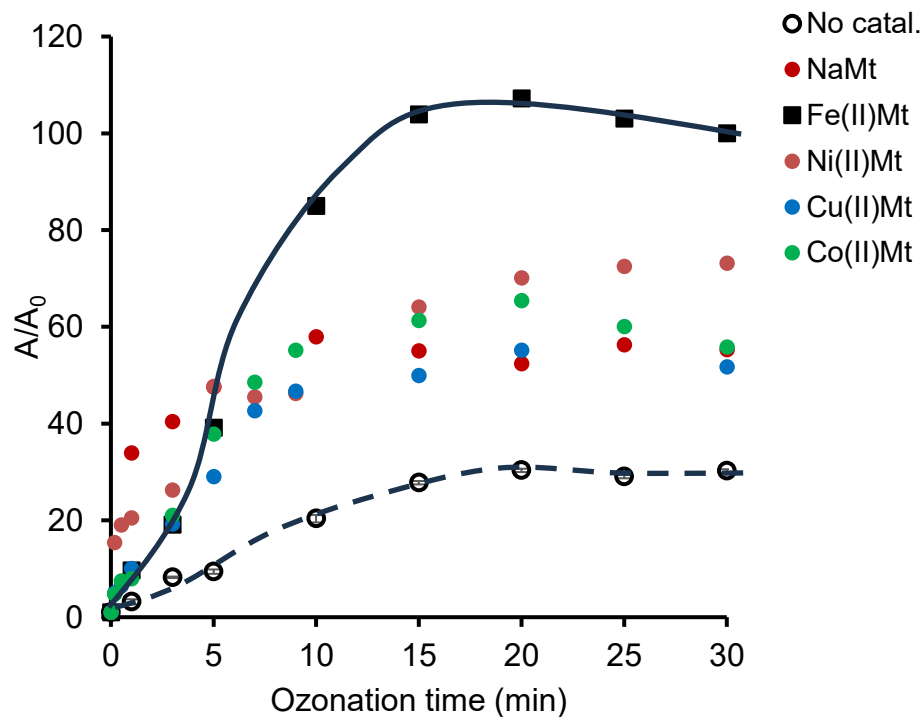
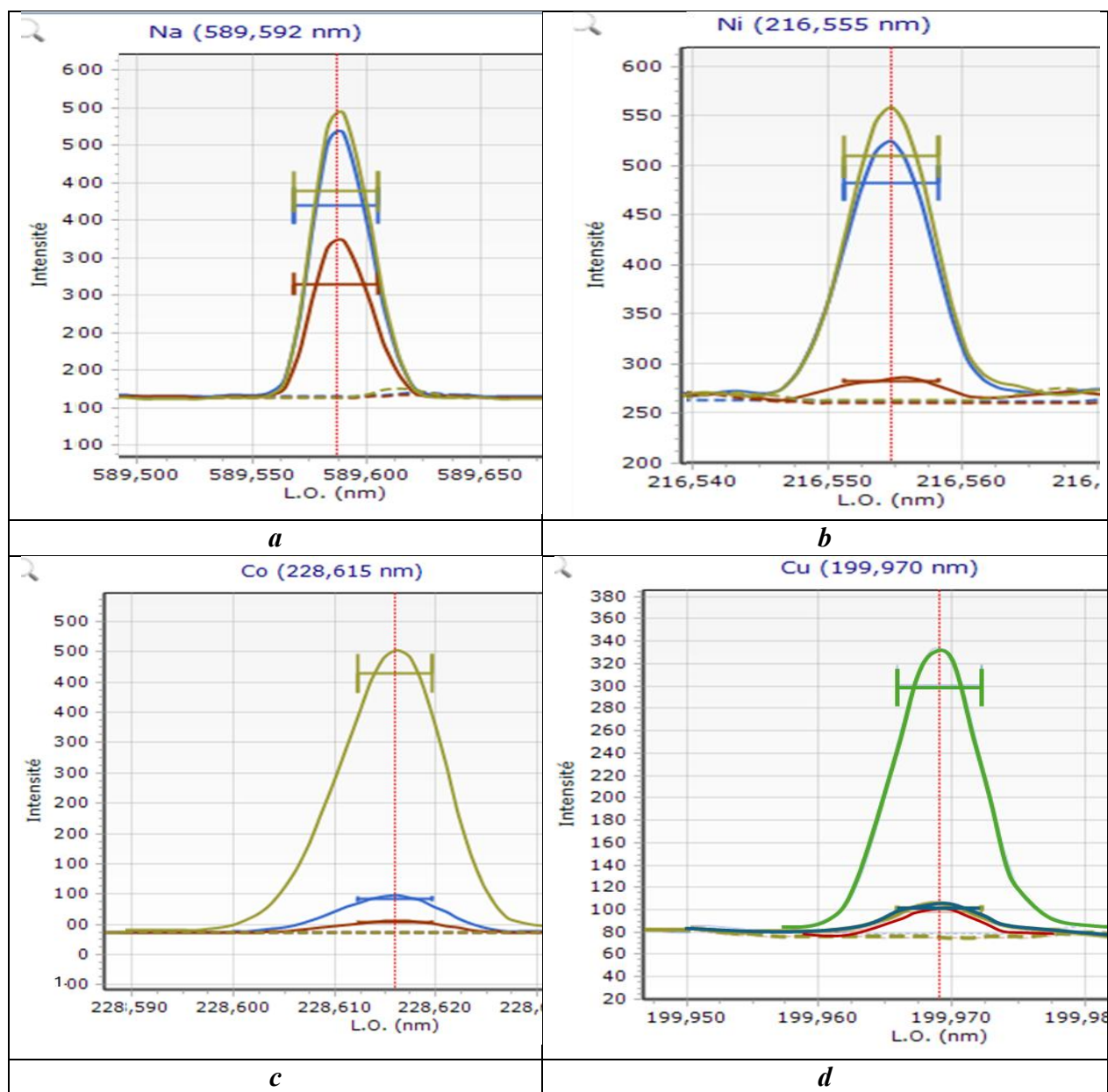


Fig. S5. Evolution of the relative absorbance of the ozonized NOF solution at 200 nm (a).

7. ICP-OES measurements-operating conditions

Table S1. Instrumental parameters of ICP-OES

Parameter	value
Power	1.5 kW
Plasma argon flow rate	12 L/min
Auxiliary argon flow rate	1 L/min
Nebulizer argon flow rate	1 L/min
Wavelengths (nm)	Na (589.592), Fe (238.204), Ni (216.555), Cu (199.970) and Co (228.615).



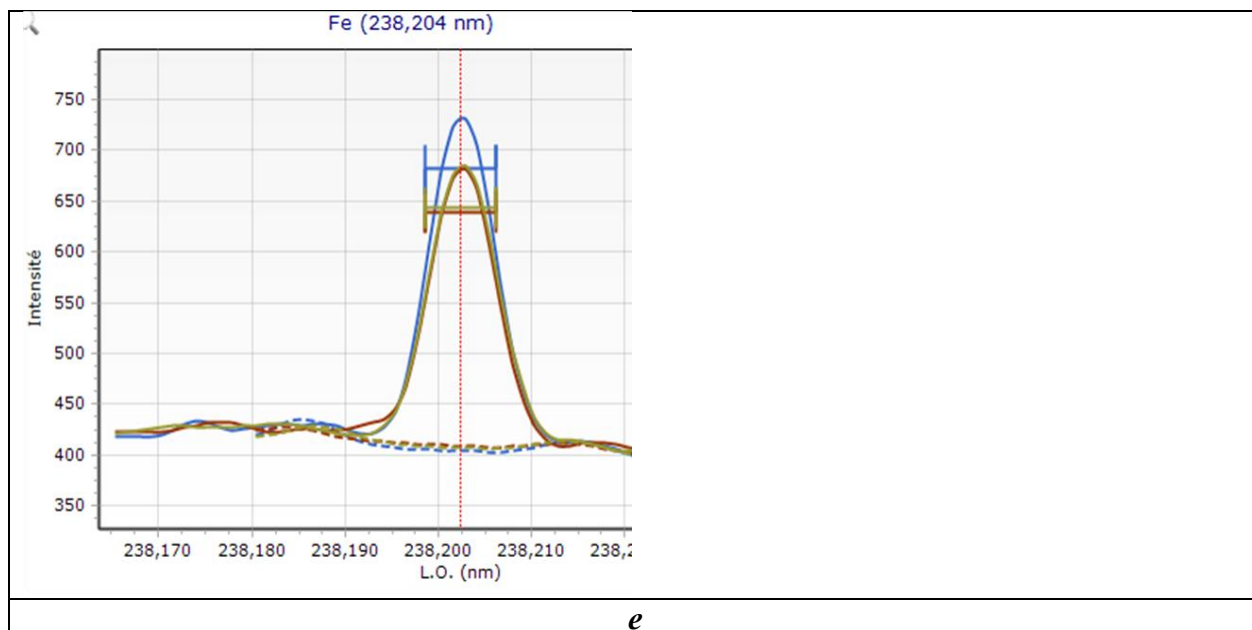


Fig. S6. Emission spectra of different cations detected in the NOF solution by ICP-OES before and after ozonation. The blue curve corresponds to the initial time (0 min) before ozonation. The red and green curves represent the emission after 30 s and 30 min of ozonation, respectively.

8. Liquid-phase chromatography measurements-operating conditions

Table S2. Parameters of HPLC-UV analysis

Parameter	Specification
Apparatus model	Agilent Technologies model 1290 equipment
Column specification	C18 column 4.6 ×150 mm,5 μm particle size
Mobile phases composition	A: water with 0.1% Formic Acid B: methanol
Detection wavelength	275 nm
Gradient / isocratic	0-0.5 min, 10% B; 0.5-2.0 min, 10-15% B; 2.0-8.0 min, 15-35% B; 8.0-10.0 min, 35-90% B; 10.0-11.0 min, 90% B; 11.0-11.2 min, 90-10% B; 11.2-18.0 min, 10%B.
Flow rate	1 mL. min ⁻¹
Temperature	20 °C
Pump pressure	18 MPa
Injected volume	20 uL

9. HPLC-UV measurements of residual NOF

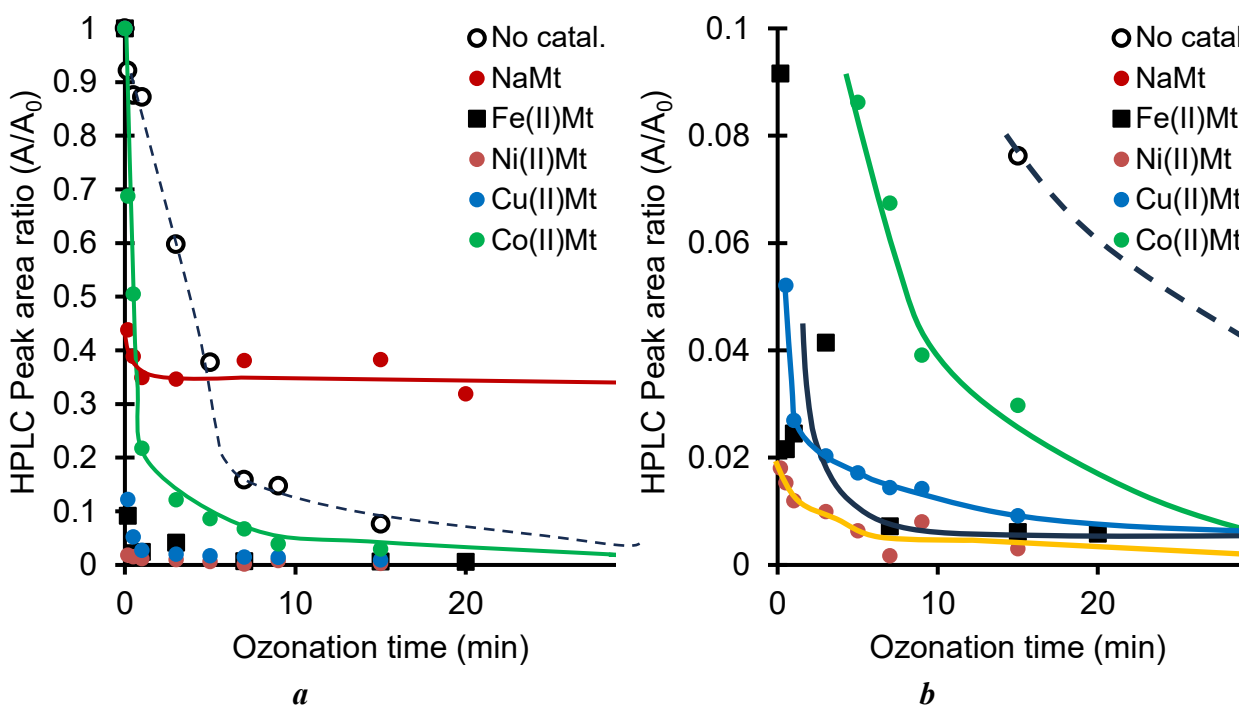
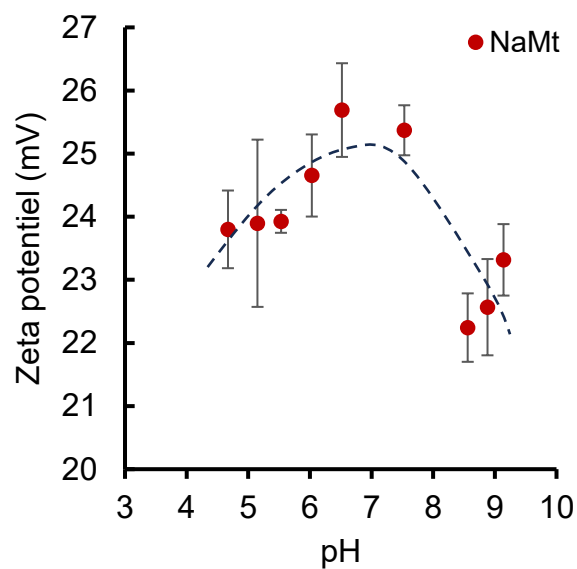
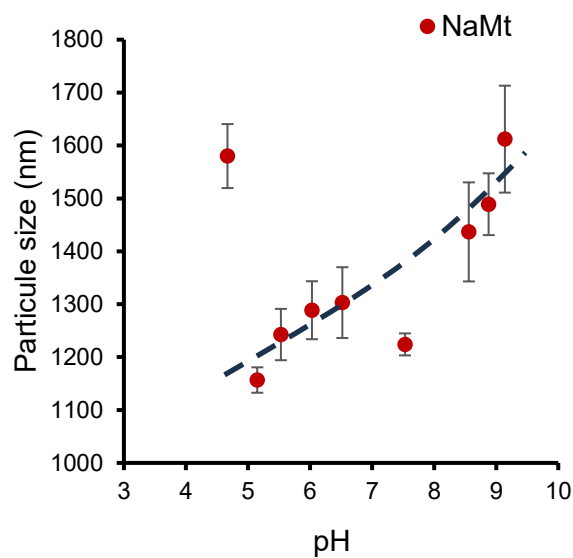


Fig. S7. Evolution of the relative area of the HPLC-UV peak of NOF during the catalytic ozonation (a) and closeup on the A/A_0 ratio range 0-0.2 (b). Ozone flow rate: 600 mg/h. Catalyst amount: 50 mg. Sample volume: 25 mL. The relative peak area (A/A_0) was calculated as the instant/initial peak area ratio ($A/A_0 \times 100\%$) obtained by HPLC-UV measurements.

