French Priority Research Programme (PPR) on antibiotic resistance:

Call for junior and senior researcher positions (chairs)

ILS SONT PRÉCIEUX, UTILISONS-LES MIEUX.

Managing **E**nvironmental **H**otspots and **T**ransmission of **A**MR

MEHTA Project

Porteur : Ed Topp – INRAE Agroécologie - Ex Agriculture and Agri-Food Canada

À ECOSYS : P. Benoit, N. Bernet, G. Delarue, M. Deschamps, C.-S. Haudin, V. Serre

Projet MEHTA - Partenaires

Programme prioritaire de recherche (PPR, chair d'excellence)

Managing Environmental Hotspots and Transmission of Antimicrobial resistance (AMR)

Senior Research Chair **Ed Topp**
Durée : 36 mois (2023-2026)

Unités partenaires du projet MEHTA

Agroécologie Dijon Unité de Recherche

Ed Topp, Alain Hartmann, Fabrice Martin-Laurent, Daniel Martak

ECOSYS Ecologie fonctionnelle et écotoxicologie des agroécosystèmes

Pierre Benoit, Marjolaine Deschamps, Claire-Sophie Haudin

CHRONO ENVIRONNEMENT

Didier Hocquet

Scientifiques collaborant au projet MEHTA

LBE Laboratoire Biotechnologie de l'Environnement

Dominique Patureau, Nathalie Wery

OPAALE INRAE

Anne-Marie Pourcher

RÉPUBLIQUE FRANÇAISE Liberté Égalité Fraternité

anses

Caroline Le Marechal

Projet MEHTA

Etablissement Coordinateur
INRAE Centre Bourgogne-Franche-Comté

Responsable du projet
M. Edward TOPP
Responsable administratif (RFCI)
Mme Sarah HOYOUX (à confirmer)

Référence du projet
ANR-22-PAMR-0009

Dates de démarrage et de clôture
01 juillet 2023 → 30 juin 2026

Montant du projet : **1 636 537,05 €**
Montant de l'aide : **999 722 €**
Echéancier :

Notification (N)	N + 12 → + 36 mois	Solde
224 937 €	224 937 €/an	99 974 €

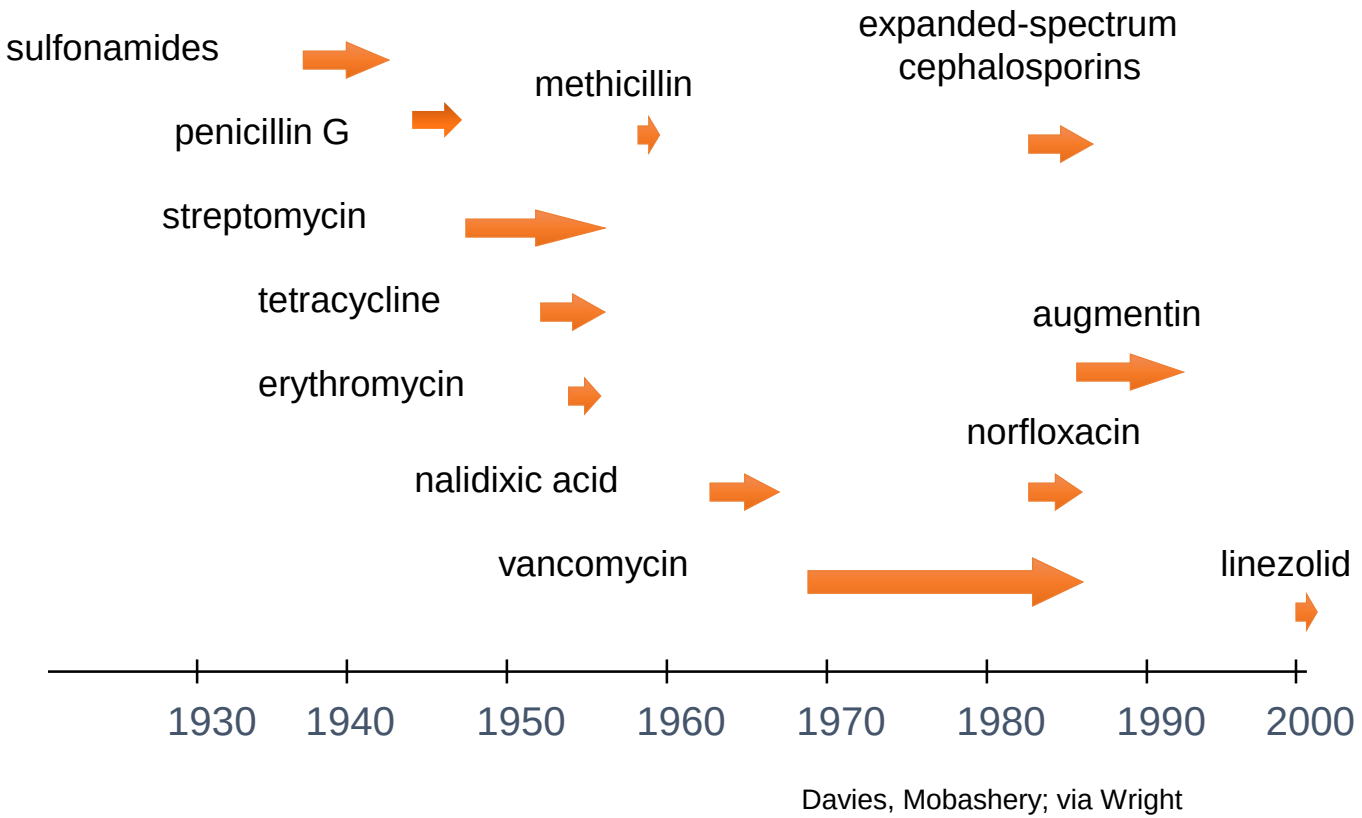
Contexte du projet MEHTA

- Antibioresistance
- Qualité des aliments par rapport à la santé humaine
- Changement climatique et manque d'eau
- La sécurité alimentaire

- Irrigation des cultures avec les effluents des eaux usées municipales traitées… reute

