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Data Article

RISMEAU dataset: Pharmaceuticals and biocides concentrations in urban and agricultural sludge, amended soil and leachate and their environmental impacts

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ABSTRACT

The RISMEAU project (*RISques liés aux résidus de Médicaments, biocides et antibiorésistance d'origine humaine et vétérinaire sur les ressources en EAU du bassin versant de l'Arve* – Risks related to residues of pharmaceuticals and biocides, and antimicrobial resistance of human and veterinary origin on the water resources of the 2083 km^2 Arve catchment located in the French Alps) was implanted from 2018 to 2024 on the SIPIBEL observatory. It was devoted to the evaluation of (i) transfers of and processes related to pharmaceutical residues and biocides from both urban sludge and manure spread on fields as fertilisers, and (ii) the environmental impacts of land spreading, in particular the ecotoxicological risks and antimicrobial resistance dissemination. The

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methodology was based on the physico-chemical, ecotoxicological and antimicrobial resistance (AMR – assessed by molecular biology) characterisation of leachate and soil matrices samples, and focused on organic waste products application at locally representative agronomic rates. This dataset can be reused by other researchers for comparison with their own investigations on this emerging topic under different contexts and conditions, and may contribute to international reviews.

The database includes in total 26,217 values, measured on 348 samples of organic waste products, soil, *in situ* lysimeter leachate, earthworms, plants with 3136 usual physico-chemical values, 15,469 values on pharmaceuticals and biocides concentrations, 6827 bioassay values (ecotoxicity and phytotoxicity) and 785 values of antimicrobial resistance indicators. The 348 samples have been collected from Nov. 2019 to Dec. 2022: 4 samples in 2019, 26 in 2020, 130 in 2021 and 188 in 2022. Regarding AMR, 96 assays coding for most abundant resistance genes in healthy humans, clinically relevant antibiotic resistance genes, biocide resistance genes, heavy metal genes, integrons (class I, II and III), and mobile genetic elements (transposase genes) have been carried out.

Specifications Table

Subject	Environmental Engineering
Specific subject area	Pollutants, pharmaceuticals, biocides, ecotoxicity indicators, antimicrobial resistance dissemination from sludge and manure by land spreading
Data format	Raw
Type of data	Tables (csv files)
Data collection	Data have been obtained applying ISO, EN, French and experimental standard methods, or laboratories' internal methods. The applied methods are listed below and described in four csv files of the RISMEAU database available on Zenodo: https://zenodo.org/records/13736625. Analyses of 33 pharmaceuticals and biocides on liquid and solid matrices: • Internal method based on HPLC - MS/MS developed by Guironnet |6] Bioassays on and analyses of Eisenia fetida: • Avoidance rate – avoidance test: ISO 11268-1 [7] and internal protocol by Roques [14–16] • Cadmium, chromium, copper, lead, nickel, zinc: Roques [14] • Cocoon produced - reproduction test on soil from lysimeter: internal protocol by Roques [14–16] • Glycogen concentration: Pelosi *et al.* [13] • Lipid concentration: Pelosi *et al.* [13] • Mortality rate – *in situ* reproduction test: Roques [14–16] • Mortality rate - lethality test: ISO 11268-1 [7] and internal protocol by Roques [14–16] • Mortality rate - reproduction test on soil from lysimeter: internal protocol by Roques [14–16] • Mortality rate - reproduction test: ISO 11268-2 [8] • Number of cocoon produced - *in situ* reproduction: Roques [14–16] • Number of juveniles - *in situ* reproduction: Roques [14–16] • Number of juveniles - reproduction test on soil from lysimeter: internal protocol by Roques [14–16]

(*continued on next page*)

- Number of juveniles - reproduction test: ISO 11268-2 [8]
- Protein concentration: BCA methods, Smith *et al.* [18]
- Weight change rate - *in situ* reproduction: Roques [14–16]
- Weight change rate - lethality test: ISO 11268-1 [7] and internal protocol by Roques [14–16]
- Weight change rate - reproduction test on soil from lysimeter: internal protocol by Roques [14–16]
- Weight change rate - reproduction test: ISO 11268-2 [8]

Bioassays on and analyses of plants:

- Cadmium, chromium, copper, lead, nickel, zinc in aerial and root parts: Roques [14]
- Dry mass of aerial parts - plant growth test: ISO 11269-2 [9]
- EC50 - plant germination test: ISO 18763 [11] and Roques [14–16]
- Germination - plant germination test: ISO 18763 [11] and Roques [14–16]
- Germination - plant growth test: ISO 11269-2 [9]
- Height of aerial parts - plant growth test: ISO 11269-2 [9]
- LOEC - plant germination test: ISO 18763 [11] and Roques [14–16]
- NOEC - plant germination test: ISO 18763 [11] and Roques [14–16]
- Root length - plant germination test: ISO 18763 [11] and Roques [14–16]
- Wet mass of aerial parts - plant growth test: ISO 11269-2 [9]
- Wet mass of root parts - plant growth test: ISO 11269-2 [9]

Analyses of water:

- Ammonical nitrogen: NF T 90-015-2
- Chemical oxygen demand: ISO 15705
- Dissolved and total organic carbon: NF EN 1484
- Nitrates: NF EN ISO 10304-1
- Nitrites: NF EN 26777
- Orthophosphates and total phosphorous: NF EN ISO 6878
- pH: NF T 90-008
- Total Kjeldahl nitrogen: NF EN 25663
- Total suspended solids: NF 872

Analyses of soils, OWP and mixture soil/OWP:

- Ammonical nitrogen: NF T 90-015-1
- ARN activity: ISO 20130, Cheviron *et al.* [3] and Roques *et al.* [15,16]
- Arsenic, cadmium, calcium, chromium, copper, iron, lead, magnesium, manganese, mercury, nickel, phosphorus, potassium, selenium, zinc: NF EN 11885 and Roques [14]
- Beta-GLU activity: ISO 20130, Cheviron *et al.* [3] and Roques *et al.* [15,16]
- COD: Jimenez *et al.* [12]
- Conductivity: ISO 11265
- Dry matter: NF EN 12880
- Mineral and organic content: NF EN 12879
- NEOM: Jimenez *et al.* [12]
- Organic carbon: NF U 44-160
- PAK activity: ISO 20130, Cheviron et al. [3] and Roques et al. [15,16]
- PEOM: Jimenez et al. [12]
- pH: ISO 10390, NF EN 15933
- PHOS activity: ISO 20130, Cheviron *et al.* [3] and Roques *et al.* [15,16]
- Proportion of fluorescence - zones 1 to 7: Jimenez *et al.* [12] and Fernandez-Dominguez *et al.* [5]
- REOM: Jimenez *et al.* [12]
- SEOM: Jimenez *et al.* [12]
- SPOM: Jimenez *et al.* [12]
- Total Kjeldahl nitrogen: NF EN 13342
- YAS, YES, anti-YAS, anti-YES tests: Routledge and Sumpter [17] and Roques [14]

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	Antibioresistance in leachate, soil, OWP and earthworms: • Bacterial concentration 16S: Stalder *et al.* [19] • PCR quantitative 16S: Stalder *et al.* [19] • PCR quantitative int1, int2 and int3: Stalder *et al.* [19] • Relative abundance int1, int2 and int3: Stalder *et al.* [19]
Data source location	Arve Valley, Haute-Savoie, France GPS coordinates of sampling points : see sampling point csv files at https://zenodo.org/records/13736625
Data accessibility	Five files are available on Zenodo in the RISMEAU dataset zip file: - the RISMEAU data set description (one PDF file) - the RISMEAU metadata (two CSV files: i) sampling points, ii) methods) - the RISMEAU data sets (two CSV files: i) samples, ii) data). Data set doi: https://zenodo.org/records/13736625
Related research article	Roques, O., Bayard, R., Le Maux, J., Patureau, D., Nélieu, S., Lamy, I., and Bedell, J.-P. 2023. « Assessing the chronic toxicity of spreading organic amendments on agricultural soil: Tests on earthworms and plants ». Ecotoxicology and Environmental Safety 265 (October): 115504. 10.1016/j.ecoenv.2023.115504.