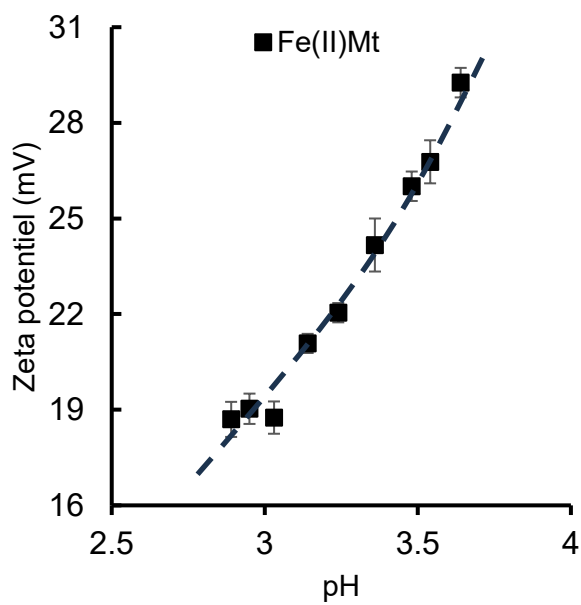
10. Time evolution of pH and Zeta potential during ozonation



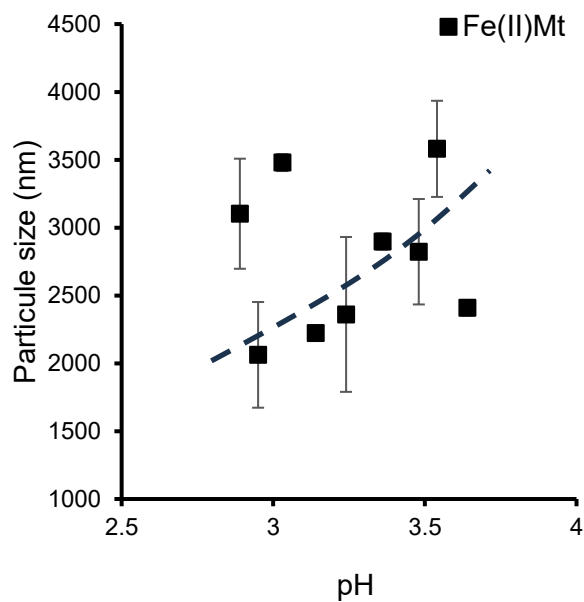
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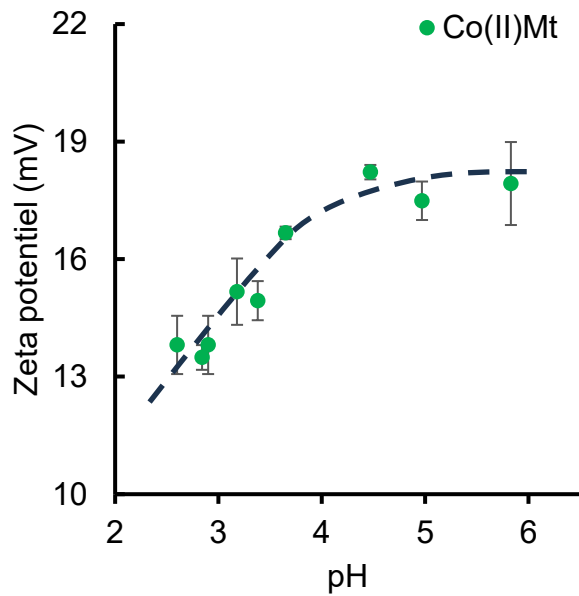
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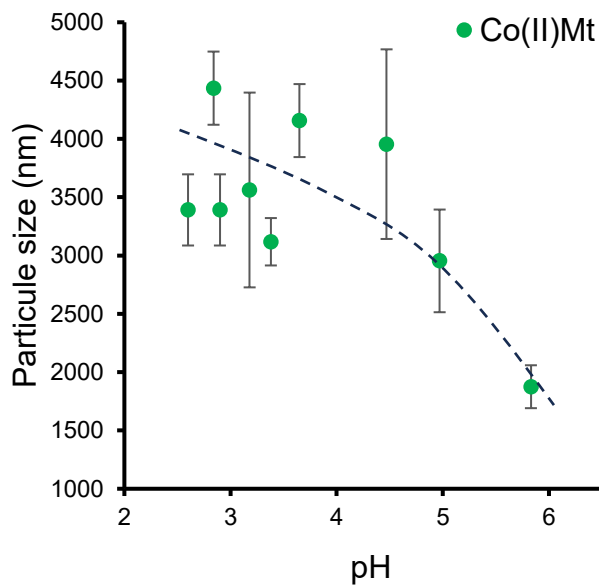
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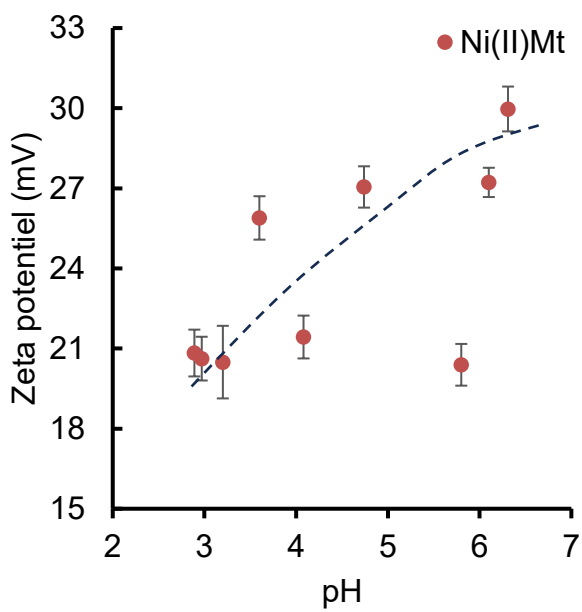
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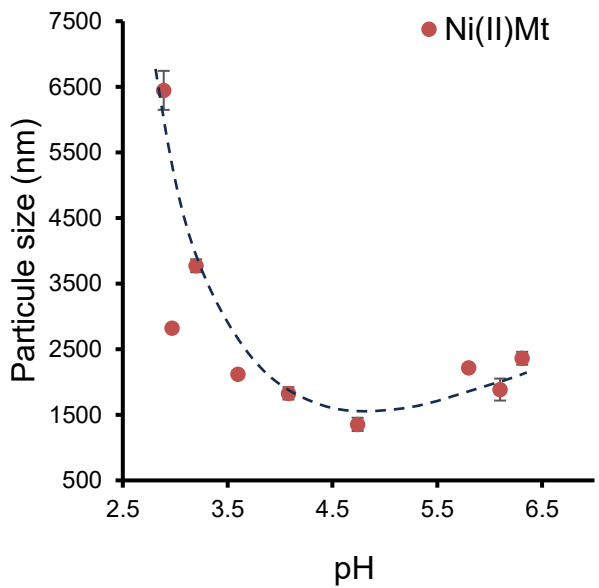
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a

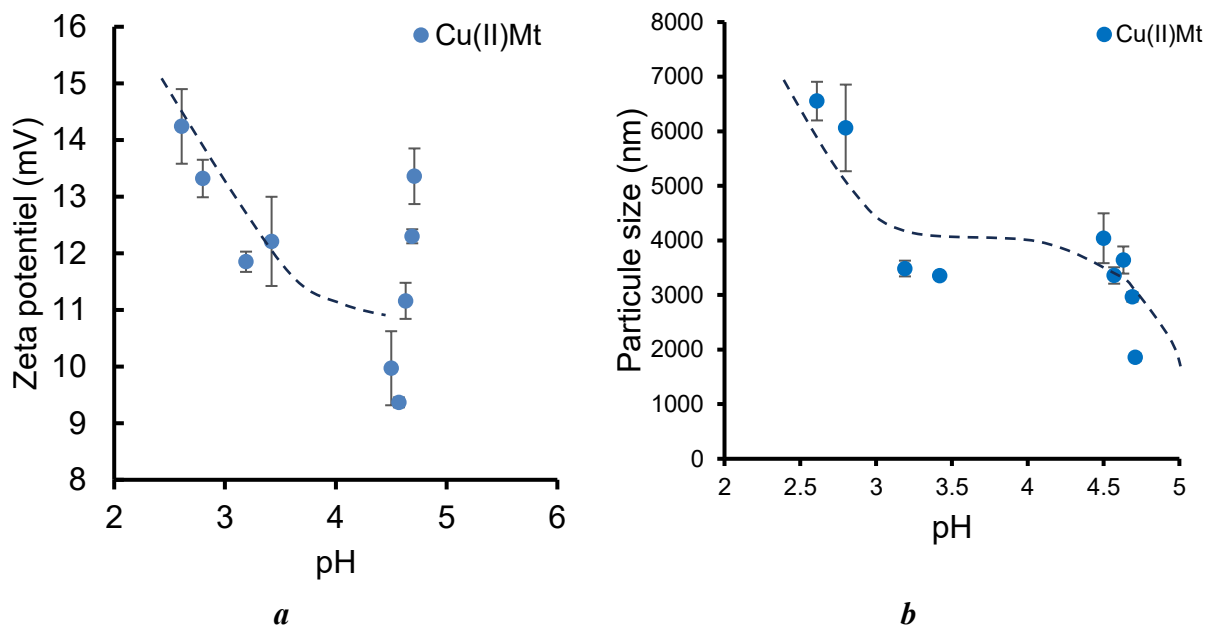


Fig. S8. Correlation between the evolution of both pH with Zeta potential (a) and with particle size (b) during clay-catalyzed ozonation of NOF up to 30 min. Ozone flow rate: 600 mg/h. Catalysts amount: 50 mg. Sample volume: 25 mL.

11. Effect of metal cation on Chlorophyll a/b ratio

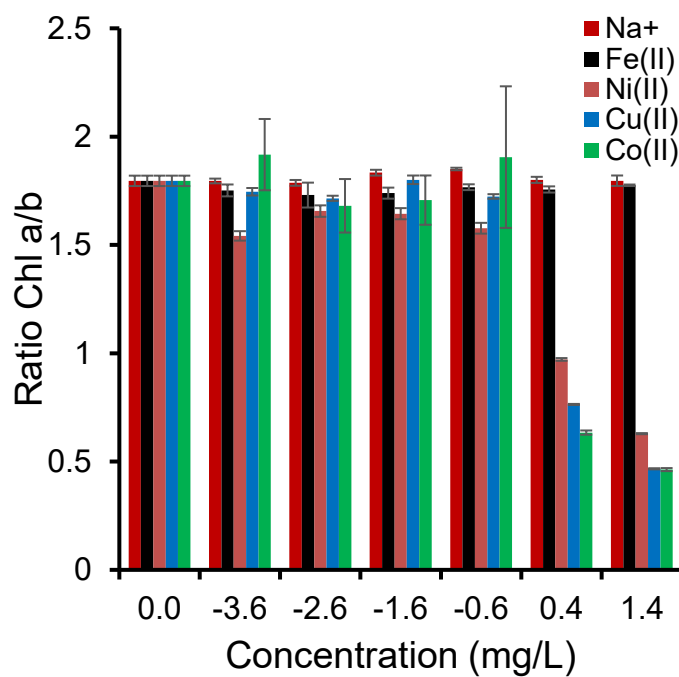


Fig. S9. Effect of cations concentration on *L. minor* through changes in the chlorophyll *a/b* ratio. These data account for the mean of triplicate measurements after plant exposure for 7 days including the control sample (*Lemna minor* in SIS medium without metals).

12. The individual effects of pH and clay catalysts

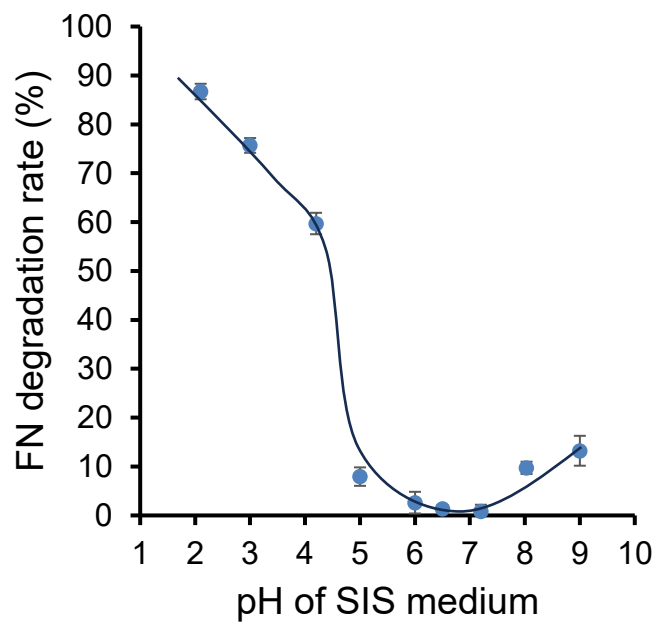


Fig. S10. Effect of adjusted initial pH of SIS medium on toxicity on *L. minor* as expressed in terms of inhibition of frond number growth as reported to the blank sample (pH = 6.5). This result is obtained after 7 days of exposure in SIS medium with different pH ranging from 2 to 9. The adjusted initial pH was measured with a ± 0.1 accuracy. The results are expressed as percentages relative to the control sample.

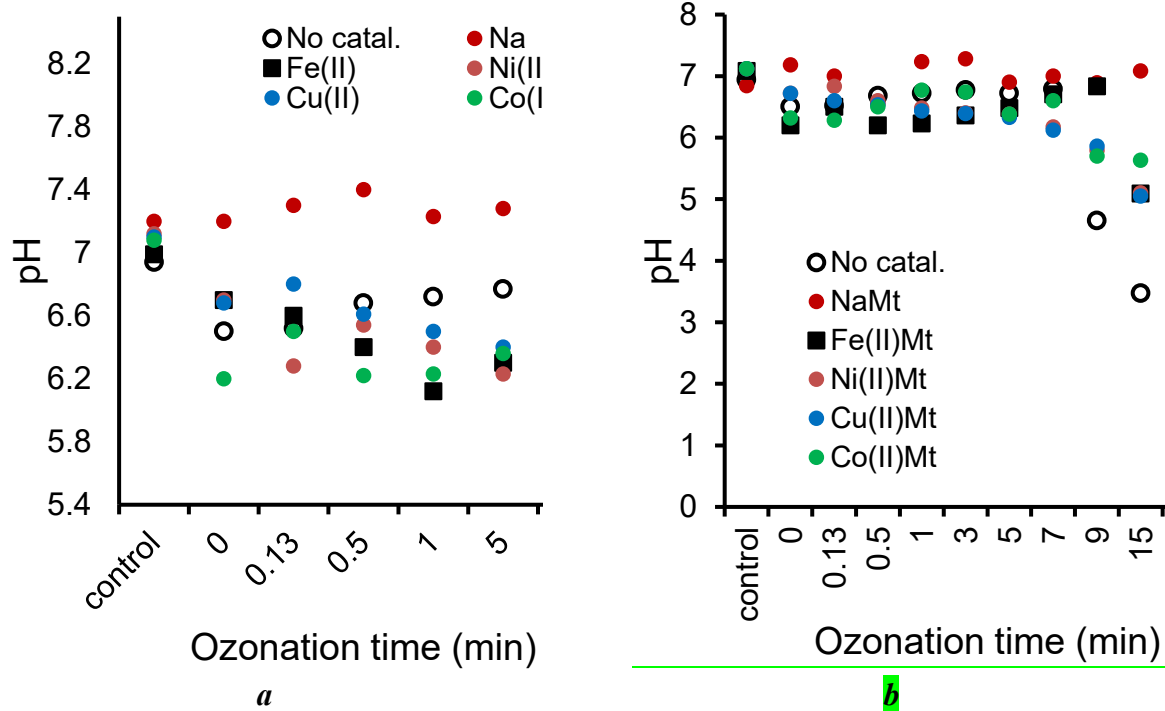


Fig. S11. pH of SIS medium and ozonated mixture of NOF with cations (a) and with clay catalyst (b). The pH was measured with a ± 0.1 accuracy.