Antibiorésistance

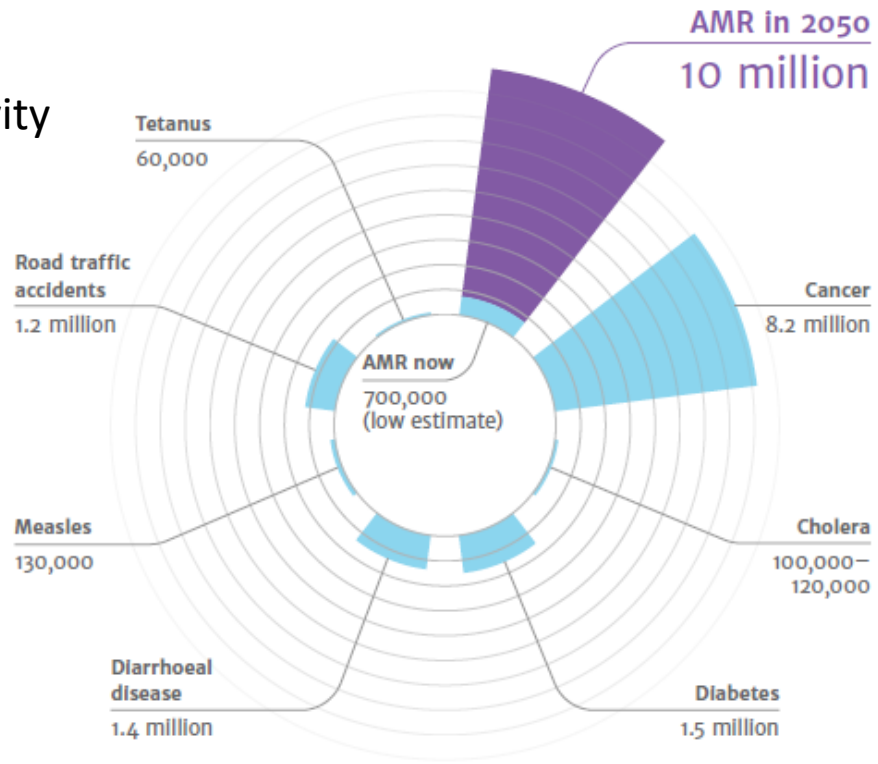
Antibiotic Resistance Develops Rapidly in Pathogens Following the Introduction of Antibiotics



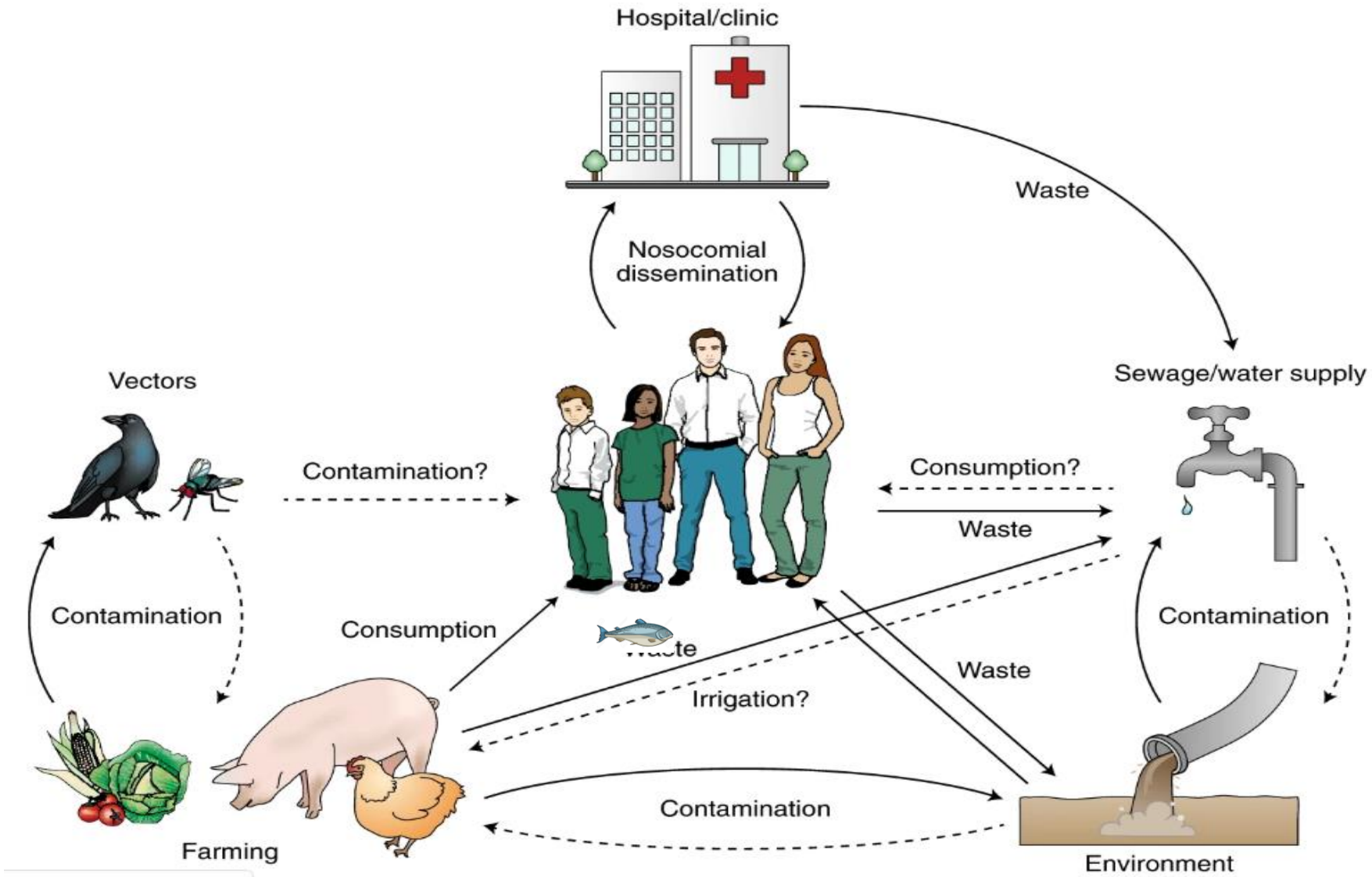
Davies, Mobashery; via Wright

Antibiorésistance (AntiMicrobial Resistance – AMR)

"The O'Neill report" 2016
100 trillion USD decline in global productivity attributable to 10 million deaths.



AMR and One Health



Questions clés

- L'**exposition** aux antibiotiques augmente-t-elle l'abondance des **gènes de résistance aux antibiotiques** ?

- L'**exposition** aux antibiotiques augmente-t-elle l'abondance d'**éléments génétiques mobiles** ?

Human and Environmental Health Risks

- Microbial pathogens (virus, parasite, bacteria) **[antibiotic resistant bacteria]**
- Endocrine-active chemicals (EDCs)
- harmaceuticals **[antibiotics],** personal care products.
- Metals (eg. Cd, Pb, Hg)
- N and P