1. Value of the Data

- The RISMEAU data set contains values of numerous indicators (usual physico-chemical indicators of groundwater and soil contamination, major and trace elements, pharmaceuticals, biocides, bioassays, abundance of mobile genetic element (MGE) and antimicrobial resistance (AMR) genes, etc.) measured in i) urban sludge and manure, ii) amended soil, and iii) *in situ* lysimeter leachate (also named “infiltrated water” in the data set csv files), allowing a global analysis of impacts of land spreading practices in terms of contaminant concentrations, ecotoxicity and antimicrobial resistance dissemination. The interest of this dataset also lies in the study of *in situ* land spreading practices over two years of monitoring.
- These data contribute to a better assessment of i) concentrations of thirty-three pharmaceuticals and biocides in urban sludge and livestock effluent, amended soil, *in situ* lysimeter leachate, and exposed earthworms, ii) their ecotoxicological impacts, and iii) their impact on dissemination of antimicrobial resistance.
- These data can be useful and reused by researchers working on pharmaceuticals and biocides in the agricultural field for comparisons and statistical analyses. They can also be used by regulators to inform their decisions about new policies and regulations related to pharmaceuticals and biocides in sludges and livestock effluents, and in the environment or to prioritise contaminants to monitor.
- The data can also be used in modelling works.

2. Background

The compilation of data from the RISMEAU project is motivated by the acquisition of interdisciplinary data on i) the transfer of pharmaceuticals and biocides residues through soils in dissolved and particulate fractions, and ii) the environmental impacts of spreading of sewage sludge and livestock effluent. The various characterisation methods, combining physico-chemistry, ecotoxicology and AMR gene abundance, were applied on common matrices (soil, *in situ* lysimeter leachate, organic waste products (OWP)) to provide an overall understanding of one health issue.

3. Data Description

The RISMEAU dataset provides data on i) usual indicators of sludge, manure, soil and *in situ* lysimeter leachate) quality and contamination, (ii) pharmaceuticals, biocides and transformation products concentrations in sludge, manure, soil and *in situ* lysimeter leachate, (iii) ecotoxicolog-

ical and dissemination of AMR genes indicators that enable the assessment of risks for environment and health:

Usual indicators:

OWP (sludge and manure): dry weight content, pH, organic carbon content, organic matter content, dissolved organic carbon, ammoniacal nitrogen, total Kjeldahl nitrogen, phosphorous, calcium, magnesium, potassium, cadmium, chromium, copper, mercury, lead, nickel, selenium, zinc content.

Soil: dry weight content, pH, organic carbon content, organic matter content, dissolved organic carbon, ammoniacal nitrogen, total Kjeldahl nitrogen, phosphorous, calcium, magnesium, potassium, cadmium, chromium, copper, lead, zinc, manganese, arsenic, iron content.

In situ lysimeter leachate : dissolved organic carbon, total Kjeldahl nitrogen, total suspended solid, total phosphorous, orthophosphates, pH, ammoniacal nitrogen, nitrates, chemical oxygen demand.

Organic matter fractionation: soluble fraction from particular extractable organic matter, readily extractable organic matter, slowly extractable organic matter, hardly extractable organic matter, non-extractable organic matter, fluorescence.

Pharmaceuticals, biocides and transformation products:

Antibiotics: ciprofloxacin, erythromycin, ofloxacin, sulfaguanidin, sulfamethoxazole, sulfathiazole, tetracycline, trimethoprim.

Non-steroidal anti-inflammatory: ketoprofen.

Antihelmintics: doramectin, eprinomectin, flubendazole, levamisole, oxfendazole, pyrantel, pyrimethamine, thiabendazole.

Biocides: bromacil, chlorhexidine, dimethyl-didecyl ammonium, methamidophos, sulfoxaflor, tebuconazole, triclosan.

Transformation products: 3-amino-5-methylisoxazole, 5-hydroxythiabendazole, acetyl-sulfamethoxazole, albendazole-2-aminosulfone, anhydroerythromycin, desmethyl-ofloxacin, epitetracycline, hydroxy-flubendazole, hydroxy-levamisole.

The above residues of pharmaceuticals, biocides and transformation products have been analysed in dissolved and particulate fractions of solid matrices (soil, manure, earthworms) by means of analytical methods specifically developed by Guironnet [6].

Antimicrobial resistance genes indicators: bacterial concentrations (16S copy number), relative abundance of resistance genes, and mobile genetic elements (class 1, 2 and 3 integrons) (Barraud *et al.* [1]; Buelow *et al.* [2]; Stalder *et al.* [19]). They are listed in Table 1.