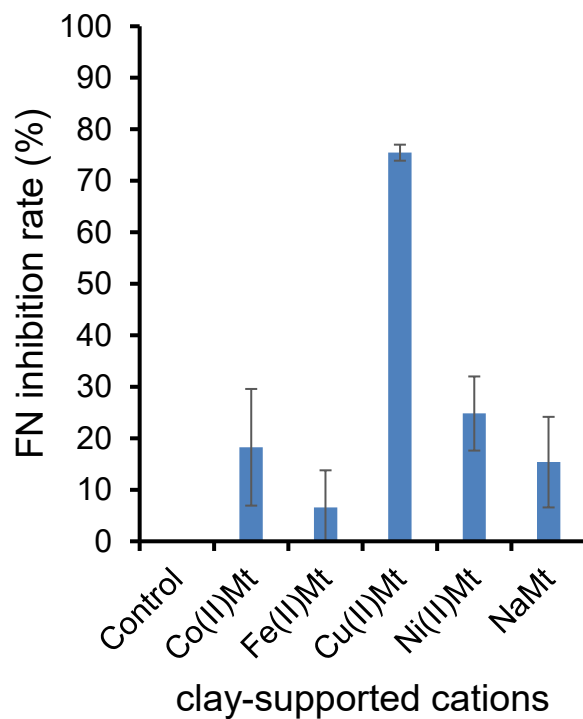
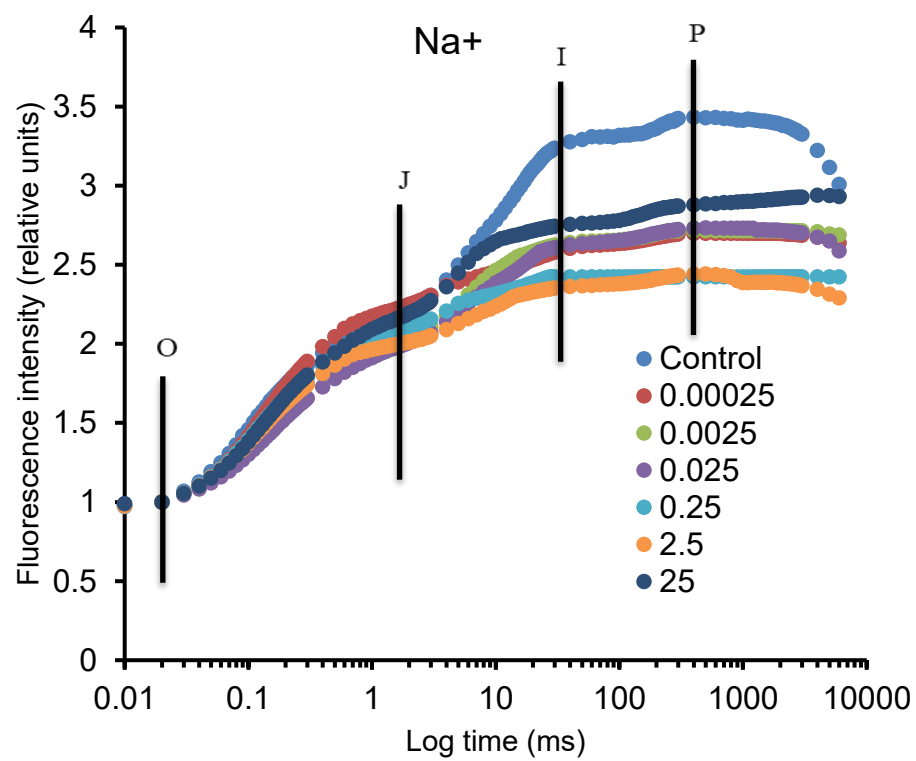
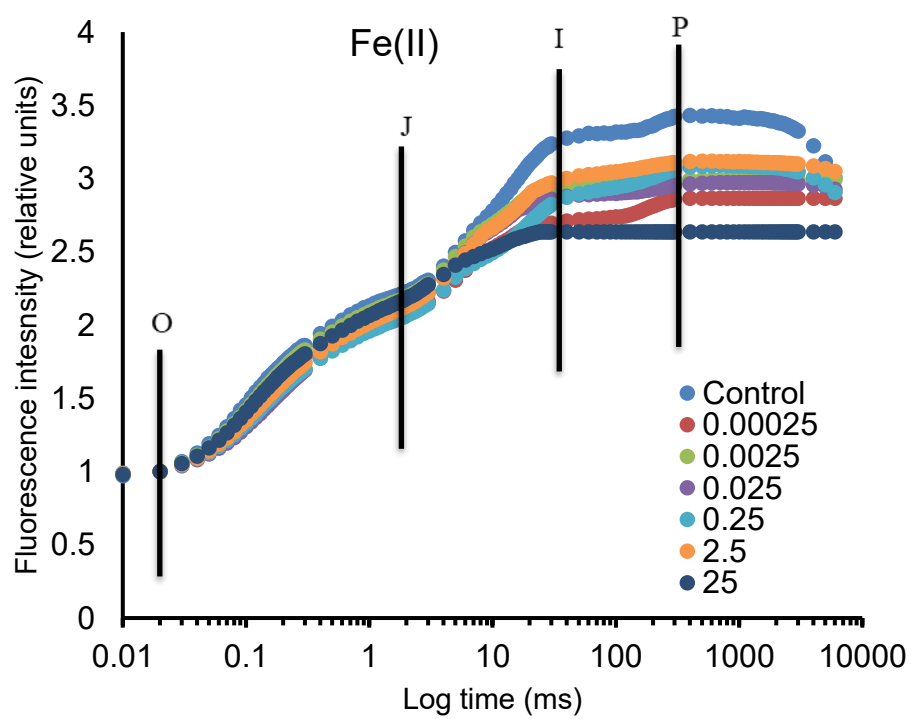


Fig. S12. Effect of clay catalyst alone on toxicity on *L. minor* as expressed in terms of inhibition of frond number growth as reported to the blank sample (without clay catalyst). This result is obtained after 7 days of exposure in SIS medium. The results are expressed as percentages relative to the control sample.

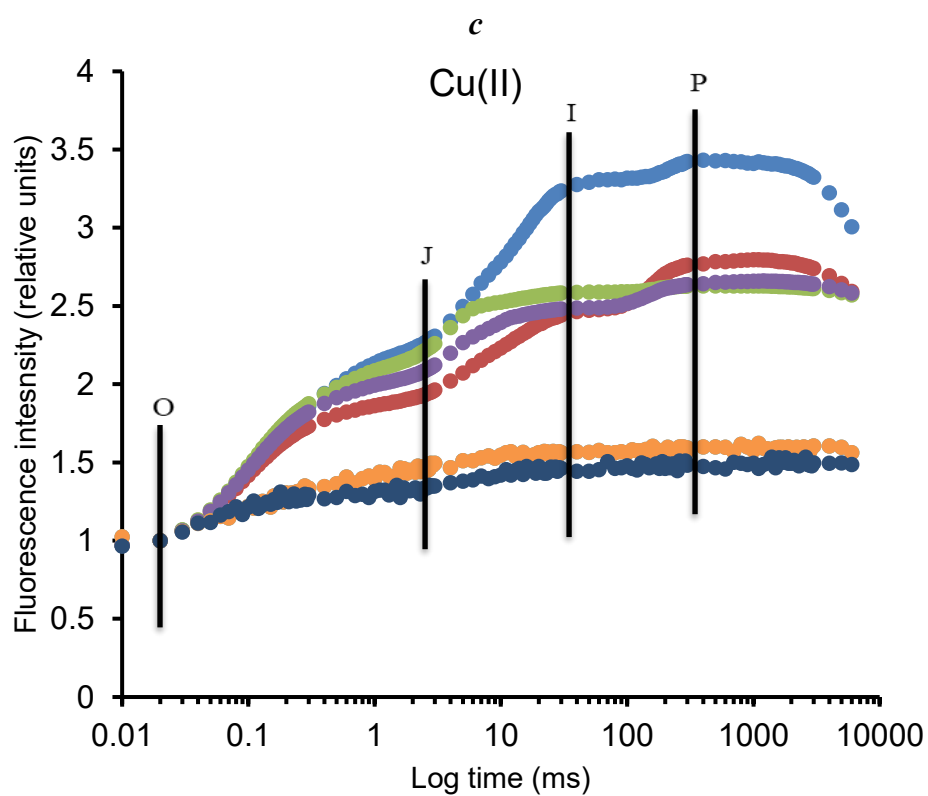
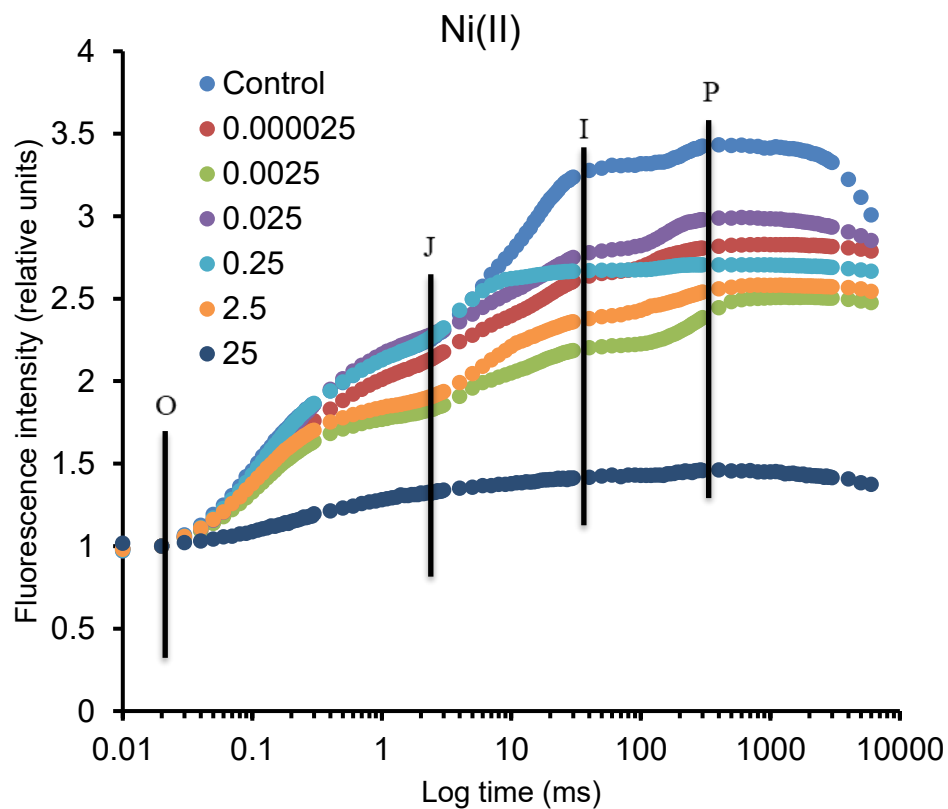
13. Changes in the fast and polyphasic fluorescence kinetics of chlorophyll a of *L. minor*



a



b



d

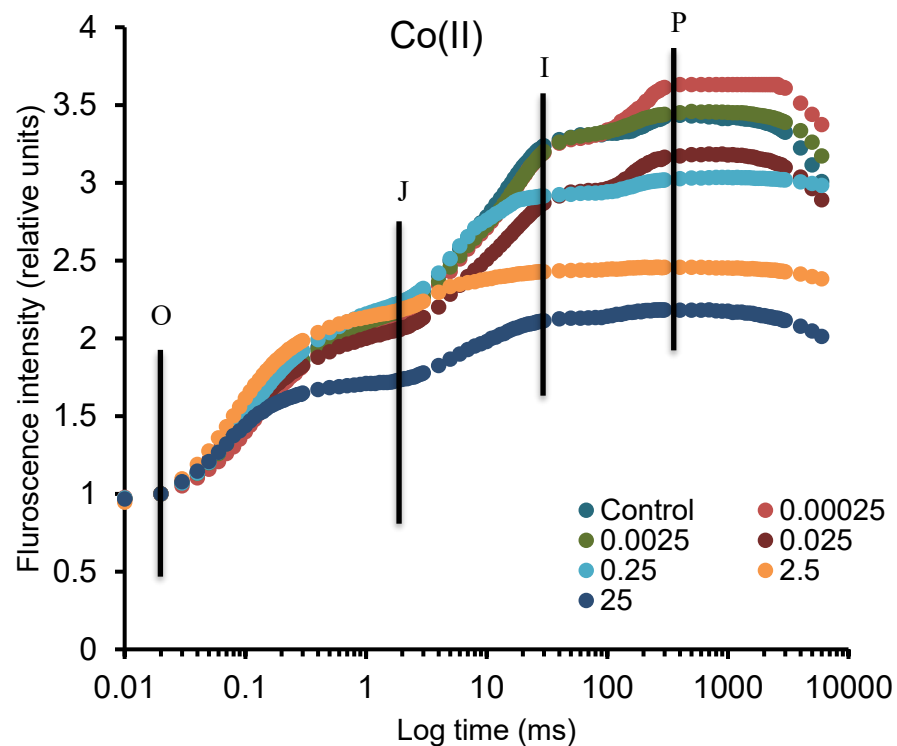


Fig. S13. Change in the fast and polyphasic fluorescence kinetics of chlorophyll *a* of *L. minor*. Plants were exposed for 7 days to different cation concentrations (mg/L). Symbols O, J, I and P represent, respectively, the fluorescence intensities at 50 μ s, 2ms, 30ms, and 200ms.

14. Changes in the variable fluorescence relative to the reduction of quinone A

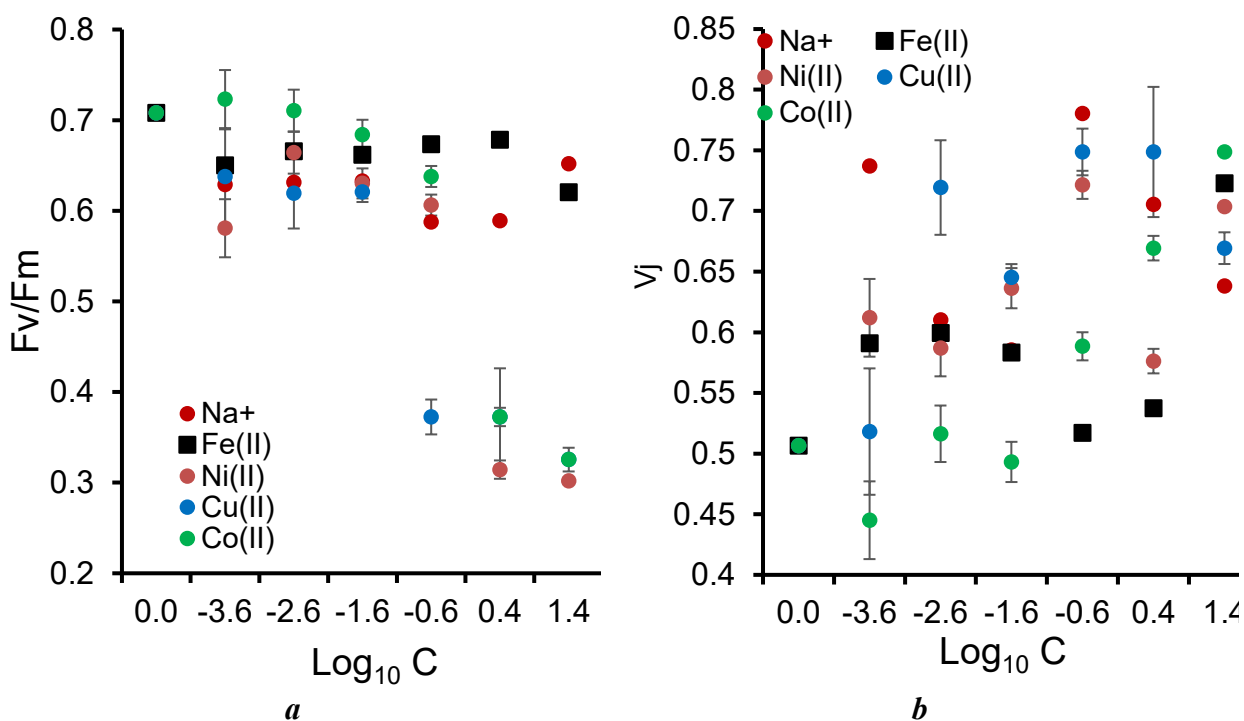


Fig. S14. Change in the variable fluorescence relative to the reduction of quinone A Q_A (V_j). *L. minor* were exposed for 7 days to different cation concentrations (mg/L). Triplicates measurements were achieved including the metals-free control sample. The control consisted of plants grown in SIS medium, an average fresh weight of 96 mg and 66 fronds.

15. Effect of cation-catalyzed NOF ozonation on chlorophyll *a/b* ratio of *L. minor*

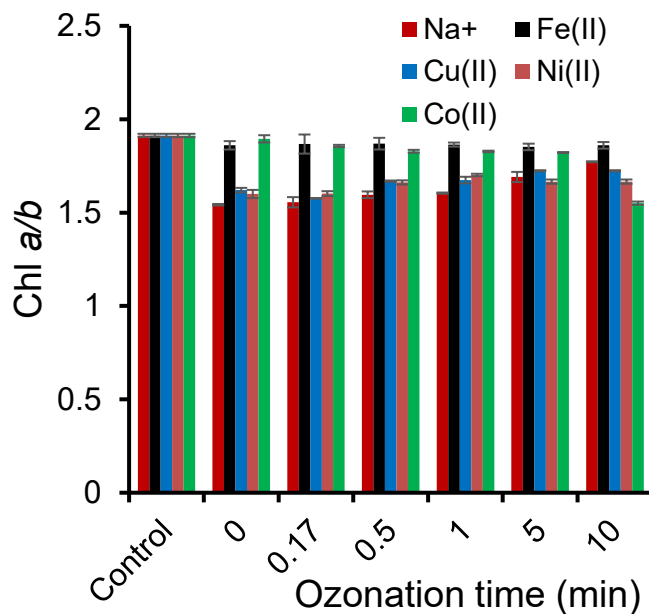


Fig. S15. Effect of cation-catalyzed NOF ozonation on chlorophyll *a/b* ratio of *L. minor*. Triplicates measurements were achieved including the metals-free control sample that consisted in plants growth in SIS medium, an average fresh weight of 96 mg and 66 fronds.