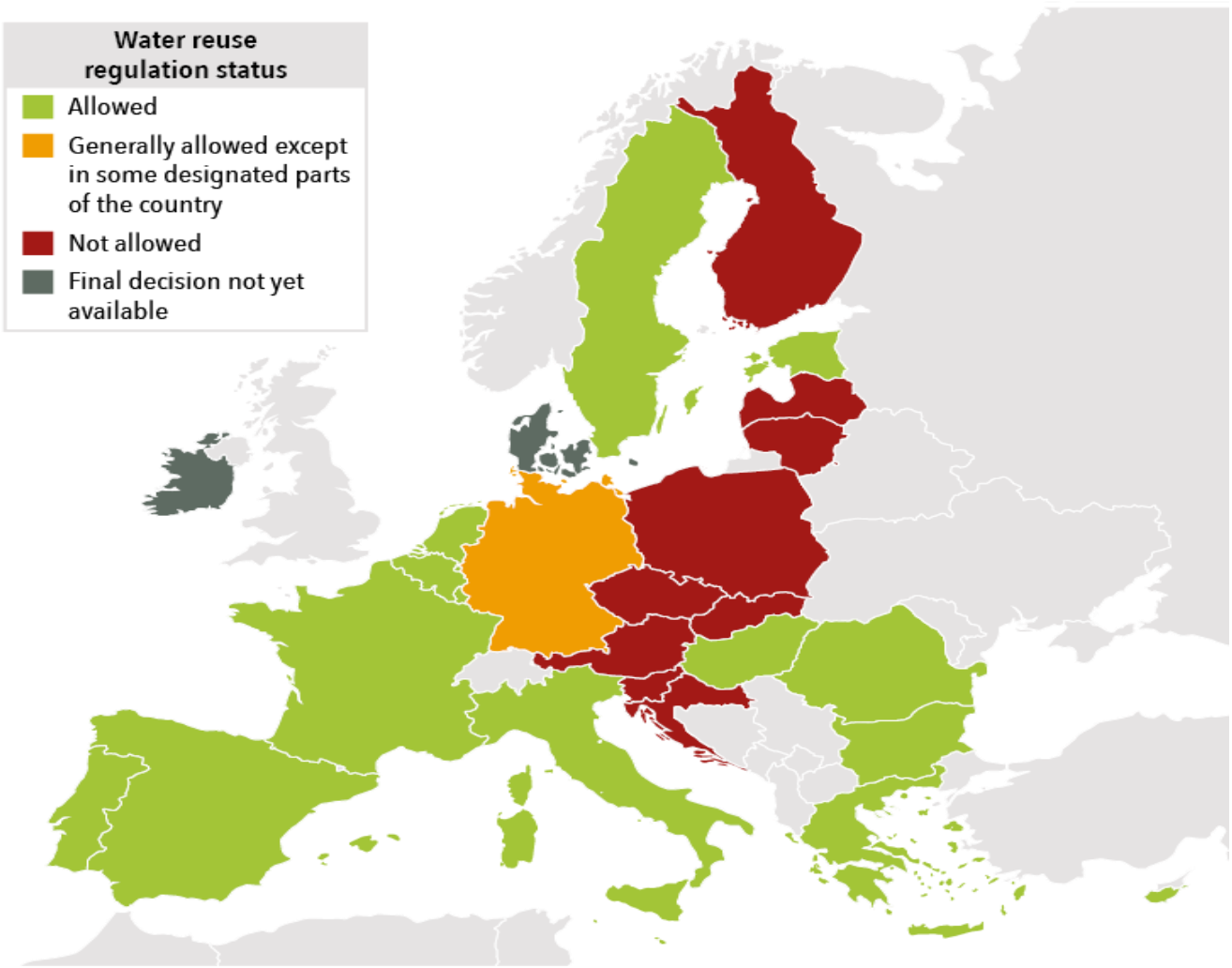
Principales voies d'entrée dans les sols agricoles

- MAFOR, PRO comme amendements ou fertilisants
  - Lisier, fumier, boues d'épuration, urines
  - Effet traitement : compostage, digestion anaérobie
- Les antibiotiques comme pesticides dans la production agricole
  - Eg. Streptomycine et oxytetracycline pour traiter des maladies de tomate
- Irrigation avec des effluents d'eaux usées traitées
  - La charge d'antibiotiques va varier en fonction du degré de traitement

MEHTA

https://www.gouvernement.fr/actualite/plan-eau-le-gouvernement-simplifie-la-reutilisation-des-eaux-usees-traitees