Endocrine disruption potential: androgenic and oestrogenic disrupting effects (YES, YAS, anti-YES and anti-YAS tests).

Ecotoxicology: plant germination and growth test (*Cucurbita Pepo, Medicago Sativa* and *Sinapis Alba*), lethality, avoidance, bioaccumulation and reproduction tests with *Eisenia fetida*.

The RISMEAU dataset on Zenodo includes 5 files listed in Table 2. The column separator of the csv files is the semicolon ';'. In the following sections, each csv file is briefly described.

File 01_RISMEAU_dataset_description

This pdf file is a paper providing a very short presentation of the Rismeau project and a detailed description of the content of the four csv files containing all metadata (files 02 to 04) and data (file 05).

File 02_RISMEAU_metadata_sampling_points.csv

This csv file presents the 18 sampling points, with their characteristics (ID code, name, type of matrix to be sampled, and their coordinates of in situ experimental sites).

File 03_RISMEAU_metadata_methods.csv

This csv file presents the details of all the methods used for samples and analyses and *in situ* and laboratory experiments (ID code, name of parameter or quantity of interest, sample matrix, fraction to be analysed, standard or guideline applied, LoD, LoQ, uncertainties, etc.).

File 04_RISMEAU_dataset_samples.csv

This csv file presents all samples of the data set, with their ID code, sampling point (referring to file 02), date of sampling and specific treatment or preparation if relevant.

File 05_RISMEAU_dataset_data.csv

Table 1
List of the genes and families of genes for assessment of antimicrobial resistance.

Family	Genes							
Chloramphenicol	catB3	cat						
Aminoglycosides	cm1A1	aac(3)-Iid	aadE	aac(6′)-IB	aadA	aac(6′)-IIa	spc	aph'3)-III
	aph(2)-I(de)	aph(2)-Ib	aadB	AAC(3)-Ib	strB			
β-lactamase	aac(6)-aph(2)	bacA_2	bacA_1	cepA	cblA	cfxA	blaTEM	blaIMP
	blaAMPC	blaDHA	blaCMY-2	blaACC	blaSHV	blaVIM	blaKPC	blaIMI
	blaBIC-1	blaGES	blaNDM	blaOXA	blaCTX-M	blaPer-1		
Macrolides	ermF	ermB	ermC	ermY	mfsA	mefA_10	macB	ermX
Heavy metals	acrA	mdtO	tolC	mdtL	mdtF	qnrA	qnrB	qnrS
	qnrC	cusF	copA	copD	cadA	merA	czcA	
QAC	qacA	qacC	qacE					
Glycopeptide	vanA	vanB						
Tetracycline	tetQ	tetW	tetM	tetO	tetB			
Polymixine	arnA	mrc-1						
Sulfonamides	sul1	sulA						
mecA	mecA							
Trimethoprime	dfrA27	dfrF	dfrB1					
Streptogramine	vatB	vat(A)						
MGE	ISSW1	ISS1N	IS6_IS6100	Tp614	IS613	IS6 group	tn3_tnpA	ISEc9
	intI1	intI2	intI3	inc-P1				

Table 2
List of the 5 files of the RISMEAU data set available on Zenodo.

File name	Content
01_RISMEAU_dataset_description.pdf	Presentation of RISMEAU project and dataset
02_RISMEAU_metadata_sampling_points.csv	List and description of sampling points
03_RISMEAU_metadata_methods.csv	List and description of methods
04_RISMEAU_dataset_samples.csv	List and description of samples
05_RISMEAU_dataset_data.csv	Full set of data

This csv file contains the Rismeau data. It includes 26217 lines providing, for each value, the corresponding sample ID code (referring to file 04), the identification code of the replicates (if relevant), the ID code of the method applied (referring to file 03), the measured value and a few additional characteristics.

4. Experimental Design, Materials and Methods

The RISMEAU project is based on both laboratory and *in situ* experiments.