16. Effect of cation-catalyzed NOF ozonation on frond number inhibition and ROS production

Table S3. ANOVA-Test of multiple comparison for concentration effect of cations alone dissolved in water on toxicity on *L. minor* as expressed in terms of frond number inhibition rate and relative ROS production

	Na ⁺ cation							
	Frond number inhibition rate (%)				ROS relative production (%)			
	MeanDiff	Prob	Alpha	Sig	MeanDiff	Prob	Alpha	Sig
-3.6 0	16.66667	<0.0001	0.05	1	-56.1778	0.59172	0.05	0
-2.6 0	27.77778	<0.0001	0.05	1	-54.28077	0.62647	0.05	0
-6.2	11.11111	0.00328	0.05	1	1.89703	1	0.05	0
-1.6 0	12.12121	0.00149	0.05	1	-73.164	0.31178	0.05	0
-5.2	-4.54545	0.44853	0.05	0	-16.9862	0.99779	0.05	0
-4.2	-15.65657	1.11E-04	0.05	1	-18.88322	0.99606	0.05	0
-0.6 0	17.67677	<0.0001	0.05	1	-42.05377	0.83327	0.05	0
-4.2	1.0101	0.99916	0.05	0	14.12403	0.99921	0.05	0
-3.2	-10.10101	0.00735	0.05	1	12.227	0.99965	0.05	0
-2.2	5.55556	0.24485	0.05	0	31.11023	0.95168	0.05	0
0.4 0	19.19192	<0.0001	0.05	1	42.18887	0.83132	0.05	0
0.4 -3.6	2.52525	0.91218	0.05	0	98.36667	0.08959	0.05	0
0.4 -2.6	-8.58586	0.02492	0.05	1	96.46965	0.09918	0.05	0
0.4 -1.6	7.07071	0.08235	0.05	0	115.35287	0.03499	0.05	1
0.4 -0.6	1.51515	0.99231	0.05	0	84.24264	0.18604	0.05	0
1.4 0	24.74747	<0.0001	0.05	1	30.72668	0.95433	0.05	0
1.4 -3.6	8.08081	0.03734	0.05	1	86.90448	0.16295	0.05	0
1.4 -2.6	-3.0303	0.82196	0.05	0	85.00746	0.17914	0.05	0
1.4 -1.6	12.62626	0.00101	0.05	1	103.89068	0.06634	0.05	0
1.4 -0.6	7.07071	0.08235	0.05	0	72.78045	0.31703	0.05	0
1.4 0.4	5.55556	0.24485	0.05	0	-11.46219	0.99976	0.05	0
	Fe ²⁺ cation							
	Frond number inhibition rate (%)				ROS relative production (%)			
	MeanDiff	Prob	Alpha	Sig	MeanDiff	Prob	Alpha	Sig
-3.6 0	4.84092	0.91362	0.05	0	22.22785	0.99956	0.05	0
-2.6 0	18.11516	0.0128	0.05	1	-49.0475	0.96987	0.05	0
-6.2	13.27424	0.094	0.05	0	-71.27536	0.85027	0.05	0
-1.6 0	6.44788	0.74985	0.05	0	11.11619	0.99999	0.05	0
-5.2	1.60696	0.99972	0.05	0	-11.11166	0.99999	0.05	0
-4.2	-11.66728	0.17331	0.05	0	60.16369	0.92407	0.05	0
-0.6 0	4.70719	0.92324	0.05	0	-62.0553	0.91348	0.05	0
-4.2	-0.13373	1	0.05	0	-84.28315	0.73361	0.05	0
-3.2	-13.40797	0.08917	0.05	0	-13.0078	0.99998	0.05	0
-2.2	-1.74069	0.99955	0.05	0	-73.17149	0.83504	0.05	0

0.4 0	8.53208	0.47567	0.05	0	34.00555	0.99534	0.05	0
0.4 -3.6	3.69116	0.97469	0.05	0	11.7777	0.99999	0.05	0
0.4 -2.6	-9.58308	0.3514	0.05	0	83.05305	0.74568	0.05	0
0.4 -1.6	2.0842	0.99876	0.05	0	22.88936	0.99948	0.05	0
0.4 -0.6	3.82489	0.96998	0.05	0	96.06085	0.61243	0.05	0
1.4 0	5.93301	0.81083	0.05	0	-38.82035	0.99062	0.05	0
1.4 -3.6	1.0921	0.99997	0.05	0	-61.04821	0.91922	0.05	0
1.4 -2.6	-12.18215	0.14316	0.05	0	10.22715	0.99999	0.05	0
1.4 -1.6	-0.51487	1	0.05	0	-49.93654	0.96719	0.05	0
1.4 -0.6	1.22582	0.99994	0.05	0	23.23495	0.99944	0.05	0
1.4 0.4	-2.59907	0.99583	0.05	0	-72.82591	0.83787	0.05	0
Ni ²⁺ cation								
	Frond number inhibition rate (%)				ROS relative production (%)			
	MeanDiff	Prob	Alpha	Sig	MeanDiff	Prob	Alpha	Sig
-3.6 0	19.69697	<0.0001	0.05	1	73.44753	0.99651	0.05	0
-2.6 0	24.74747	<0.0001	0.05	1	11.3901	1	0.05	0
-6.2	5.05051	0.12456	0.05	0	-62.05743	0.99862	0.05	0
-1.6 0	27.27273	<0.0001	0.05	1	31.2925	0.99997	0.05	0
-5.2	7.57576	0.00951	0.05	1	-42.15503	0.99985	0.05	0
-4.2	2.52525	0.77108	0.05	0	19.9024	1	0.05	0
-0.6 0	37.37374	<0.0001	0.05	1	350.42298	0.15341	0.05	0
-4.2	17.67677	<0.0001	0.05	1	276.97545	0.36233	0.05	0
-3.2	12.62626	<0.0001	0.05	1	339.03288	0.17712	0.05	0
-2.2	10.10101	7.38E-04	0.05	1	319.13048	0.22586	0.05	0
0.4 0	77.77778	<0.0001	0.05	1	774.88272	4.24E-04	0.05	1
0.4 -3.6	58.08081	<0.0001	0.05	1	701.43519	0.00113	0.05	1
0.4 -2.6	53.0303	<0.0001	0.05	1	763.49262	4.92E-04	0.05	1
0.4 -1.6	50.50505	<0.0001	0.05	1	743.59022	6.41E-04	0.05	1
0.4 -0.6	40.40404	<0.0001	0.05	1	424.45974	0.05677	0.05	0
1.4 0	86.36364	<0.0001	0.05	1	-75.84853	0.99585	0.05	0
1.4 -3.6	66.66667	<0.0001	0.05	1	-149.29606	0.89208	0.05	0
1.4 -2.6	61.61616	<0.0001	0.05	1	-87.23864	0.99128	0.05	0
1.4 -1.6	59.09091	<0.0001	0.05	1	-107.14104	0.97545	0.05	0
1.4 -0.6	48.9899	<0.0001	0.05	1	-426.27151	0.05536	0.05	0
1.4 0.4	8.58586	0.00335	0.05	1	-850.73126	1.60E-04	0.05	1
Cu ²⁺ cation								
	Frond number inhibition rate (%)				ROS relative production (%)			
	MeanDiff	Prob	Alpha	Sig	MeanDiff	Prob	Alpha	Sig
-3.6 0	5.05051	0.40689	0.05	0	67.72055	0.99864	0.05	0
-2.6 0	11.61616	0.00399	0.05	1	124.5853	0.96719	0.05	0
-6.2	6.56566	0.16383	0.05	0	56.86474	0.99949	0.05	0
-1.6 0	11.61616	0.00399	0.05	1	74.26326	0.99774	0.05	0
-5.2	6.56566	0.16383	0.05	0	6.54271	1	0.05	0
-4.2	-3.02E-14	1	0.05	0	-50.32204	0.99975	0.05	0
-0.6 0	27.77778	<0.0001	0.05	1	667.01915	0.00407	0.05	1

-4.2	22.72727	<0.0001	0.05	1	599.2986	0.00982	0.05	1
-3.2	16.16162	1.62E-04	0.05	1	542.43386	0.0207	0.05	1
-2.2	16.16162	1.62E-04	0.05	1	592.75589	0.0107	0.05	1
0.4 0	80.30303	<0.0001	0.05	1	-100	0.98893	0.05	0
0.4 -3.6	75.25253	<0.0001	0.05	1	-167.72055	0.88032	0.05	0
0.4 -2.6	68.68687	<0.0001	0.05	1	-224.5853	0.67548	0.05	0
0.4 -1.6	68.68687	<0.0001	0.05	1	-174.26326	0.86124	0.05	0
0.4 -0.6	52.52525	<0.0001	0.05	1	-767.01915	0.00114	0.05	1
1.4 0	86.86869	<0.0001	0.05	1	-100	0.98893	0.05	0
1.4 -3.6	81.81818	<0.0001	0.05	1	-167.72055	0.88032	0.05	0
1.4 -2.6	75.25253	<0.0001	0.05	1	-224.5853	0.67548	0.05	0
1.4 -1.6	75.25253	<0.0001	0.05	1	-174.26326	0.86124	0.05	0
1.4 -0.6	59.09091	<0.0001	0.05	1	-767.01915	0.00114	0.05	1
1.4 0.4	6.56566	0.16383	0.05	0	0	1	0.05	0
Co ²⁺ cation								
	Frond number inhibition rate (%)				ROS relative production (%)			
	MeanDiff	Prob	Alpha	Sig	MeanDiff	Prob	Alpha	Sig
-3.6 0	-3.0303	0.96629	0.05	0	-206.76957	0.73486	0.05	0
-2.6 0	2.52525	0.9862	0.05	0	-299.11749	0.36039	0.05	0
-6.2	5.55556	0.65416	0.05	0	-92.34792	0.99207	0.05	0
-1.6 0	9.59596	0.13127	0.05	0	-499.70275	0.03271	0.05	1
-5.2	12.62626	0.02702	0.05	1	-292.93318	0.38236	0.05	0
-4.2	7.07071	0.4007	0.05	0	-200.58525	0.75934	0.05	0
-0.6 0	21.21212	3.01E-04	0.05	1	4.98032	1	0.05	0
-4.2	24.24242	<0.0001	0.05	1	211.74989	0.71466	0.05	0
-3.2	18.68687	0.00106	0.05	1	304.09781	0.34327	0.05	0
-2.2	11.61616	0.04643	0.05	1	504.68307	0.03063	0.05	1
0.4 0	57.57576	<0.0001	0.05	1	517.78574	0.02575	0.05	1
0.4 -3.6	60.60606	<0.0001	0.05	1	724.5553	0.00169	0.05	1
0.4 -2.6	55.05051	<0.0001	0.05	1	816.90323	5.31E-04	0.05	1
0.4 -1.6	47.9798	<0.0001	0.05	1	1017.48848	<0.0001	0.05	1
0.4 -0.6	36.36364	<0.0001	0.05	1	512.80541	0.02751	0.05	1
1.4 0	83.33333	<0.0001	0.05	1	365.99016	0.17603	0.05	0
1.4 -3.6	86.36364	<0.0001	0.05	1	572.75972	0.01239	0.05	1
1.4 -2.6	80.80808	<0.0001	0.05	1	665.10765	0.00365	0.05	1
1.4 -1.6	73.73737	<0.0001	0.05	1	865.6929	2.94E-04	0.05	1
1.4 -0.6	62.12121	<0.0001	0.05	1	361.00983	0.18644	0.05	0
1.4 0.4	25.75758	<0.0001	0.05	1	-151.79558	0.91507	0.05	0

Table S4. ANOVA-Test of multiple comparison with control samples for Toxicity of ozonized NOF solution in presence of cations on frond number inhibition rate and ROS production.

Na ⁺ cation								
	Frond number inhibition rate (%)				ROS relative production (%)			
	MeanDiff	Prob	Alpha	Sig	MeanDiff	Prob	Alpha	Sig
0 control	46.52958	<0.0001	0.05	1	142.1334	0.01617	0.05	1
0.17 control	57.17653	<0.0001	0.05	1	-52.55932	0.74427	0.05	0
0.17 0	10.64695	0.06663	0.05	0	-194.69272	0.00112	0.05	1
0.5 control	58.11688	<0.0001	0.05	1	234.59696	1.69E-04	0.05	1
0.5 0	11.5873	0.04	0.05	1	92.46355	0.18945	0.05	0
0.5 0.17	0.94036	0.99993	0.05	0	287.15628	<0.0001	0.05	1
1 control	56.59452	<0.0001	0.05	1	-43.41082	0.86968	0.05	0
1 0	10.06494	0.09071	0.05	0	-185.54422	0.00175	0.05	1
1 0.17	-0.58201	1	0.05	0	9.1485	0.99996	0.05	0
1 0.5	-1.52237	0.99896	0.05	0	-278.00777	<0.0001	0.05	1
5 control	25.73834	<0.0001	0.05	1	-61.11065	0.60536	0.05	0
5 0	-20.79125	2.82E-04	0.05	1	-203.24406	7.35E-04	0.05	1
5 0.17	-31.43819	<0.0001	0.05	1	-8.55133	0.99998	0.05	0
5 0.5	-32.37855	<0.0001	0.05	1	-295.70761	<0.0001	0.05	1
5 1	-30.85618	<0.0001	0.05	1	-17.69984	0.99838	0.05	0
10 control	56.06542	<0.0001	0.05	1	-57.86752	0.65912	0.05	0
10 0	9.53583	0.11928	0.05	0	-200.00093	8.61E-04	0.05	1
10 0.17	-1.11111	0.99983	0.05	0	-5.3082	1	0.05	0
10 0.5	-2.05147	0.99464	0.05	0	-292.46448	<0.0001	0.05	1
10 1	-0.5291	1	0.05	0	-14.45671	0.99948	0.05	0
10 5	30.32708	<0.0001	0.05	1	3.24313	1	0.05	0
Fe ²⁺ cation								
	Frond number inhibition rate (%)				ROS relative production (%)			
	MeanDiff	Prob	Alpha	Sig	MeanDiff	Prob	Alpha	Sig
0 control	13.52573	0.1071	0.05	0	-49.73304	0.80846	0.05	0
0.17 control	16.75565	0.03062	0.05	1	33.63441	0.96174	0.05	0
0.17 0	3.22992	0.9896	0.05	0	83.36745	0.31008	0.05	0
0.5 control	12.46513	0.15756	0.05	0	-59.76746	0.65818	0.05	0
0.5 0	-1.06061	0.99998	0.05	0	-10.03442	0.99995	0.05	0
0.5 0.17	-4.29052	0.95818	0.05	0	-93.40187	0.20642	0.05	0
1 control	16.67869	0.03157	0.05	1	-4.36682	1	0.05	0
1 0	3.15296	0.99082	0.05	0	45.36623	0.86315	0.05	0
1 0.17	-0.07696	1	0.05	0	-38.00123	0.9337	0.05	0
1 0.5	4.21356	0.96154	0.05	0	55.40064	0.72659	0.05	0
5 control	2.09235	0.99901	0.05	0	-26.3936	0.98834	0.05	0
5 0	-11.43338	0.22488	0.05	0	23.33944	0.99386	0.05	0
5 0.17	-14.6633	0.06961	0.05	0	-60.02801	0.65402	0.05	0
5 0.5	-10.37278	0.31577	0.05	0	33.37386	0.96311	0.05	0
5 1	-14.58634	0.0717	0.05	0	-22.02678	0.99549	0.05	0

10 control	14.7595	0.06707	0.05	0	-59.61038	0.66069	0.05	0
10 0	1.23377	0.99995	0.05	0	-9.87734	0.99995	0.05	0
10 0.17	-1.99615	0.99924	0.05	0	-93.24479	0.2078	0.05	0
10 0.5	2.29437	0.99835	0.05	0	0.15708	1	0.05	0
10 1	-1.91919	0.99939	0.05	0	-55.24357	0.72898	0.05	0
10 5	12.66715	0.1466	0.05	0	-33.21678	0.96391	0.05	0
Ni ²⁺ cation								
	Frond number inhibition rate (%)				ROS relative production (%)			
	MeanDiff	Prob	Alpha	Sig	MeanDiff	Prob	Alpha	Sig
0 control	30.53391	<0.0001	0.05	1	718.51112	<0.0001	0.05	1
0.17 control	27.91727	2.30E-04	0.05	1	83.36841	0.92862	0.05	0
0.17 0	-2.61664	0.99548	0.05	0	-635.14272	<0.0001	0.05	1
0.5 control	25.3824	6.09E-04	0.05	1	252.76956	0.06954	0.05	0
0.5 0	-5.15152	0.88497	0.05	0	-465.74156	5.50E-04	0.05	1
0.5 0.17	-2.53487	0.99619	0.05	0	169.40116	0.37116	0.05	0
1 control	18.34536	0.01091	0.05	1	33.22784	0.99938	0.05	0
1 0	-12.18855	0.13751	0.05	0	-685.28328	<0.0001	0.05	1
1 0.17	-9.57191	0.34412	0.05	0	-50.14056	0.99399	0.05	0
1 0.5	-7.03704	0.66622	0.05	0	-219.54172	0.14292	0.05	0
5 control	33.88648	<0.0001	0.05	1	489.28062	3.34E-04	0.05	1
5 0	3.35257	0.9836	0.05	0	-229.23051	0.11644	0.05	0
5 0.17	5.96922	0.80117	0.05	0	405.91221	0.00205	0.05	1
5 0.5	8.50409	0.47033	0.05	0	236.51105	0.09952	0.05	0
5 1	15.54113	0.03558	0.05	1	456.05277	6.78E-04	0.05	1
10 control	84.10293	<0.0001	0.05	1	-59.61038	0.98526	0.05	0
10 0	53.56902	<0.0001	0.05	1	-778.1215	<0.0001	0.05	1
10 0.17	56.18567	<0.0001	0.05	1	-142.97879	0.55338	0.05	0
10 0.5	58.72054	<0.0001	0.05	1	-312.37994	0.01771	0.05	1
10 1	65.75758	<0.0001	0.05	1	-92.83822	0.8885	0.05	0
10 5	50.21645	<0.0001	0.05	1	-548.891	<0.0001	0.05	1
Cu ²⁺ cation								
	Frond number inhibition rate (%)				ROS relative production (%)			
	MeanDiff	Prob	Alpha	Sig	MeanDiff	Prob	Alpha	Sig
0 control	31.64502	0.00108	0.05	1	411.8555	0.00503	0.05	1
0.17 control	24.12939	0.01132	0.05	1	810.90537	<0.0001	0.05	1
0.17 0	-7.51563	0.83255	0.05	0	399.04987	0.00655	0.05	1
0.5 control	22.60462	0.01843	0.05	1	469.434	0.00157	0.05	1
0.5 0	-9.0404	0.69237	0.05	0	57.57849	0.99325	0.05	0
0.5 0.17	-1.52477	0.99996	0.05	0	-341.47137	0.02155	0.05	1
1 control	16.12314	0.13691	0.05	0	17.34731	0.99999	0.05	0
1 0	-15.52189	0.16253	0.05	0	-394.50819	0.00719	0.05	1
1 0.17	-8.00625	0.79082	0.05	0	-793.55806	<0.0001	0.05	1
1 0.5	-6.48148	0.90567	0.05	0	-452.08668	0.00222	0.05	1
5 control	21.37326	0.0273	0.05	1	-20.8978	0.99998	0.05	0