Current status of water reuse in Europe



Comprendre et évaluer les risques – transfert à l'homme

via les plantes et la chaine alimentaire

via les eaux, l'air

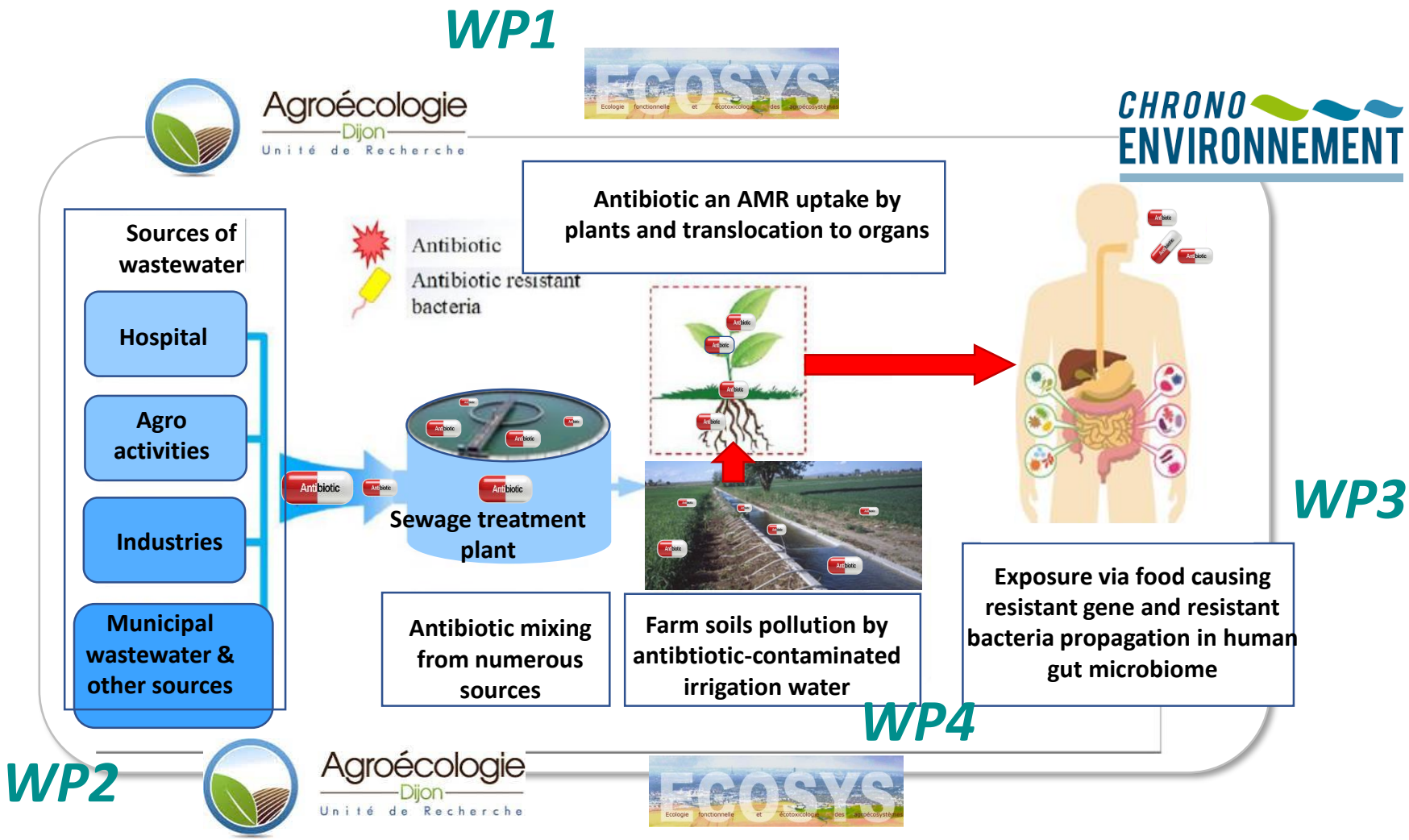
Major outputs of the MEHTA project

- An evaluation of **crop quality** following irrigation with treated wastewater, and an evaluation of the impact of various tertiary treatment technologies on the chemical and microbial composition of effluent.
- An ***ex vivo* model** will be used to evaluate the **evolution of the human gut resistome** following exposure to foodstuffs irrigated with reused wastewater or clean water
- An estimation of **bioavailable antibiotic concentrations** in soils receiving wastewater, biosolids or manures, and whether or not these are within the range that may select for antimicrobial resistance. Evaluate how these concentrations **vary for different antibiotics and with soil properties.**

Organization of the MEHTA project

- WP0. Coordination of the project.
- WP1. Microbial and chemical contaminants of crops irrigated with wastewater. ← ECOSYS
  - Greenhouse and field plot experiments will be undertaken to evaluate potential contamination of leafy and root vegetables with micropollutants, ARGs and MGEs.
- WP2. Validation of methods for decontaminating waste streams.
  - A comparison of the composition and dynamics post-irrigation will be made of wastewater following secondary and various tertiary treatment methods.
- WP3. Contamination of food products - consequences in the gut microbiome.
  - An ex-vivo gut model/bioreactor will be fed with crops alone (irrigated with clean water, wastewater treated or not by tertiary treatment) or in combination with major epidemic ARBs (ESBL-producing E. coli, carbapenemase-producing K. pneumoniae…) +/- antibiotics.
- WP4. Soil No Effect Concentrations. ← ECOSYS
  - Some antibiotic resistance genes still confer a fitness advantage at antibiotic concentrations that are significantly below the minimum selective concentration (MIC) for a given bug-drug combination. the relationship between antibiotic concentration and impacts on the microbiome will be explored to establish measured no observed effects concentration (NOECs)
- WP5. Knowledge transfer
  - Teaching, supervision, publication, conferences

Organization of the MEHTA project



WP1 - Microbial and chemical contaminants of crops irrigated with wastewater

Tomates

Spray d'antibiotiques
Streptomycine +
Oxytetracycline
(concentrations et
fréquence à définir)

X8

X8

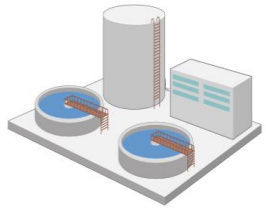
X8

X8

Eau osmosée
+ solution
nutritive

Eaux usées

Récupération
des eaux
traitées de la
station
d'épuration



Radis

Eau osmosée
+ solution nutritive

X8

X8

Eaux usées

Post-Doc Daniel Martak
Started Sept 2023

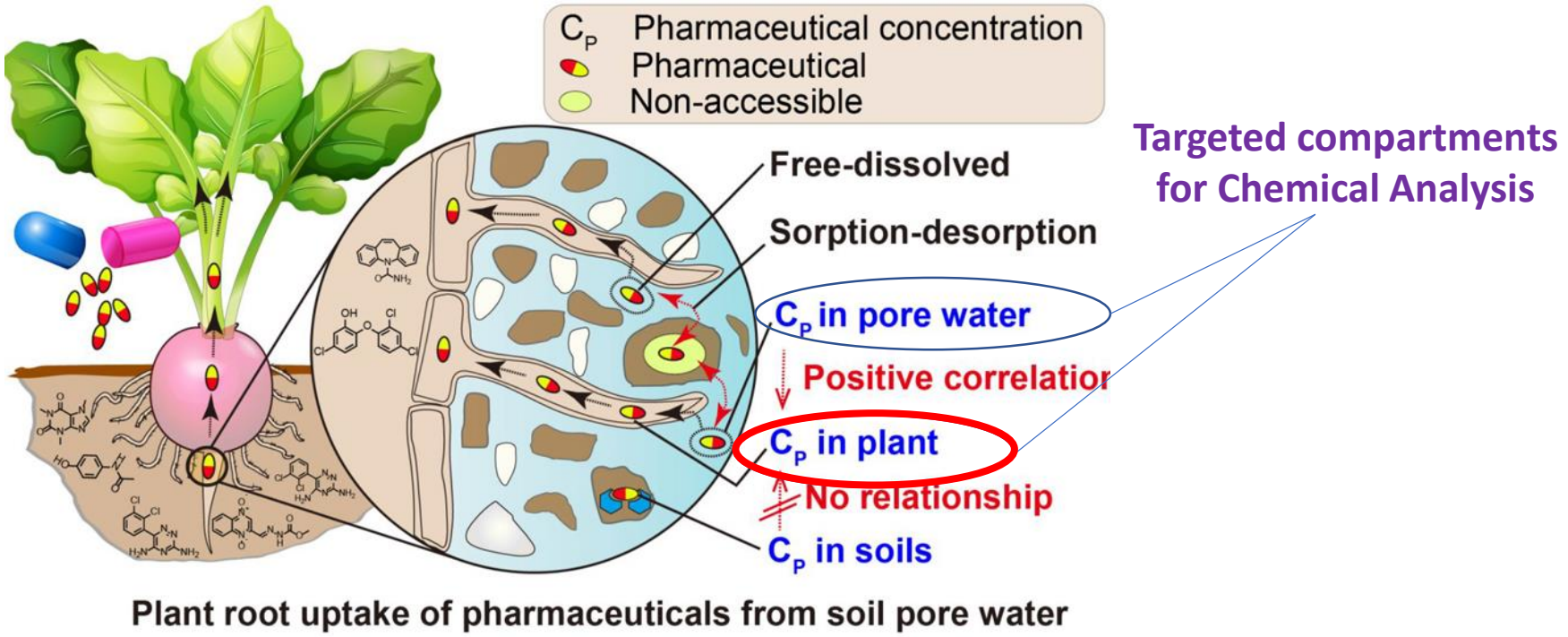
Agroécologie
Dijon
Unité de Recherche

WP1 - Microbial and chemical contaminants of crops irrigated with wastewater

Volet Chemical residue analytical methods

M. Deschamps, N. Bernet, G. Delarue, V. Serre, C.-S. Haudin, P. Benoit

Assessing the risk of ATB plant uptake by crops



Li et al, Environ. Sci. Technol. 2022, 56, 9346−9355

WP1 - Volet ATB residues in Plants

Travaux préliminaires à ECOSYS (Projets ReUSE **APIC, NEREIDES** 2020-2021 – Collab CEREGE)