4.1. In situ *lysimeters*

The selected plot is located in Scientrier, French Alps, and belongs to the local wastewater public utility. The site was chosen for three main reasons. First, the grass field is a part of a drinking water supply pumping station, where land spreading is strictly forbidden since 2012, hence there was no previous land spreading of any fertiliser reported on this plot. Secondly, a fence surrounds the field to control the access. Finally, the WWTP where the sludge is sampled and the dairy cow farm providing manure for the project are in the vicinity.

The location of the *in situ* pilot lysimeters within the plot was based on preliminary excavations done in July 2019 and selected according to two criteria: i) the representativeness of the local homogeneous soil profiles and ii) the presence of earthworms.

The experimental system is made of six lysimeters and a central access shaft, built with 2 m $\times$ 2 m squared prefabricated concrete units of 50 cm high each (Fig. 1). For lysimeters Ma,

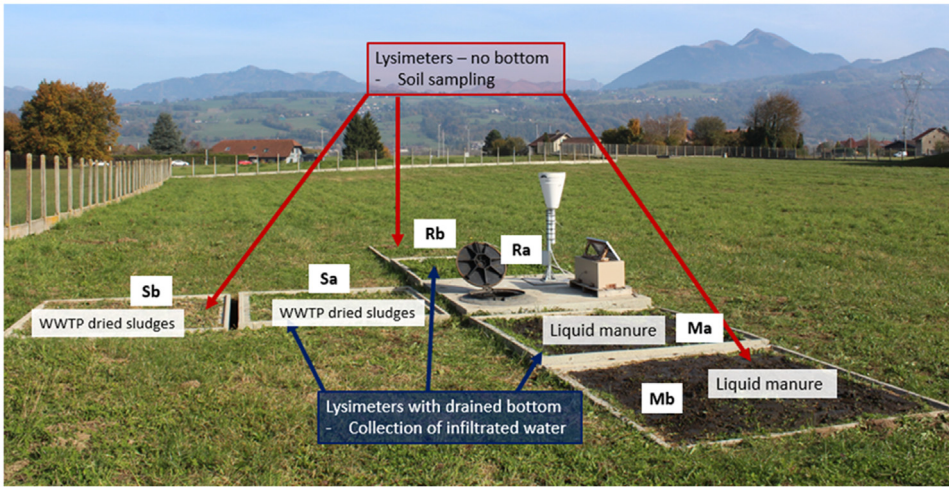

Fig. 1. *In situ* lysimeters.

Sa, Ra, two units are superposed on a watertight concrete sole slightly inclined and drained at the bottom. *In situ* lysimeter leachate is collected by gravity at the bottom (at 1 m depth) in a HDPE barrel placed in the central shaft. Lysimeters Mb, Sb, Rb are composed of a single concrete unit, with no watertight bottom. They duplicate the first three lysimeters Ma, Sa and Ra but without sampling of the *in situ* lysimeter leachate. The soil is sampled in lysimeters Mb, Sb and Rb only to not disturb the hydrological and infiltration conditions of lysimeters Ma, Sa and Ra where *in situ* lysimeter leachate is collected.

All lysimeters are placed 5 cm above the surrounding soil surface to precisely define the surface for land spreading, and also to avoid any cross contamination (i.e. run off) between lysimeters and from and to the surrounding field.

During the building phase, the soil was removed with a backhoe loader according to defined layers: four layers for lysimeters Ma, Sa and Ra (0–40 cm, 40–60 cm, 60–80 and 80–100 cm) and two layers for lysimeters Mb, Sb, and Rb (0–40 cm and 40–60 cm). The soil was then repacked layer by layer to reach approx. 95 % of its original compaction. For Mb, Sb, and Rb, the soil below 60 cm depth was undisturbed.

Lysimeters Ma and Mb receive liquid manure from a dairy cow farm, lysimeters Sa and Sb receive WWTP dried sludge from the Bellecombe WWTP. Lysimeters Ra and Rb are used as reference with no application.