5 0	-10.27177	0.56676	0.05	0	-432.7533	0.00328	0.05	1
5 0.17	-2.75613	0.9987	0.05	0	-831.80317	<0.0001	0.05	1
5 0.5	-1.23136	0.99999	0.05	0	-490.33179	0.00104	0.05	1
5 1	5.25012	0.96268	0.05	0	-38.24511	0.99929	0.05	0
10 control	84.10293	<0.0001	0.05	1	-59.61038	0.9919	0.05	0
10 0	52.45791	<0.0001	0.05	1	-471.46589	0.00151	0.05	1
10 0.17	59.97354	<0.0001	0.05	1	-870.51575	<0.0001	0.05	1
10 0.5	61.49832	<0.0001	0.05	1	-529.04438	4.92E-04	0.05	1
10 1	67.9798	<0.0001	0.05	1	-76.95769	0.97081	0.05	0
10 5	62.72968	<0.0001	0.05	1	-38.71258	0.99924	0.05	0
Co ²⁺ cation								
	Frond number inhibition rate (%)				ROS relative production (%)			
	MeanDiff	Prob	Alpha	Sig	MeanDiff	Prob	Alpha	Sig
0 control	27.50601	0.00192	0.05	1	55.29931	0.80159	0.05	0
0.17 control	25.20683	0.00417	0.05	1	119.6975	0.10387	0.05	0
0.17 0	-2.29918	0.99927	0.05	0	64.39819	0.67824	0.05	0
0.5 control	23.18903	0.00834	0.05	1	3.80722	1	0.05	0
0.5 0	-4.31698	0.97871	0.05	0	-51.49209	0.84639	0.05	0
0.5 0.17	-2.0178	0.99965	0.05	0	-115.89028	0.12192	0.05	0
1 control	21.22174	0.01647	0.05	1	106.63631	0.17771	0.05	0
1 0	-6.28427	0.88564	0.05	0	51.33699	0.8481	0.05	0
1 0.17	-3.98509	0.98571	0.05	0	-13.0612	0.99986	0.05	0
1 0.5	-1.96729	0.9997	0.05	0	102.82909	0.20625	0.05	0
5 control	5.17316	0.94998	0.05	0	-39.77824	0.94689	0.05	0
5 0	-22.33285	0.01121	0.05	1	-95.07755	0.27563	0.05	0
5 0.17	-20.03367	0.02483	0.05	1	-159.47574	0.01767	0.05	1
5 0.5	-18.01587	0.04949	0.05	1	-43.58546	0.92089	0.05	0
5 1	-16.04858	0.09509	0.05	0	-146.41454	0.03199	0.05	1
10 control	68.86243	<0.0001	0.05	1	228.12899	8.25E-04	0.05	1
10 0	41.35642	<0.0001	0.05	1	172.82968	0.00961	0.05	1
10 0.17	43.6556	<0.0001	0.05	1	108.43149	0.16544	0.05	0
10 0.5	45.6734	<0.0001	0.05	1	224.32177	9.72E-04	0.05	1
10 1	47.64069	<0.0001	0.05	1	121.49269	0.09623	0.05	0
10 5	63.68927	<0.0001	0.05	1	267.90723	1.60E-04	0.05	1

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ANNEXE C

SUPPORTING INFORMATION DE L'ARTICLE 3

Study of metal interaction and valence on Norfloxacin adsorption and ozonation in clay suspensions

Roumaissa Djidja^a, David Dewez^{a,*} and Abdelkrim Azzouz^{a,b,*}

1. Operating conditions for HPLC-DAD and ICP-OES measurements

Table S1. Parameters of HPLC-DAD analysis

Parameter	Specification
Apparatus model	Agilent Technologies model 1290 equipment
Column specification	C18 column 4.6 ×150 mm, 5 µm particle size
Mobile phases composition	A: water with 0.1% Formic Acid B: methanol
Detection wavelength	275 nm
Gradient / isocratic	0-0.5 min, 10% B; 0.5-2.0 min, 10-15% B; 2.0-8.0 min, 15-35% B; 8.0-10.0 min, 35-90% B; 10.0-11.0 min, 90% B; 11.0-11.2 min, 90-10% B; 11.2-18.0 min, 10%B.
Flow rate	1 mL. min ⁻¹
Temperature	20 °C
Pump pressure	18 MPa
Injected volume	20 µL

Table S2. Instrumental parameters of ICP-OES

Parameter	value
Power	1.5 kW
Plasma argon flow rate	12 L/min
Auxiliary argon flow rate	1 L/min
Nebulizer argon flow rate	1 L/min
Wavelengths (nm)	Na (589.592), Fe (238.204), Ni (216.555), Cu (199.970) and Co (228.615).

2. Factorial design for the 27 NOF ozonation attempts

Table S3. Parameter variation ranges for 3^2 factorial designs at $T = 25\text{ }^{\circ}\text{C}$

Parameter	Symbol (Coded Variation)	Variation levels ^a		
		Minimum (-1)	Center (0)	Maximum (+1)
pH	X_1	3.2	4.2	5.2
Clay material amount (g. L^{-1})	X_2	1	2	3
Ozonation time (min)	X_3	1	5	9

^a In brackets: the coded value of the variation levels for expressing the reduced (non-dimensional) value of each parameter

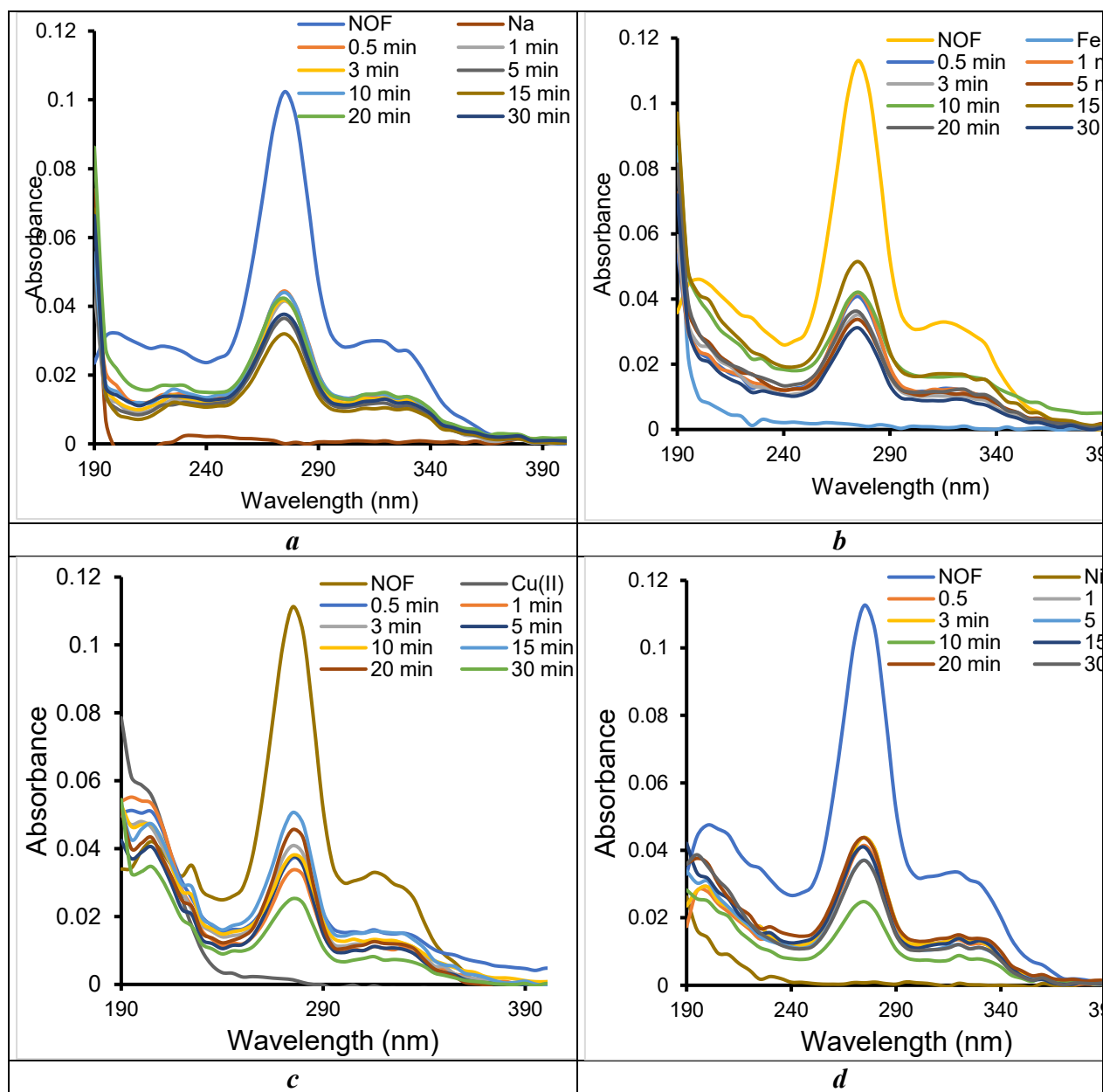
3. SIS media preparation

Table S4. Preparation and composition according to Swedish Standard for *Lemna* growth medium (SIS).

Stock solution	Chemicals	Concentration in		Manufacturer
		Stock solution (g/L)	SIS medium* (mg/L)	
I	Na NO ₃	8.500	85.000	Anachemia, ACS grade Anachemia
	KH ₂ PO ₄	1.340	13.400	
II	MgSO ₄ .7H ₂ O	15.000	75.000	Anachemia
III	CaCl ₂ .2H ₂ O	7.200	36.000	Anachemia, ACS grade
IV	Na ₂ CO ₃	4.000	20.000	Fisher Scientific
V	ZnSO ₄ .7H ₂ O	0.050	0.050	Anachemia, ACS grade
	H ₃ BO ₃	1.000	1.000	Anachemia, ACS grade
	MnCl ₂ .4H ₂ O	0.200	0.200	Anachemia, ACS grade
	Na ₂ MoO ₄ .2H ₂ O	0.010	0.010	Fisher Chemicals
	CuSO ₄ .5H ₂ O	0.005	0.005	Anachemia, ACS grade
	Co(NO ₃).6H ₂ O	0.010	0.010	Anachemia, ACS grade
VI	FeCl ₃ .6H ₂ O	0.170	0.840	Anachemia
	Na ₂ -EDTA.2H ₂ O	0.280	1.400	
VII	MOPS (buffer)	490.000	490.000	Anachemia

* The SIS medium was prepared through the dissolution of 10 mL of solution I, 5 mL of solution II, 5 mL of solution III, 5 mL of solution IV, 1 mL of solution V, 5 mL of solution VI, 1 mL of solution VII in a 1 L flask containing ca 900 mL of ultrapure water and dissolution of the following stock solutions. The pH was adjusted to 6.5 ± 0.2 with either 0.1 M HCl or 0.1 M NaOH. The volume was finally adjusted to 1 litre.

4. Metal cation:NOF interaction



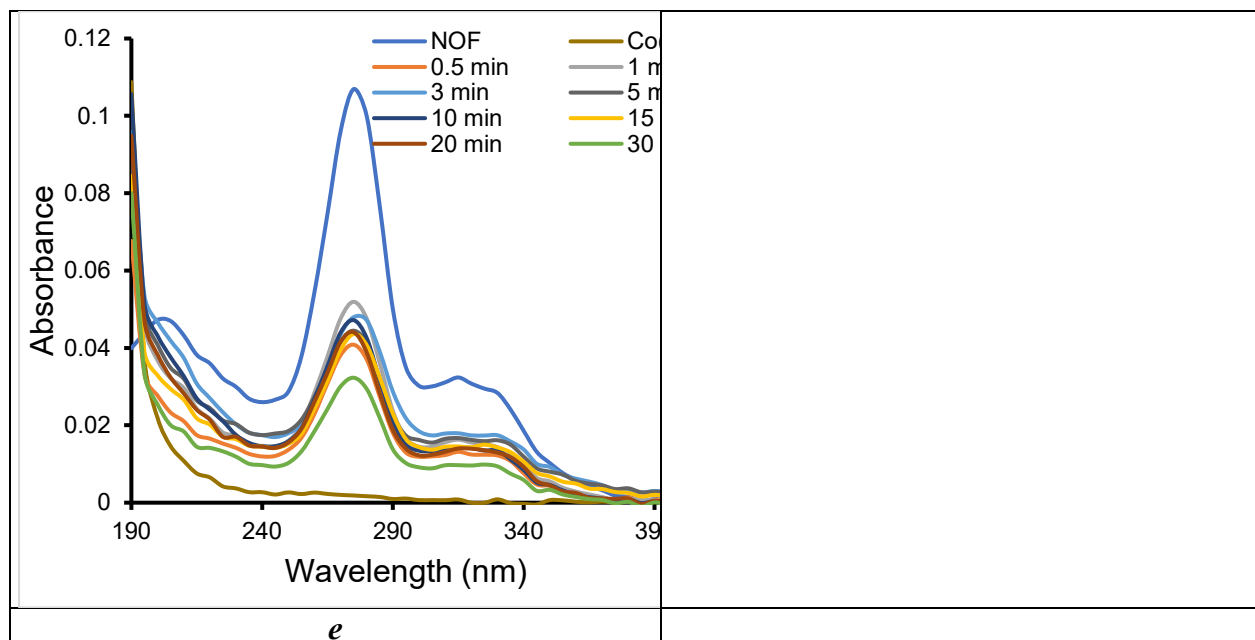
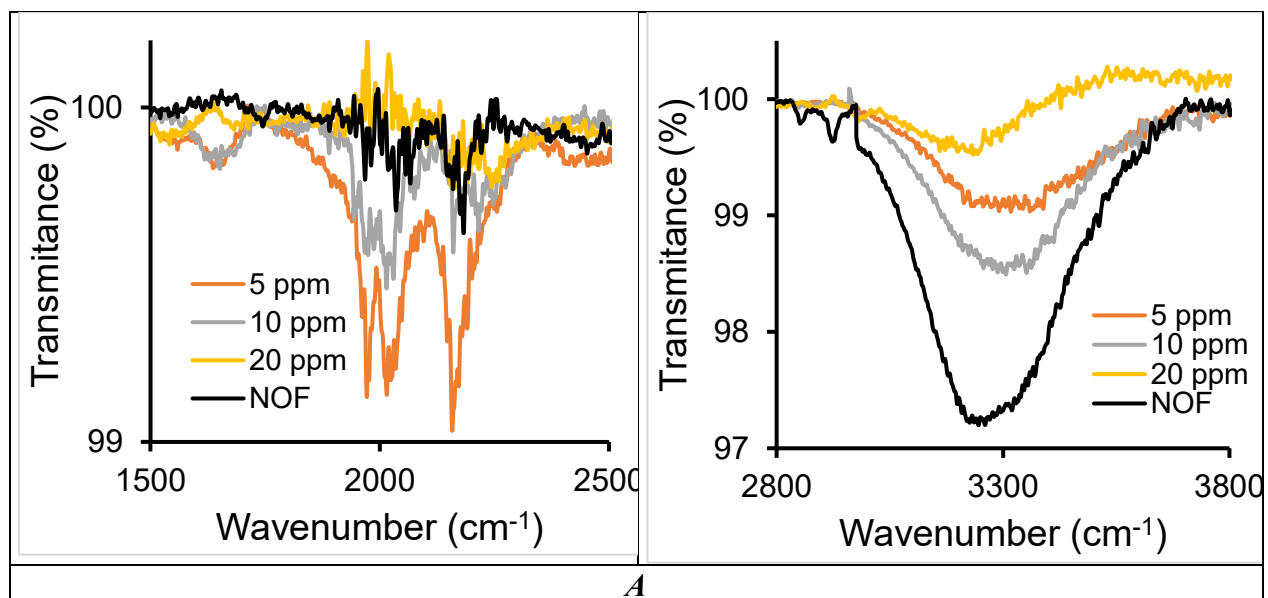
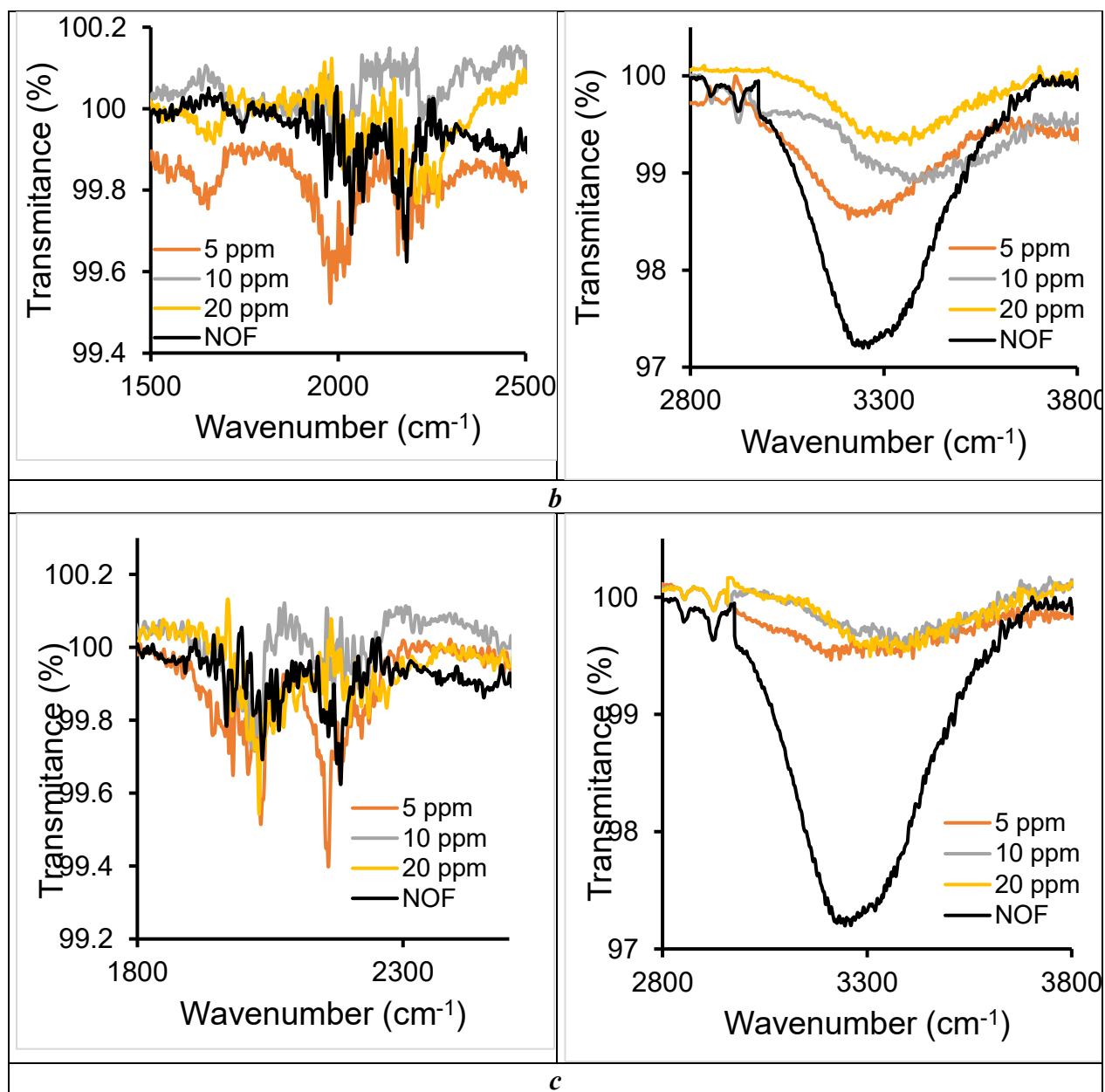