- Mise au point de l'extraction de produits pharmaceutiques d'une matrice végétale : **la laitue**
- Ofloxacine, Tetracycline, Sulfamethoxazole, Carbamazépine, Aténolol, Diclofenac, Ketoprofen, Ibuprofen, 1-OH-ibuprofen, 2-OH-ibuprofen, Gemfibrozil, Triclocarban, Caféine
- QuEChERS = Quick, Easy, Cheap, Effective, Rugged and Safe / Rapide, facile, bon marché, efficace, robuste et sûr

➡ Topo Ghislaine TS Contaminants 24/11/2023

WP1 - Volet ATB residues in Plants

Salade lyophilisée broyée

Prise d'essai : 200 mg

Ré-hydratation : ajout 1,8 ml eau
Attendre 1 heure

Dopage et/ou ajout étalons internes 40 µl
Temps de contact

MO Boue

MO Sol

MO QuEChERS Original et AOAC

Extraction
Ajouter 1,5 ml Mc Ilvaine pH 3,5 +
Vortex
Ajouter 5 MeCN + Vortex

Extraction 1
Ajouter 0,9 ml Mc Ilvaine pH 9 + Vortex
Ajouter 2,5 MeCN + Vortex

Ajouter Acétonitrile 2 ml + Vortex

Ultrasons : Deux cycles de 10'
Vortex entre chaque cycle et changer
eau du bac si nécessaire

Ajouter les sels QueCHERS + Vortex

Extraction 2
Reprendre le culot avec :
1 ml Mc Ilvaine pH 2 + Vortex
Ajouter 2,5 MeCN + Vortex

Original
0,8 g MgSO4 remplacé
Na2SO4, 0,2 g NaCl

AOAC
0,8 g MgSO4 remplacé
Na2SO4, 0,2 g NaOAc

Centrifuger 20' à 4000 rpm, et 20°C

Récupérer le surnageant dans un tube
Falcon de 15 ml
Pour MO sol : ajouter à l'extrait 1

Ajouter les sels QueCHERS + Vortex

Centrifuger 20' à 4000 rpm, et 20°C

Centrifuger 20' à 4000 rpm, et 20°C

Prélever 0,520 ml de la phase supérieur
Mettre en vial HPLC
Evaporer à la goutte sous flux d'azote

Prélever 1,3 ml de la phase supérieur
Mettre en vial HPLC
Evaporer à la goutte sous flux d'azote

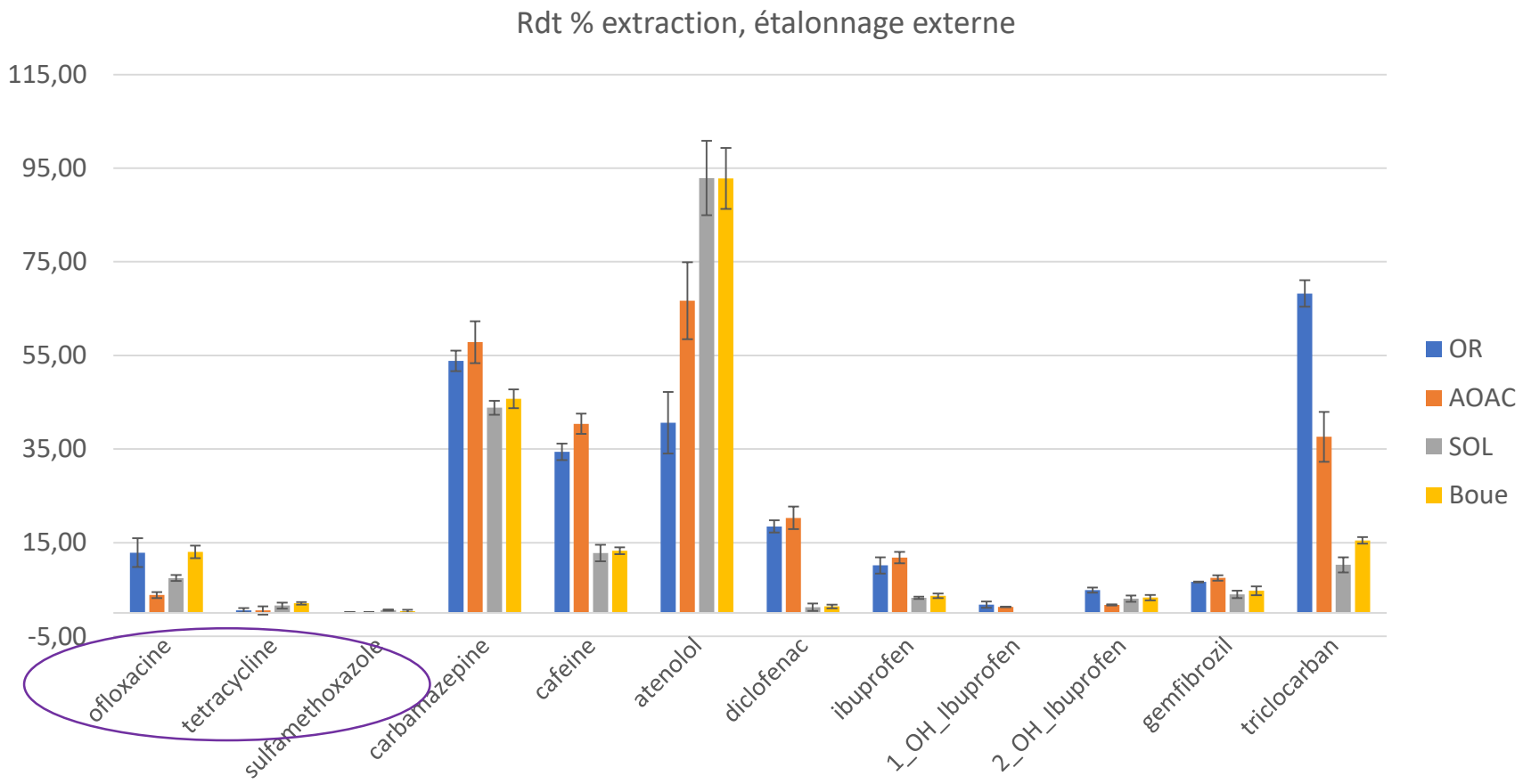
Reprendre avec 90 µl MeCN +vortex
Ajouter 2 x 605 µl eau pH 2,5 + vortex