5. Land Spreading and Sampling Campaigns

5.1. Land spreading campaigns

Bi-annual land spreading campaigns were carried out in respect of local practices: one at the end of spring and one at the end of autumn. The first two campaigns (campaign 1: 05 November 2020 and campaign 2: 03 June 2021) followed local pasture land spreading practices. The doses of WWTP dried sludge and liquid manure applied were chosen according to information provided by local WWTP operators and farmers. For the second year of experiments, application rates were multiplied by five for the next two campaigns (campaign 3: 27 October 2021 and campaign 4: 17 May 2022). A fifth campaign was carried out on 05 October 2022. Land spreading was carried out only on the lysimeters Ma and Sa where the soil had not been sampled during the first four campaigns. Dried sludge from Bellecombe WWTP was applied at a high rate (five times higher than local practices), as in campaigns 3 and 4. This was the only

batch of sludge applied that was digested before being dried in the WWTP greenhouse. For the lysimeter Ma receiving liquid manure (MA), the input was first spiked with a solution containing ofloxacin, flubendazole, levamisole and pyrantel, with final concentrations estimated at 60 µg/g DW of liquid manure, 10 µg /g DW, 10 µg/g DW and 80 µg/g DW respectively. This spiked liquid manure was applied by respecting the local practices (as in campaigns 1 and 2).

5.2. Sampling campaigns

Sampling of *in situ* lysimeter leachate started more than one year after installation of the lysimeters to allow sufficient time for soil structuration and drainage conditions to establish. Leachate collected at 1 m depth at the bottom of lysimeters Ma, Ra and Sa was sampled regularly, depending on the volume collected: a minimum of 5 L sample was required for physico-chemical analyses and AMR quantification.

Three soil cores were taken from each lysimeter Mb, Sb and Rb at the end of the three first campaigns, using a manual auger. The cores were divided into four layers (0–10 cm, 10–40 cm, 40–60 cm and 60–100 cm depth). The soil taken from each layer was homogenised and the final sample was obtained by quartering. During the third campaign (28 October 2021–17 May 2022), soil samples were taken in triplicate from the first layer (0–10 cm) of each lysimeter at monthly intervals. At the end of the fourth campaign, soil samples were taken in triplicate only from the first layer (0–10 cm) of each lysimeter. As drought occurred during this campaign, we supposed that no vertical transport of contaminants occurred. Finally, for the fifth campaign, triplicate core sampling was carried out on Sa lysimeter. Similarly, sixteen soil cores were sampled from lysimeter Ma to better assess the spatial variability of soil concentrations of antibiotic residues. The sixteen sampling points were taken randomly within a regular 4 by 4 mesh (i.e. 50 cm by 50 cm areas).

More information on the *in situ* experiments is available in Etienne [4].

In total, 348 samples have been collected on 18 sampling points: details are given in csv files named respectively “04 RISMEAU data set samples” and “02 RISMEAU metadata sampling points”.

6. Ecotoxicological Tests on OWP (Organic Waste Products)

Ecotoxicological tests were carried out on several terrestrial model organisms to assess the impact of the WWTP dried sludge and the liquid manure used for spreading on *in situ* lysimeters. The soil used in the tests was either sampled from the experimental plot or laboratory made. In addition to WWTP dried sludge and liquid manure, manure from a dairy cow farm was also studied. All tests are described in Table 3. Specific methods are detailed in the file 03_RISMEAU_metadata_methods.csv.

6.1. Laboratory and in situ *reproduction tests*

Reproduction tests were adapted from the standard procedure ISO 11268-2 [8] and performed on *Eisenia fetida* over 56 days (Fig. 2). Bellecombe soil/OWP mixtures were prepared using the following rate: 0, 20 and 50 g/kg (DW/DW) for the sewage sludge and the manure; 5, 10, and 20 g/kg (DW/DW) for the liquid manure, and maintained with a water content closed to 70 % of the water holding capacity (WHC). Each treatment was tested in triplicates except for soil/liquid manure which was tested in four replicates and a negative control was performed in an artificial ISO soil [10].

After five days of soil/OWP acclimatisation, 450 g of soil or soil/OWP mixture were introduced in one-litre jars (Le Parfait©), covered by an aluminium foil to maintain darkness around

Table 3
Summary of ecotoxicological tests made on soil or soil/OWP mixture, performed on plants and earthworms.