Fig. S1. UV–vis absorption spectra for Norfloxacin, metal ions and their complexes at different contact time. Na (a), Fe (b), Cu (c), Ni (d) and Co (e)





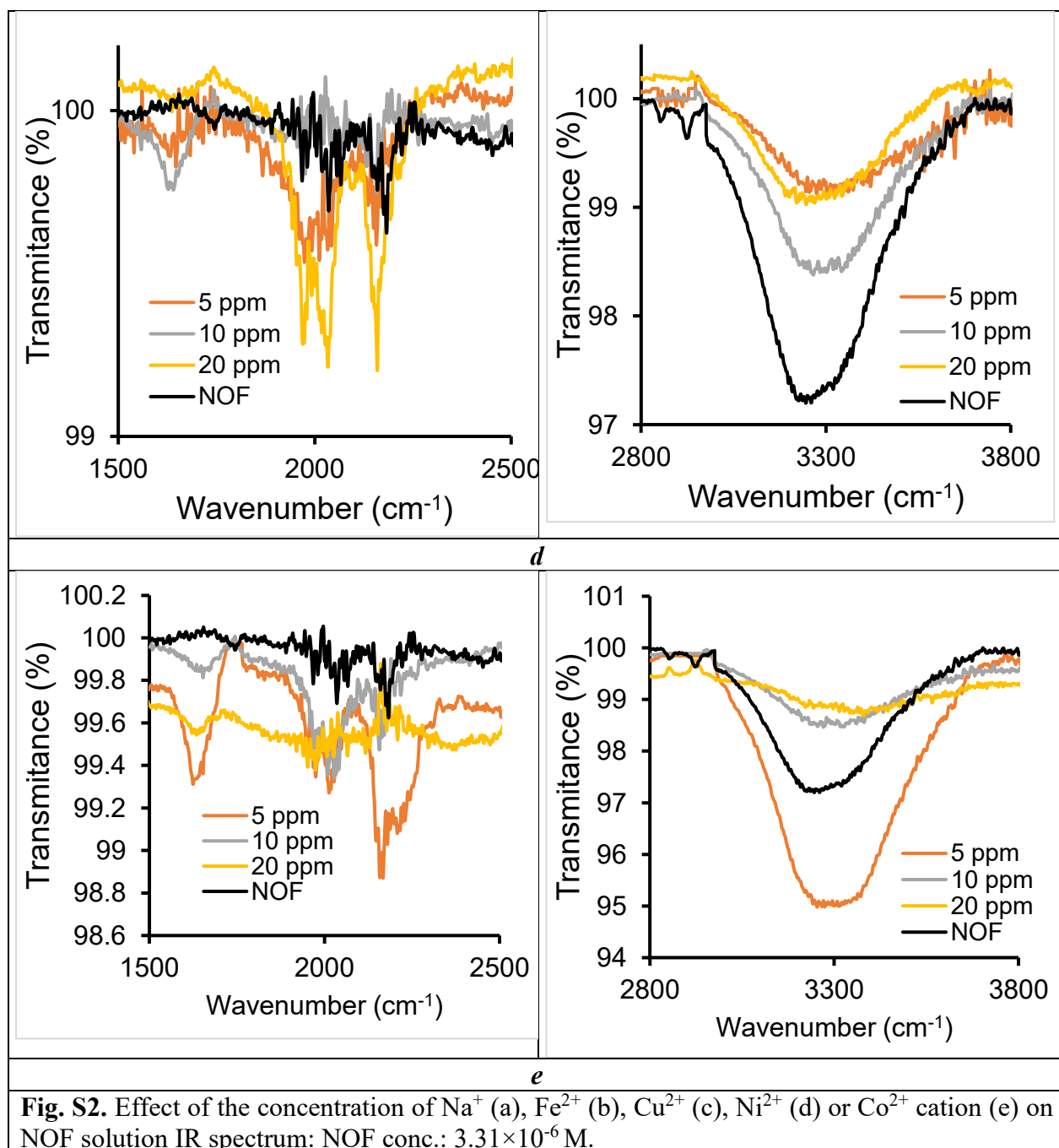


Table S5. FTIR vibrational changes of norfloxacin upon metal addition

Complex	Conc. (ppm)	N-H stretching O-H stretching	C=O stretching	N-H bending
		Wavenumber (cm ⁻¹)		
NOF	1	3260	1740	-
	5	3320	-	1650
	10	3320	-	1650
NOF:Na	20	3270	-	-
	5	3240	-	1650
	10	3420	-	1670
NOF:Fe	20	3370	-	1670
	5	3280	-	-
	10	3380	-	1650
NOF:Cu	20	3380	-	1650
	5	3320	-	1660
	10	3300	-	1640
NOF:Ni	20	3280	-	-
	5	3290	-	1620
	10	3300	-	1650
NOF:Co	20	3320	-	1630

5. Parameter interaction and effect on NOF degradation and toxicity

Table S6. NOF relative absorbance (Y_{1,2,3}) for 200, 275, 315 nm respectively, fresh wight (Y₄), fronds number (Y₅), number of plants (Y₆), Chl *a/b* (Y₇), ROS (Y₈) and final pH measurements (Y₉) for NaMt and Fe(II)Mt catalyst system

NaMt											
X ₁	X ₂	X ₃	A/A _{0_200} Y ₁	A/A _{0_275} Y ₂	A/A _{0_315}	Fresh weight Y ₄	Fronds numbe Y 5	Number of plants Y ₆	Chl <i>a/b</i> Y ₇	ROS Y ₈	Fina l pH Y ₉
-1	-1	-1	17,9491	1,6233	1,9700	0.098	54	15	0,8838	168,9828	
-1	-1	0	35.8687	1.7021	2.0211	0.079	50	10	0.8838	168.9828	
-1	-1	1	45.0644	1.5136	1.8097	0.009	9	9	0.4184	321,9287	
-1	0	-1	24.3342	2.3581	2.8636	0.090	46	8	1,2682	646,8543	
-1	0	0	46.9685	2.8231	3.3335	0.073	47	15	1,2431	1237,417 0	
-1	0	1	9.3204	0.6182	1.5317	0.082	44	12	1,3142	423,5099	
-1	1	-1	23.9497	2.5182	2.9579	0.065	38	12	1,3423	644,0141	
-1	1	0	41.4197	3.5855	4.4993	0.055	30	11	1,2992	1977,465 0	

-1	1	1	16,4598	1,0420	2,5281	0.064	33	7	1,2787	349,2958	
0	-1	-1	13,2365	1,1648	1,4708	0.075	50	12	1,2358	6,9549	
0	-1	0	40.2056	1.4840	1.9228	0.064	46	10	1.2183	242,1321	
0	-1	1	45,2579	1,0638	1,1678	0.048	40	9	1,2474	103,8668	
0	0	-1	33,2118	3,6164	3,5118	0.060	33	10	1,2648	765,3496	
0	0	0	39.1972	2.2873	2.5990	0.069	48	11	1.0875	83,6273	
0	0	0	41.1368	2.0928	2.3546	0.087	45	13	1.0875	84.2756	
0	0	0	40.4841	2.0884	2.4649	0.084	53	13	1.0778	86,6549	
0	0	1	53.3965	2.7666	3.2864	0.066	40	9	1.2992	316,8693	
0	1	-1	28.3953	2.7059	3.2392	0.065	44	11	1.4345	548,6280	
0	1	0	45.4518	4.7815	4.6840	0.078	40	11	1.4062	781,5549	
0	1	1	14,7772	0,9314	2,1056	0.061	36	10	1,3704	1366,3110	
1	-1	-1	20.8299	1.4111	1.7579	0.038	28	8	1.1553	418,3673	
1	-1	0	40.4083	1.7101	2.0554	0.061	32	10	1.3757	81,2777	
1	-1	1	50.9514	3.0569	3.4698	0.080	56	17	1.6233	287,0452	
1	0	-1	43.3925	2.5703	3.5222	0.019	23	9	1.2079	85,4326	
1	0	0	40.7216	2.4962	2.9241	0.042	28	9	1.4310	258,5980	
1	0	1	44.2105	1.7574	2.1017	0.063	28	9	1.3288	79,8832	
1	1	-1	35.9819	5.0071	5.0071	0.076	36	11	1.5347	57,6768	
1	1	0	45.1411	4.6585	4.6584	0.064	33	11	1.3843	194,2424	
1	1	1	51,2096	4,3533	4,3533	0.030	26	9	1,2544	591,4647	
Fe(II)Mt											
X ₁	X ₂	X ₃	A/A _{0_200} Y ₁	A/A _{0_275} Y ₂	A/A _{0_315} Y ₃	Fresh weight Y ₄	Fronds numbe Y 5	Number of plants Y ₆	Chl <i>a/b</i> Y ₇	ROS Y ₈	Fina l pH Y ₉
-1	-1	-1	7.4360	0.3324	0.9358	0.085	55	17	1.7016	474.2496	
-1	-1	0	29.9904	0.3468	0.9770	0.032	43	12	1.4882	621.9589	
-1	-1	1	41.2761	0.2928	0.8233	0.001	9	9	0.4960	572.9589	
-1	0	-1	7.8839	0.3037	0.8322	0.099	65	23	1.6880	3.3592	
-1	0	0	27,9383	0,3663	0,9991	0.029	35	11	1,6165	16,3204	
-1	0	1	39.7397	0.3122	0.8595	0.003	9	9	0.5996	0.2187	
-1	1	-1	8.8689	0.3326	0.8697	0.086	57	17	1.6784	1723.8727	
-1	1	0	27,3644	0,3278	0,9095	0.018	27	8	1,3579	3073,2091	
-1	1	1	45.9466	0.4112	1.1190	0.004	12	12	0.5132	2557.0292	
0	-1	-1	8.01741	0.5565	1.5360	0.092	64	20	1.1118	143,1301	
0	-1	0	29,2932	0,5178	1,4731	0.099	71	22	1,3104	46,0163	
0	-1	1	29.3615	0.3247	0.8827	0.001	9	9	0.3922	286,9106	

0	0	-1	7.5331	0.5493	1.4338	0.088	57	18	0.8776	1866.9064	
0	0	0	26.9505	0.5422	1.4189	0.088	74	23	1.0862	1269,7842	
0	0	0	26,2587	0.5391	1.3974	0.083	69	21	1,04840	1264.5458	
0	0	0	25.9573	0.5179	1.3806	0.086	71	21	1,07985	1262.3795	
0	0	1	40.3938	0.5776	1.5277	0.002	9	9	0.4840	906,4748	
0	1	-1	7.6516	0.6171	1.5684	0.088	56	18	1.1273	3,5235	
0	1	0	24.8021	0.6307	1.6019	0.083	62	21	1.1531	32,1216	
0	1	1	36.8586	0.4700	1.2020	0.028	24	9	0.8301	151.8456	
1	-1	-1	8,64501	0,4925	1,3279	0.113	68	24	0,0794	182,4687	
1	-1	0	25.0314	0.5158	1.4389	0.081	63	21	1.2131	843.8283	
1	-1	1	37,2302	0,5189	1,4481	0.006	11	11	0,0914	293,3810	
1	0	-1	8,46069	0,5748	1,5394	0.103	68	22	1,3212	3076,6917	
1	0	0	27.1959	0.6301	1.6326	0.073	62	19	1.2267	3485.7143	
1	0	1	32.3008	0.5224	1.3610	0.003	8	8	0.7668	6022,5564	
1	1	-1	7,82108	0,6092	1,5242	0.096	62	20	1,0088	313,3735	
1	1	0	25.0325	0.6519	1.6417	0.093	64	21	1.0593	336.5060	
1	1	1	35,3533	0,3755	0,9256	0.006	9	9	0,3957	985,3012	

Table S7. Polynomial models for the response-functions in NOF ozonation with NaMt and Fe(II)Mt.