Analyse UPLC-MSMS

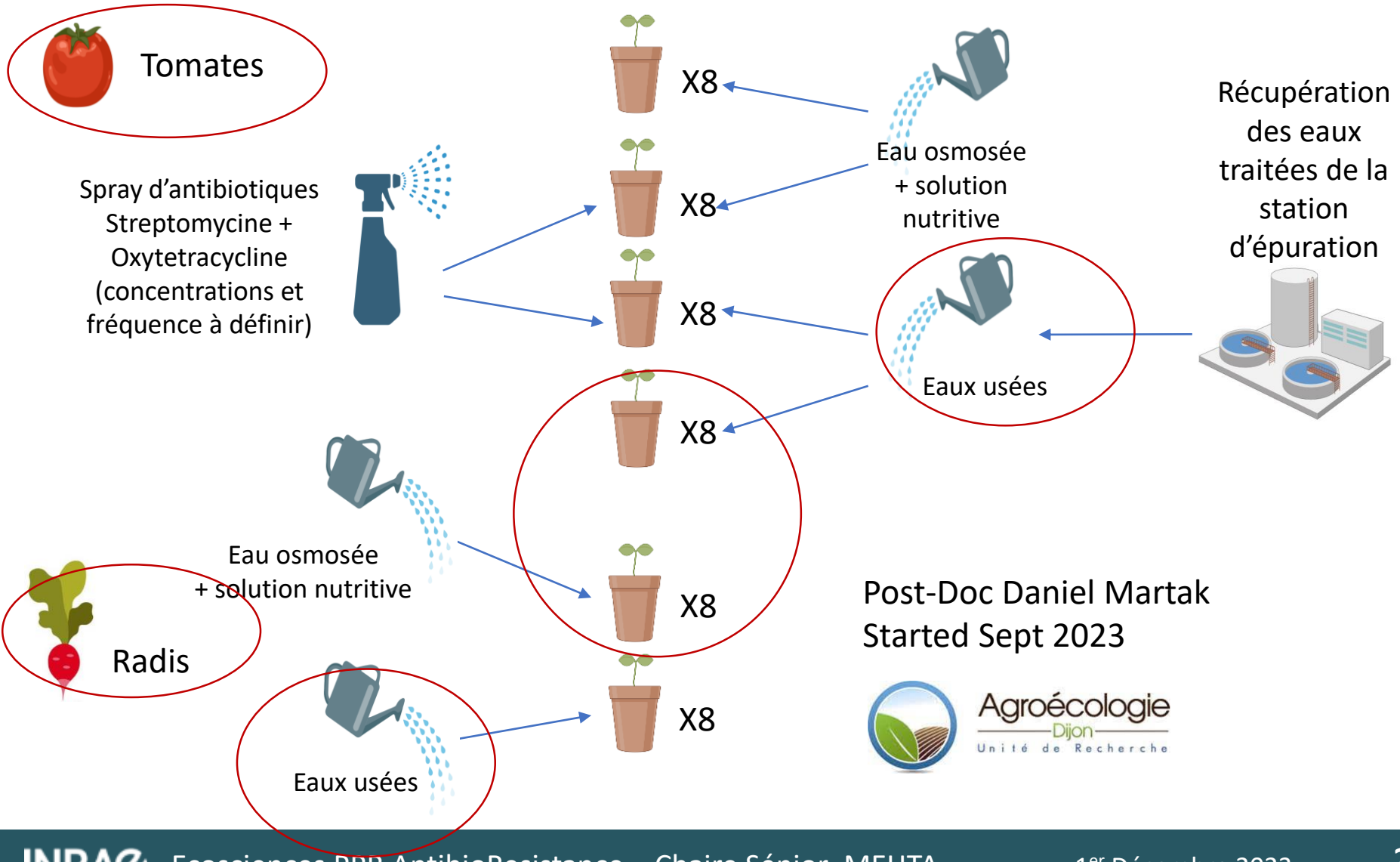
WP1 - Volet ATB residues in Plants

Travaux préliminaires à ECOSYS

Extraction Yields for antibiotics on salad leaves (lettuce) (**Project NEREIDES - REUSE**)



WP1 - Microbial and chemical contaminants of crops irrigated with wastewater



Post-Doc Daniel Martak
Started Sept 2023

Agroécologie
Dijon
Unité de Recherche

WP1 - Volet ATB residues in Plants

Litterature review on the quantification of plant uptake of ATB : Cp in plant

- **Methods to be adapted and tested at ECOSYS with the new UPLC_MS_MS analytical chain**
  - Focus on Radish and Tomato as plant species (more publications for radish than tomato)
  - Focus on Streptomycine, Oxytetracycline and other ATB molecules
  - Focus on extraction and purification steps

From previous experience : strong variability between publications using very similar methods

➡ Topo Ghislaine TS Contaminants 24/11/2023

- **Sample pre-treatment:**
  radish : generaly freeze-dryed + grounded. Storage at -20°C
  tomato : generaly frozen (-20°C), then cut in pieces and mixed

WP1 - Volet ATB residues in Soils

Litterature review initiated on plant bioavailability assessment : Cp in soil pore water

- **Bioavailable fraction for plant uptake** (and for microorganisms)
- **Proxy for ATB soil available concentration : via soft extraction approach**

Science of the Total Environment 651 (2019) 506–515

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Environmental availability of sulfamethoxazole and its acetylated metabolite added to soils via sludge compost or bovine manure

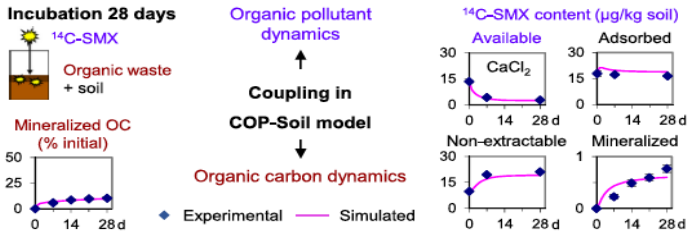
Anaïs Goulas, Nicolas Sertillanges, Khaled Brimo, Patricia Garnier, Valérie Bergheaud, Valérie Dumény, Pierre Benoit, Claire-Sophie Haudin *

UMR ECOSYS, INRA, AgroParisTech, Université Paris-Saclay, 78850 Thiverval-Grignon, France

HIGHLIGHTS

- The decomposition of the added organic matter influenced the fate of SMX and AcSMX in soil.
- SMX and AcSMX were initially more available in soil/manure than in soil/compost mixtures.
- The dynamics of SMX and its acetylated metabolite in amended soils were similar.
- Co-metabolism could originate the formation of non-extractable residues.
- $CaCl_2$ extraction could be the best method to assess the sulfonamide availability, resulting in best simulations.

GRAPHICAL ABSTRACT

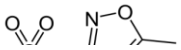


Goulas, A. et al. 2019 Science of the Total Environment 651, 506–515

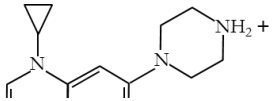
WP1 - Volet ATB residues in Soils

Soft extraction methods to assess available fraction of ATB in soil

Test et sélection de solutions aqueuses adaptées aux propriétés physicochimiques

SMX 

$CaCl_2$, EDTA, HPCD

CIP 

Borax, EDTA, HPCD

Solution aqueuse	Mécanisme d'extraction
$CaCl_2$ (chlorure de calcium) 0,01 M, pH 4,5	Solution du sol + désorption
EDTA (acide éthylène diamine tétraacétique) 0,1 M, pH 7	Chélation de cations
HPCD (2-hydroxypropyl-β-cyclodextrine) 0,05 M, pH 7	Solubilisation à l'intérieur de molécules cages
Borax (tétraborate de sodium) 0,2 M, pH 9	Solubilisation à l'intérieur de micelles + alcalinisation

Goulas, A., et al. 2016. A new extraction method to assess the environmental availability of **ciprofloxacin** in agricultural soils amended with exogenous organic matter. Chemosphere 165, 460–469.