Catalyst	Response-function		Polynomial coefficients										
			a ₀	a ₁ pH effect	a ₂ clay amount effect	a ₃ ozonation time effect	a ₁₁	a ₂₂	a ₃₃	a ₁₂	a ₁₃	a ₂₃	a ₁₂₃
NaMt	A/A ₀ (200nm)	Y ₁	43,525	6,195	-0,388	4,965	0,440	-3,164	-9,94	3,10	3,463	-7,928	2,464
	A/A ₀ (275nm)	Y ₂	2,654	0,513	0,825	-0,326	0,178	0,096	-0,61	0,461	0,292	-0,445	-0,117
	A/A ₀ (315nm)	Y ₃	2,977	0,352	0,910	-0,219	0,299	0,018	-0,49	0,213	0,130	-0,289	-0,262
	Fresh weight	Y ₄	0,069	-0,008	0,000	-0,005	-0,005	-0,001	0,004	0,005	-0,001	-0,004	-0,022
	Fronnd number	Y ₅	43,259	-3,389	-2,722	-2,222	-6,278	0,389	-2,44	-0,75	6,250	0,333	-9,750
	Number of plants	Y ₆	10,333	-0,333	-0,389	-0,278	0,333	0,500	-0,50	0,000	1,167	-0,667	-1,500
	Clh a/b	Y ₇	1,312	0,132	0,126	-0,011	-0,050	-0,030	-0,01	-0,14	0,066	-0,035	-0,144
	ROS	Y ₈	148,901	-216,9	260,54	27,66	-23,15	29,806	-162	-186	63,503	78,185	139,097
Fe(II)Mt	A/A ₀ (200nm)	Y ₁	26,290	-1,63	0,190	14,786	1,211	-0,05	-4,24	-0,5	-1,90	0,837	-0,536
	A/A ₀ (275nm)	Y ₂	0,581	0,104	0,029	-0,031	-0,092	-0,024	-0,05	0,001	-0,026	-0,005	-0,047

	A/A ₀ (315nm)	Y ₃	1,534	0,251	0,029	-0,079	-0,229	-0,056	-0,14	-0,02	-0,068	-0,006	-0,135
	Fresh weight	Y ₄	0,073	0,012	0,000	-0,044	-0,012	0,002	-0,02	0,000	-0,003	0,004	0,002
	Fron d number	Y ₅	60,593	5,722	-1,111	-25,11	-6,944	-0,444	-19,4	0,333	-1,917	2,333	0,375
	Number of plants	Y ₆	18,630	2,056	-0,556	-5,222	-1,389	-0,222	-2,89	-0,42	-0,917	0,583 inf error	-0,125
	Clh a/b	Y ₇	1,308	-0,221	0,069	-0,335	0,086	-0,129	-0,44	0,101	0,192	-0,014	-0,083
	ROS	Y ₈	1283,804	360,967	317,369	221,574	842,712	-1147	6,254	-448	233,395	108,397	-21,774

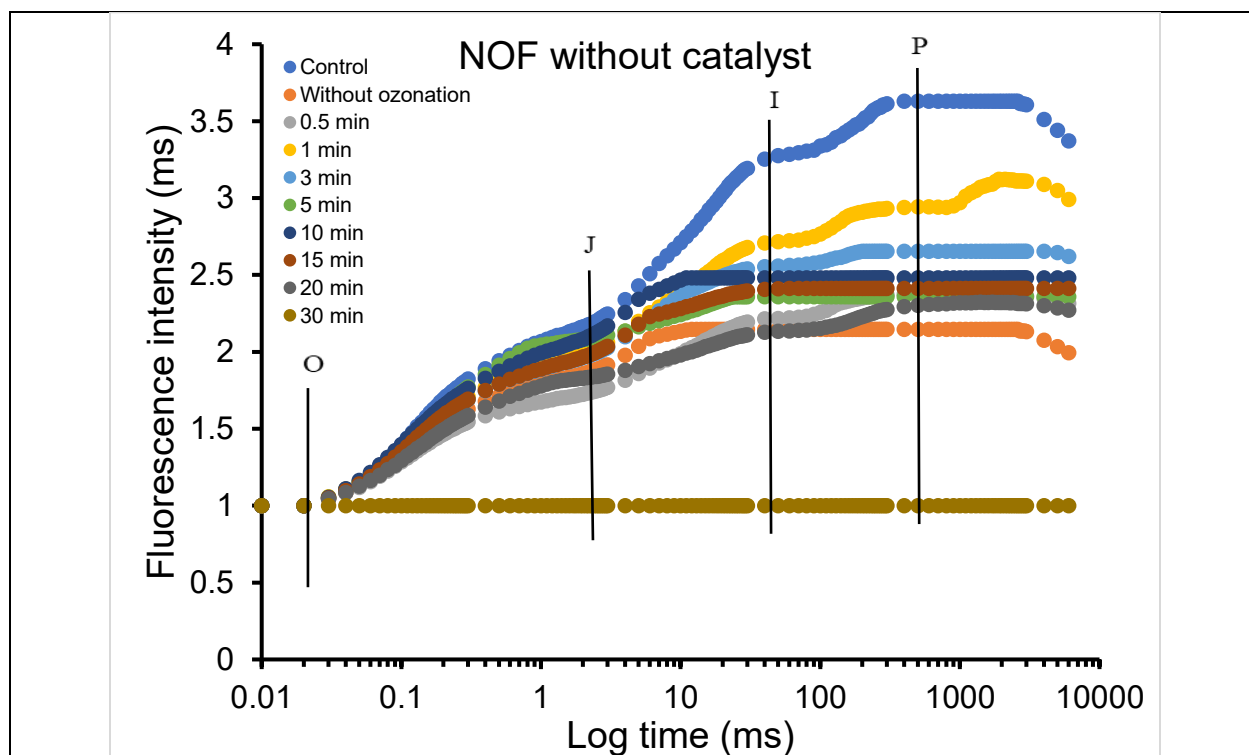
Table S8. Calculated adimensional/real optimum parameters for catalytic ozonation

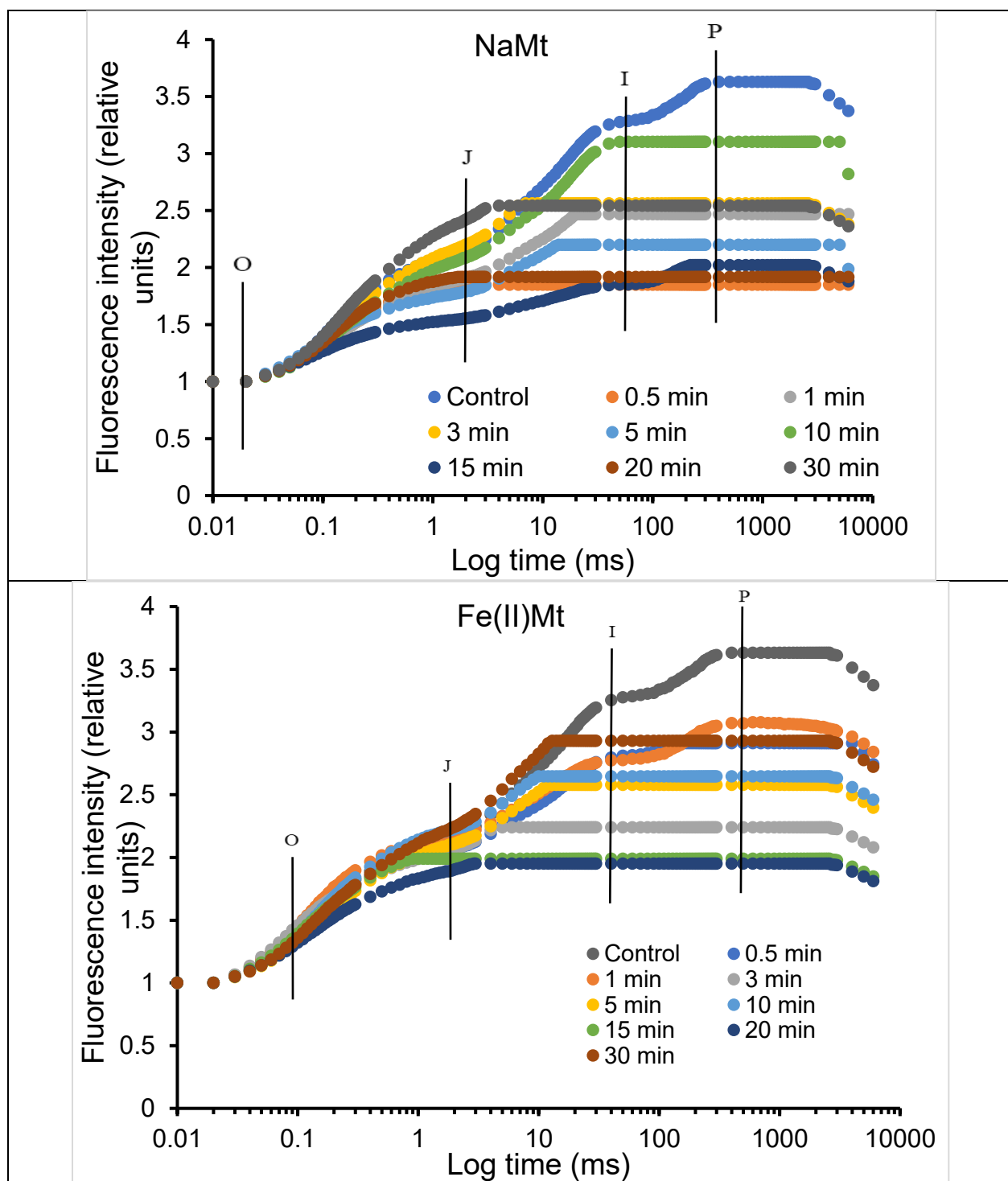
	Response function	value	NaMt			Fe(II)Mt		
			X ₁	X ₂	X ₃	X ₁	X ₂	X ₂
A/A ₀ (200nm)	Y1	Adimensional	-	0.555	0.471	-	-17.665	0
			-11.7	-	-1.789	0.749	-	0.096
			-4.079	-1.998	-	0	-3.169	-
		Real (g.L ⁻¹ ; min)	-	63.9	7	-	492	5
			16	-	12	4.9	-	5
			8.3	99	-	4.2	129	-
A/A ₀ (275nm)	Y2	Adimensional	-	-2.664	0.705	-	0.604	-0.316
			-1.021	-	-0.512	0.634	-	-0.484
			-1.955	0.397	-	0.565	0.604	-
		Real (g. L ⁻¹ ; min)	-	117	8	-	65	6
			5.2	-	7	4.8	-	7
			6.2	60	-	4.8	65	-
A/A ₀ (315nm)	Y3	Adimensional	-	11.348	3.149	-	0.259	-0.288
			-0.524	-	-0.295	0.613	-	-0.441
			-4.27	10.342	-	0.541	0.143	-
		Real (g. L ⁻¹ ; min)	-	334	18	-	56	6
			4.7	-	6	4.8	-	7
			8.5	308	-	4.7	54	-
Fresh weight	Y4	Adimensional	-	0	0	-	11	-11
			0	-	0	-14.667	-	121.333
			0	0	-	0	0.5	-
		Real (g. L ⁻¹ ; min)	-	50	5	-	325	49
			4.2	-	5	18.9	-	489
			4.2	50	-	4.2	63	-
Fron d number	Y5	Adimensional	-	0	0	-	10.763	0
			0	-	0.542	0.412	-	-0.646
			-0.27	0	-	0.412	0	-
		Real (g. L ⁻¹ ; min)	-	50	5	-	319	5
			4.2	-	5	4.6	-	8
			4.5	50	-	4.6	50	-
Number of plants	Y6	Adimensional	-	0	0	-	0	-0.301
			0	-	0	0.74	-	-0.904
			0	0	-	0.74	0	-
		Real (g. L ⁻¹ ; min)	-	50	5	-	50	6
			4.2	-	5	4.9	-	9

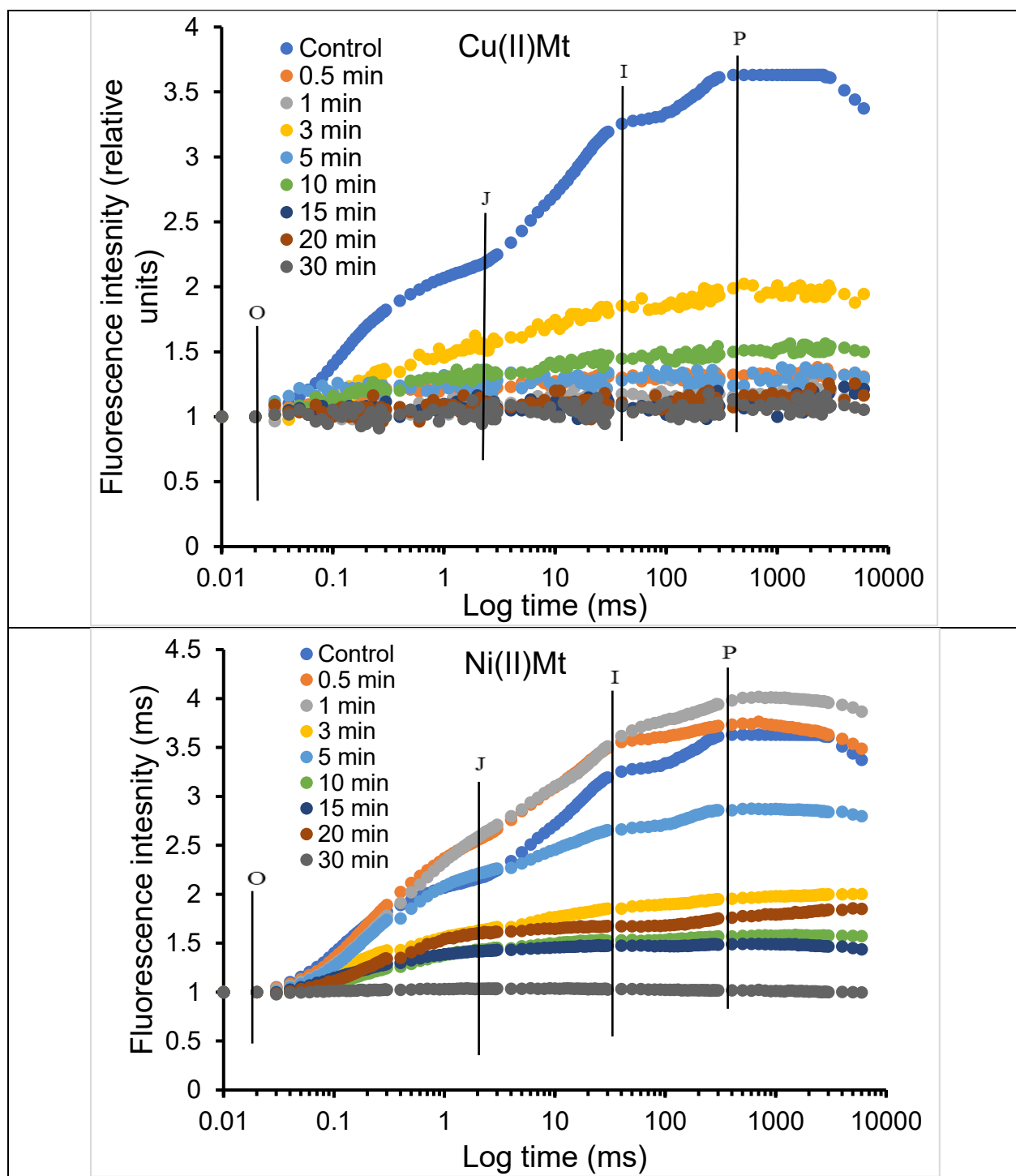
			4.2	50	-	4.9	50	-
Chl <i>a/b</i>	Y7	Adimensional	-	0	3.6	-	0.267	-0.383
			0	-	-2	1.375	-	-0.081
			0.699	0.434	-	0.017	0.626	-
		Real (g. L ⁻¹ ; min)	-	50	19	-	57	7
			4.2	-	13	5.6	-	5
			4.9	61	-	5.1	66	-
ROS	Y8	Adimensional	-	-3.404	-0.737	-	-0.496	-13.42
			6.573	-	1.376	-1.413	-	8.657
			0.951	-1.403	-	-0.169	0.171	-
		Real (g. L ⁻¹ ; min)	-	135	8	-	62	59
			10.8	-	11	5.6	-	40
			5.2	85	-	4.4	54	-

* Certain values are outside the considered range and were not considered for the determination of optimum response-functions. They are qualitatively assessed using a formula that is valid only within the considered parameter ranges.

6. Ozonation effect on photosynthetical system







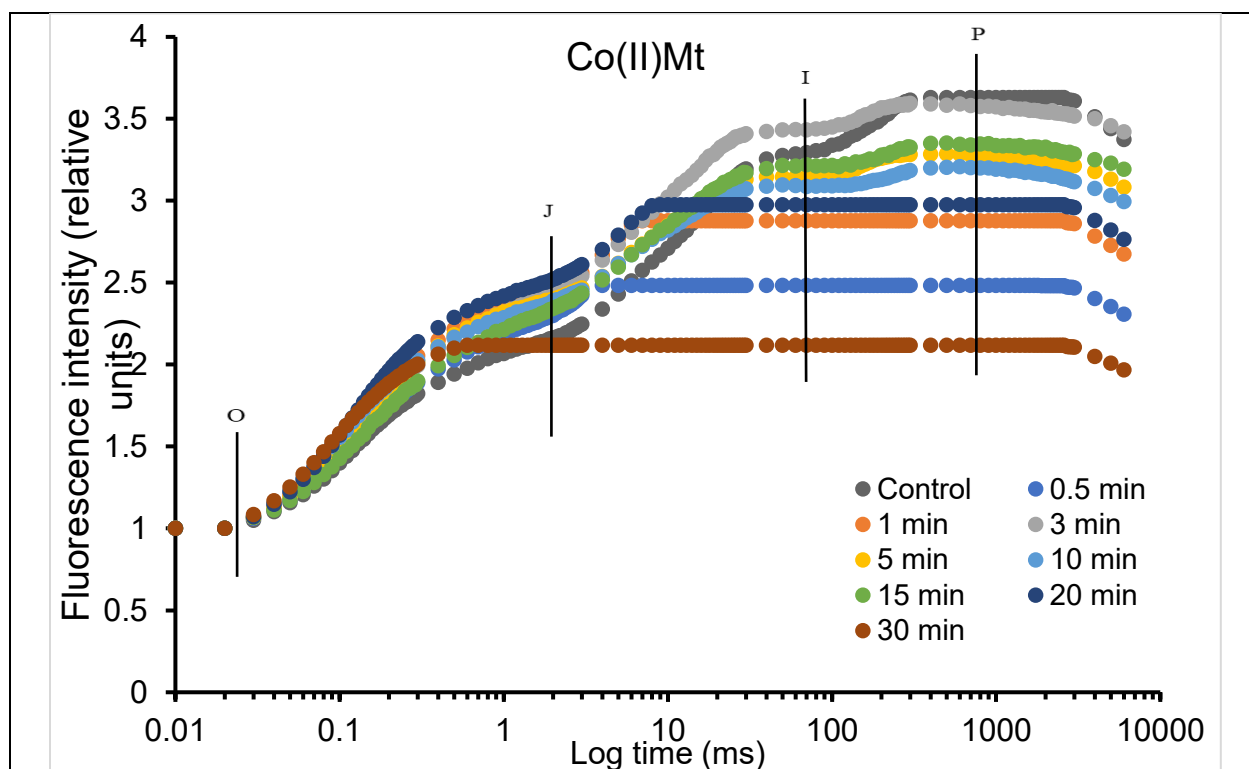
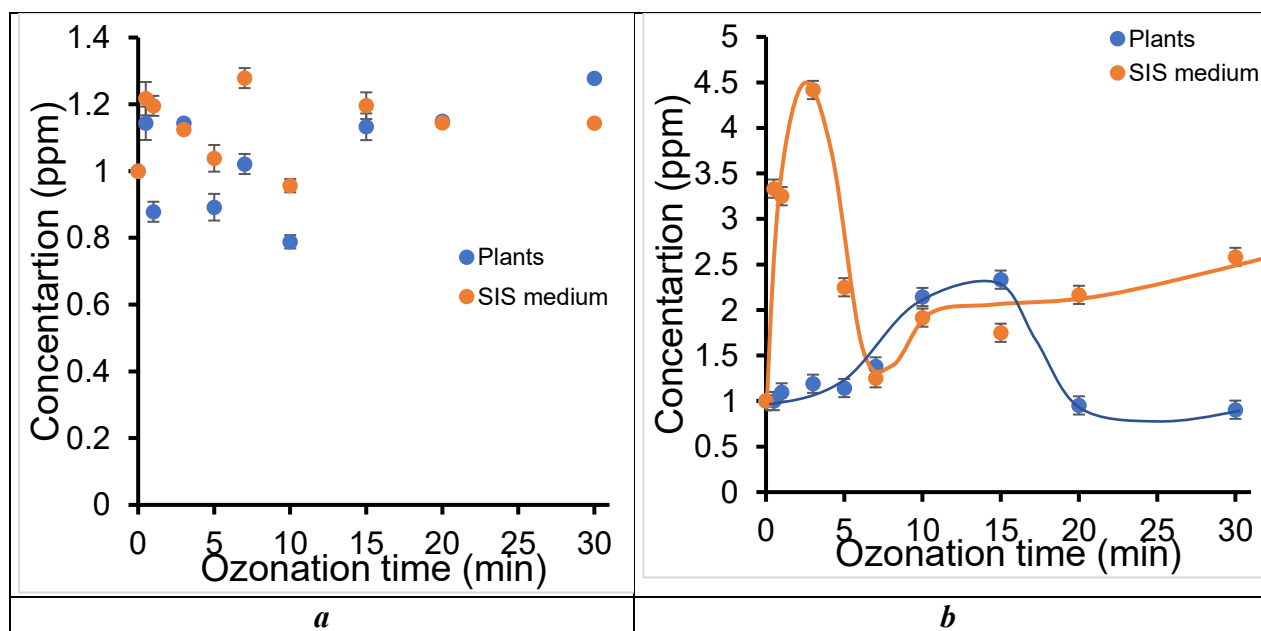
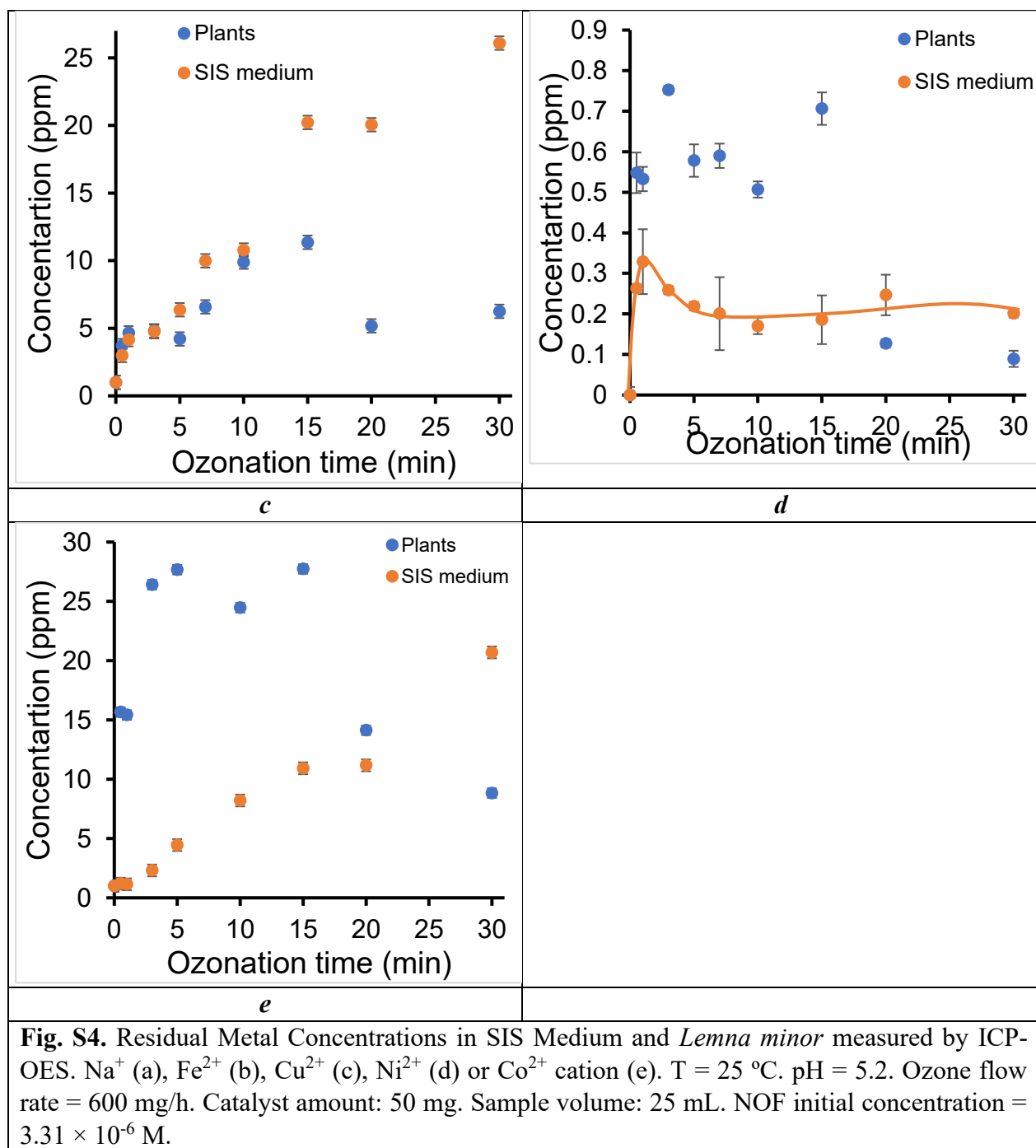


Fig.S3. Change in the fast and polyphasic fluorescence kinetics of chlorophyll *a* of *L. minor*. Plants were exposed for 7 days to different ozonised mixture with clay-catalyst. Symbols O, J, I and P represent, respectively, the fluorescence intensities at 50 μ s, 2ms, 30ms, and 200ms.





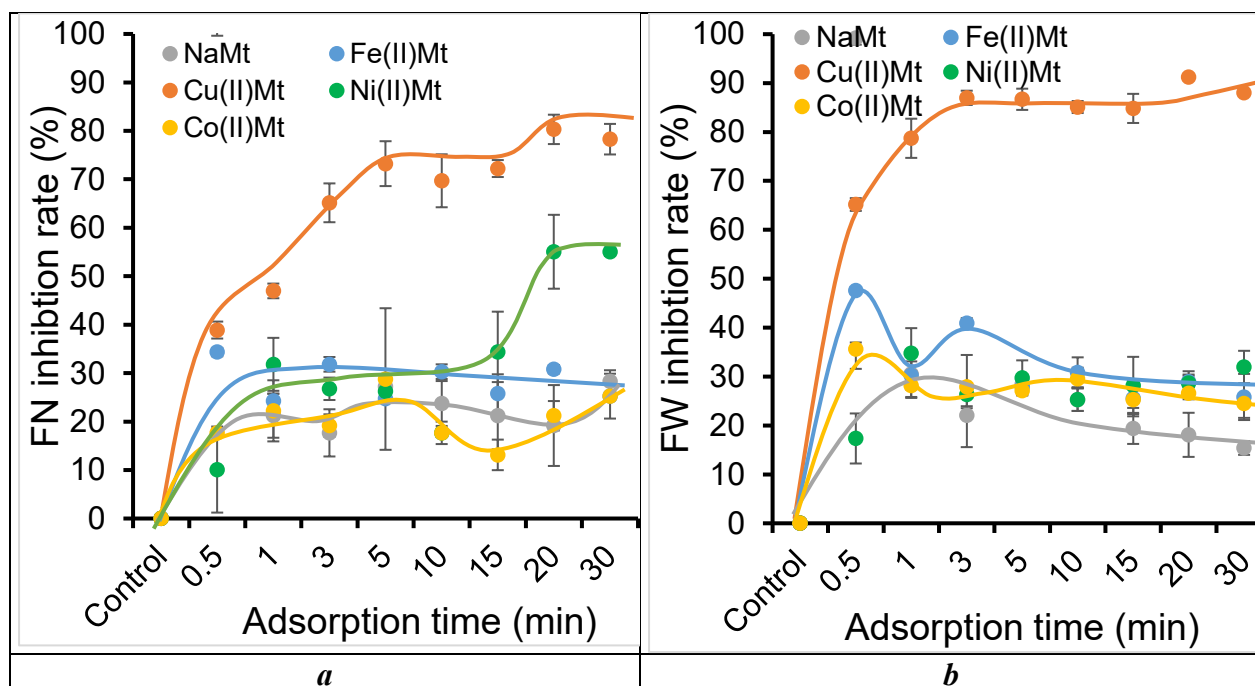


Fig. S5. Effect of combined NOF and metal ion on growth inhibition expressed in terms of frond number (a) and fresh weight after 7 days of exposure to *Lemna minor*. Triplicates measurements were achieved including the control sample. The control sample consisted of plants grown in SIS medium.

7. ZVM-loaded Mt analysis by FTIR

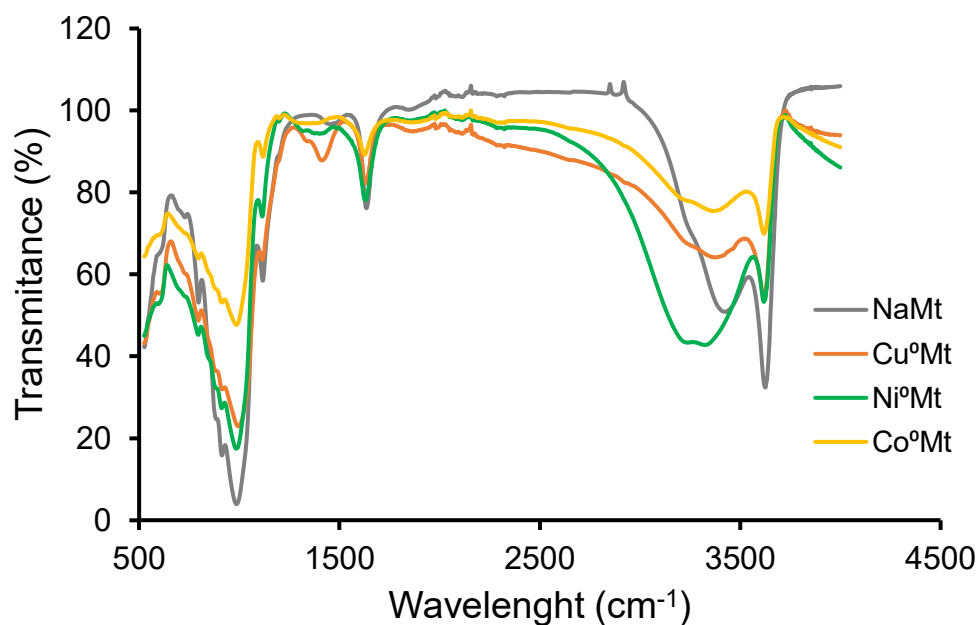
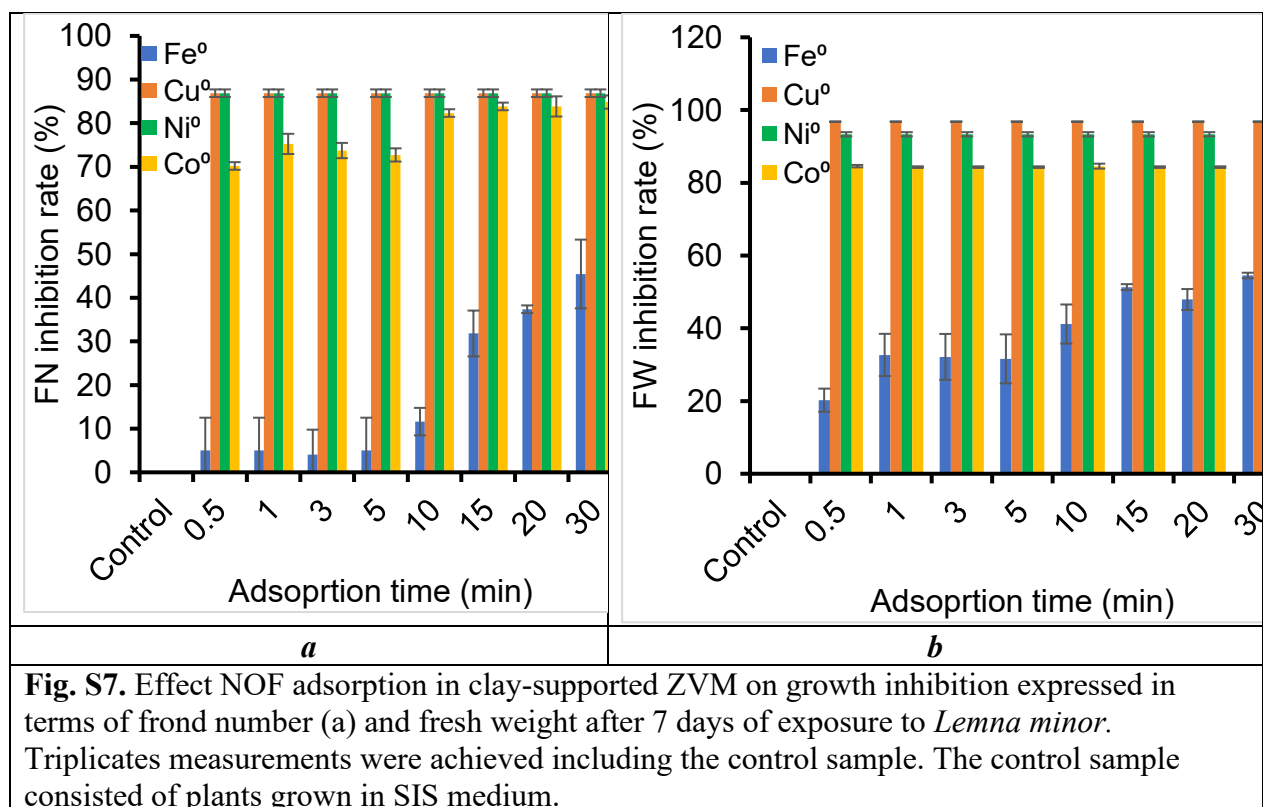


Fig. S6. IR spectra of NOF-free aqueous suspension of ZVM-montmorillonite

8. Toxicity of NOF adsorbed on ZVM



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