Goulas, A et al. 2017. Development of a soft extraction method for **sulfamethoxazole and transformation products** from agricultural soils: effects of organic matter coextraction on the environmental availability assessment. Science of the Total Environment 607, 1037–1048.

- **To be tested on tetracyclines, streptomycine**

WP4. Soil No Effect Concentrations

P. Benoit, C.-S. Haudin, M. Deschamps, N. Bernet, G. Delarue, V. Serre
11 mois CDD niveau Ingénieur

- Exploring the relationship between antibiotic concentration and impacts on the microbiome **-> <u>to establish measured no observed effects concentration</u>** (NOECs) in several conditions including in mixtures with copper
- A range of variable soil properties will be included in experiments to estimate key drivers, information that can then be used to inform predicted no effects concentrations (PNECs) for soils and **<u>to establish pedotransfer functions</u>** predicting **sorption and the environmentally available fractions** of different classes of antibiotics
- This information can be used **to establish thresholds for soil antibiotic concentrations that would represent acceptable risks**. This information will be impactful for risks from wastewater reuse but also land application of sewage sludge that contain elevated concentrations of antibiotics and other pharmaceuticals (Sabourin, Duenk et al. 2012)

WP4. Soil No Effect Concentrations

Establishing NOEC values

- Experimental **NOEC and** available concentrations estimations (soil pore water concentration)

  -> Common experiments – soil incubation to monitor over time available concentrations estimations (**ECOSYS**) and ARG **- PNEC and NOEC (AgroEcologie)**

Starting 2025

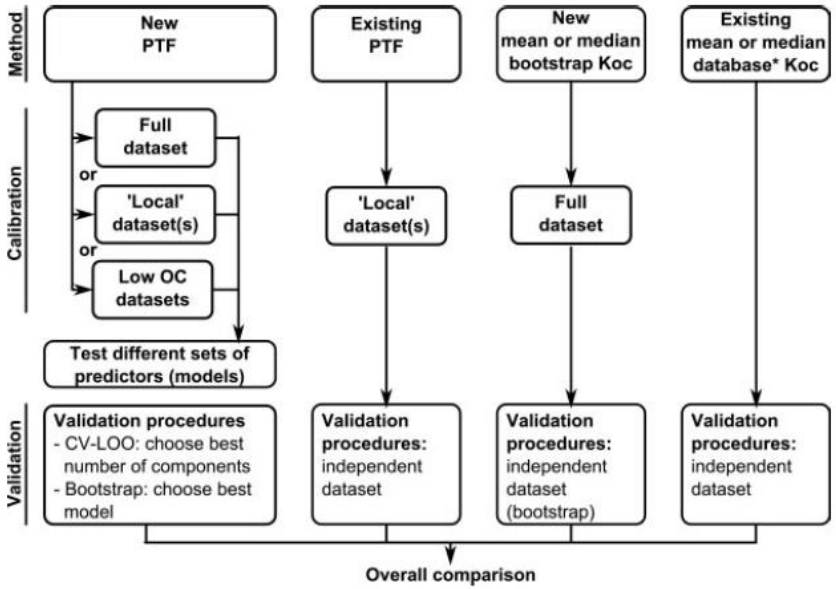
- ***Links with on going ADEME project ACV EcotoMix (Tetracycline one of the targeted compound) and a starting Ph D Thesis on plant uptake of different PPCP compounds (Thèse Antoine Spaudo – CIRAD INRAE Runéo CIFFRE collaboration)***

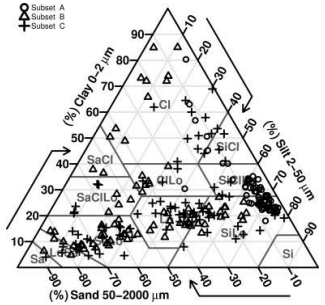
- ***Links with the litterature review on plant bioavailability assessment : Cp in soil pore water***

WP4. Soil No Effect Concentrations

Predicting sorption and the environmentally available fractions

Accounting for soil properties to explain variability of sorption





- Pedostransfer function : PTF
- CV-LOO : Leave-one-out cross validation

$$K_D = 1.7822 + 0.0162\ OC^{1.5} - 0.1958\ pH$$

$$K_D = 0.9980 + 0.0002\ clay - 0.0990\ pH$$

for low OC soil (OC < 6.1 g kg^{-1})

K_D in L kg^{-1} ; OC in g kg^{-1} ; Clay g kg^{-1}

Pedotransfer functions for isoproturon sorption on soils and vadose zone materials

Moeys, Bergheaud, Coquet, 2011

WP4. Soil No Effect Concentrations

Litterature review : only few pedotransfer functions to predict sorption available for some ATB chemical classes (Tetracyclines, Fluoroquinolones) – cf C. Godard CDD 2023

- **7 publications :** prédiction de sorption des produits pharmaceutiques (K_F) en fonction des propriétés physico-chimiques des sols

  → Conde-Cid and al., 2019 / 2020
  → Hu and al., 2022
  → Klement and al., 2018
  → Kodešová and al., 2015 / 2023
  → Li and al., 2020

- **55 molécules**

- **Souvent les mêmes paramètres physico-chimiques :** pH, fractions granulométriques, carbone organique du sol et la capacité d'échange cationique

ACV-Ecoto(Mi)x Project

WP4. Soil No Effect Concentrations

First results : Variabilité des propriétés de sorption - C. Godard CDD 2023

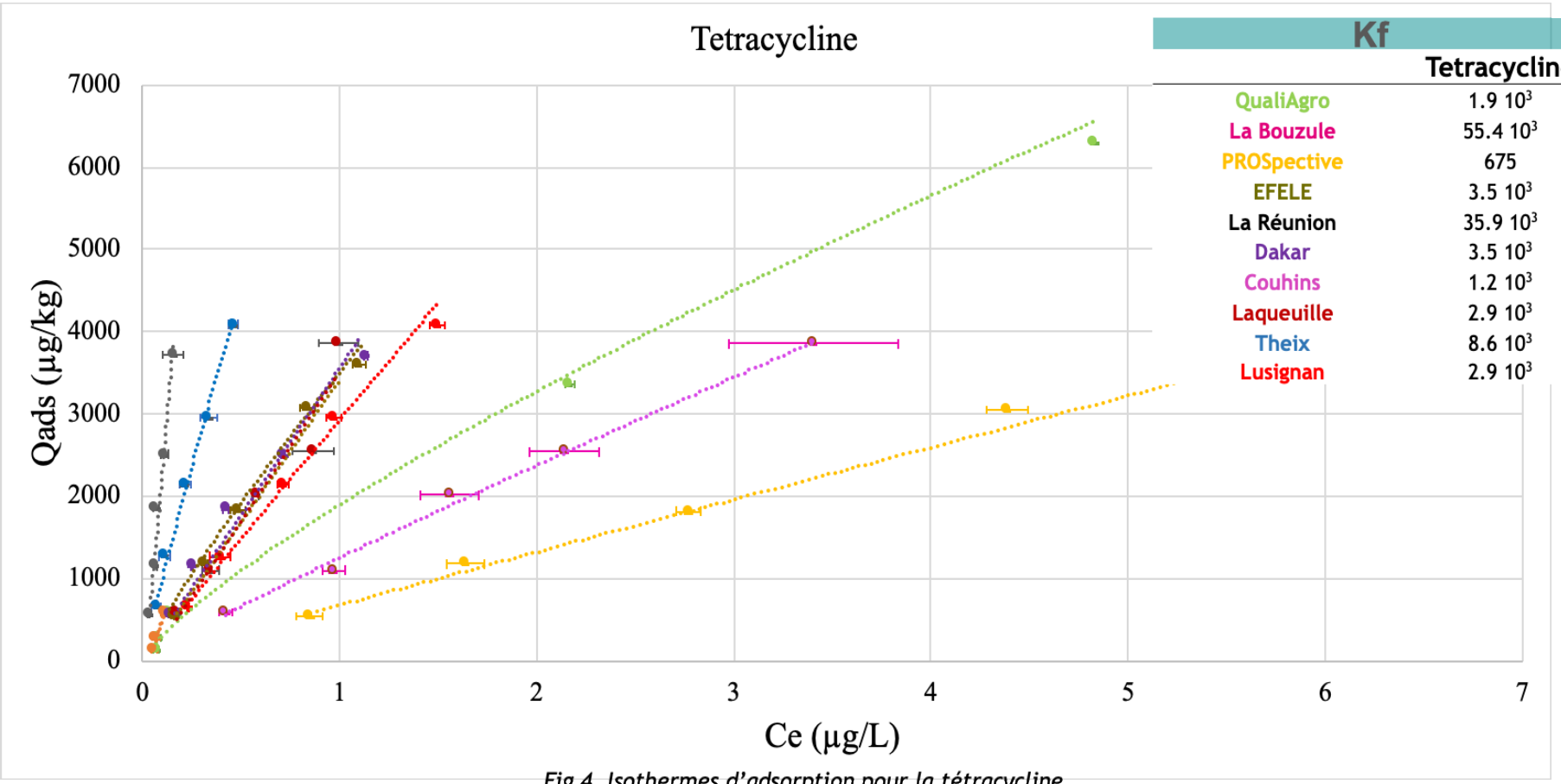
- 10 sols (tempérés et tropicaux ; 9 types de sol) : isothermes d'adsorption via experimentation

Experimental site	Soil type	pH_w	SOC	CEC	S	V	Clay	Silt	Sand
			g/kg	$cmol^+/kg$	$cmol^+/kg$	%	%	%	%
QualiAgro	Luvisol	6,43	9,74	9,11	8,31	91,26	15,63	77,83	6,55
PROSpective	Calcosol	8,36	13,86	17,25	18,28	106,00	18,20	62,50	7,30
EFELE	Luvisol-Redoxisol	6,17	9,61	5,67	5,74	101,32	14,55	70,08	15,38
La Bouzule	Calcisol rédoxique	7,08	17,60	15,48	16,60	107,25	34,93	53,38	32,68
Couhins	Luvisol dystrique	6,57	13,50	4,79	3,85	80,38	4,30	6,70	89,00
Theix	Brunisol	5,83	43,90	9,13	8,74	95,72	18,50	26,20	55,30
Laqueuille	Andosol	5,60	90,03	8,27	5,90	71,38	23,13	54,71	22,64
La Réunion	Nitisol	6,36	19,10	10,65	11,08	104,03	46,01	43,50	10,50
Dakar	Arénosol	6,46	6,82	9,66	8,08	83,64	9,99	11,22	76,52
Lusignan	Brunisol	6,30		6,70	6,55	97,76	14,00	63,00	23,00

ACV-Ecoto(Mi)x Project

WP4. Soil No Effect Concentrations

First results : Isothermes d'asorption – N. Bernet, V. Serre, C. Godard CDD 2023



Kf	Tetracycline
QualiAgro	1.9 10^3
La Bouzule	55.4 10^3
PROSpective	675
EFELE	3.5 10^3
La Réunion	35.9 10^3
Dakar	3.5 10^3
Couhins	1.2 10^3
Laqueuille	2.9 10^3
Theix	8.6 10^3
Lusignan	2.9 10^3

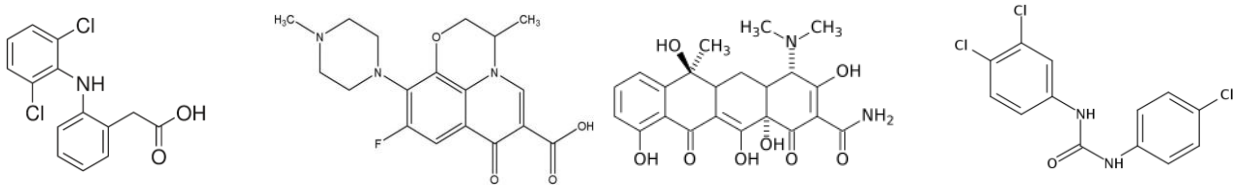
Fig 4. Isothermes d'adsorption pour la tétracycline

ACV-Ecoto(Mi)x Project

WP4. Soil No Effect Concentrations

First results : Variabilité des propriétés de sorption - N. Bernet, V. Serre, C. Godard CDD 2023

- Diclofénac / Ofloxacine / Tétracycline / Triclocarban



- Recherche de corrélations Kf et propriétés de sols

	Kf	pH	SOC	CEC	S	V	Argile	Limon	Sable
Kf	1.00	-0.49	0.92	-0.18	-0.19	-0.15	-0.03	-0.46	0.40
pH		1.00	-0.31	0.80	0.80	0.44	0.28	0.22	-0.33
SOC			1.00	0.05	0.07	0.17	0.26	-0.22	0.15
CEC				1.00	0.99	0.63	0.70	0.24	-0.37
S					1.00	0.72	0.75	0.31	-0.43
V						1.00	0.79	0.69	-0.75
Argile							1.00	0.42	-0.45
Limon								1.00	
Sable									1.00

1 0.8 0.6 0.4 0.2 0 -0.2 -0.4 -0.6 -0.8 -1

- Proposition fonctions de pédotransfert

Ex Diclofenac : Kf = 216,74 - 35,74 . pH + 10,44 . SOC - 1,29 . Limon (R^2 = 0,91)

WP4. Soil No Effect Concentrations

Establishing threshold values

- Estimating available concentrations estimations from **Kd/Kf** pedotransfer functions

-> Threshold values estimation for different ATB and in different soil types

- Explorating relationships betwen chemical structure and sorption parameters

-> Extending threshold values estimation to **other ATB compounds** from **in sillico approach** (TyPol)

Merci pour votre attention !