Organism	Plants		Earthworms			
Species	Medicago sativa		Eisenia fetida			
	Sinapis alba					
	Cucurbita pepo					
Acclimatisation	None		Dark chamber containing coco peat and organic manure (50/50)			
Test	Germination	Plant growth	Avoidance	Lethality	Reproduction test	*In situ* reproduction test
Guidelines	ISO 18763 [11]	ISO 11269-2 [9]	ISO 17512-1 [10]	ISO 11268-1 [7]	ISO 11268-2 [8] adapted	ISO 11268-2 [8] adapted
Exposure medium	Sand and Bellecombe soil	Bellecombe soil	ISO soil	Bellecombe soil	Bellecombe soil	Bellecombe soil
OWP tested	Bellecombe sludge, liquid manure and manure					Bellecombe sludge and liquid manure
Length of exposure	12 days (M. sativa) and 5 days (S. alba and C. pepo)	21 days (S. alba) to 28 days (M. sativa)	2 days	14 days	28 days (adults) than more 28 days for juveniles	28 days
Number of organisms	10/replicate	75/replicate	10/replicate	10/replicate	10/replicate	18/replicate
Replicate	3		4	3	3	3
Test temperature	20+-2 °C	20+-2 °C	20+-2 °C	20+-2 °C	20+-2 °C	12–34 °C
Photoperiod	16 h day/8 h night (400–800 lx)	16 h day/8 h night (400–800 lx)	16 h day/8h night (400–800 lx)	16 h day/8 h night (400–800 lx)	16 h day/8 h night (400–800 lx)	
Validity criterion	Germination of controls > 70 % Root size of controls > 30 mm	Germination of controls > 70 % Root size of controls > 30 mm	Mortality rate of each treatment < 10 %	Mortality rate of each treatment < 10 % Loss of biomass of controls < 20 %	Mortality rate of each treatment < 10 % Loss of biomass of controls < 20 %	Mortality rate of each treatment < 10 % Loss of biomass of controls < 20 %

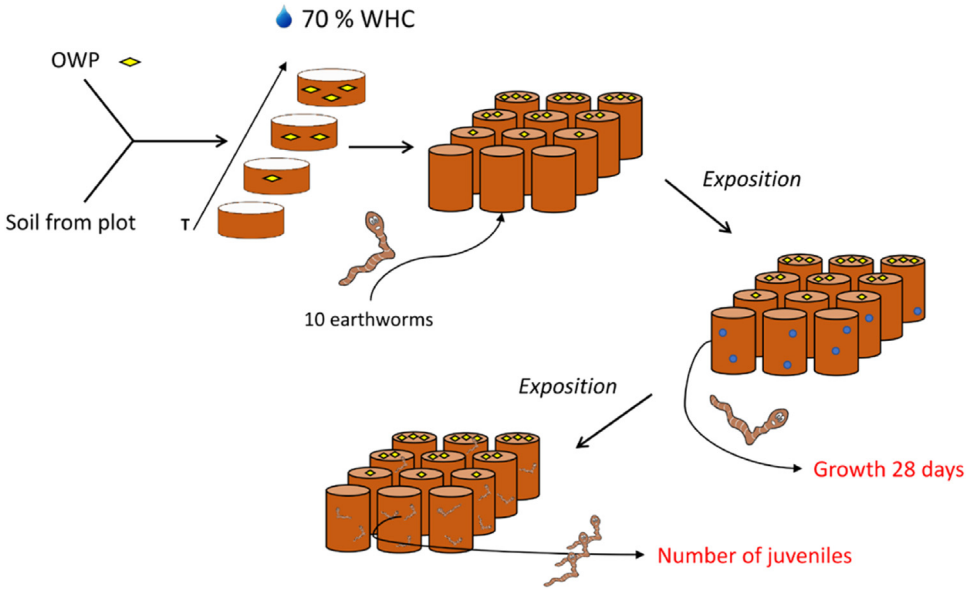

Fig. 2. Reproduction test protocol ISO 11268-2 [8].

the soil. Organic horse dung was added to equalise the organic carbon content of each jar, with a maximum of 20 g of dry organic horse dung (8.36 g of organic carbon) to the control jar (ISO soil without OWP).

Then 10 earthworms were added to each jar and exposed for 28 days. After 14 days, living adult earthworms were counted and reintroduced in jars. After 28 days, living adult earthworms were extracted and counted, weighted and frozen at −20 °C for later analysis. The tests lasted 28 days more for a total of 56 days, then the juveniles and unhatched cocoons were removed from soil and counted. The test was valid if, for control assays, i) mortality was lower than 10 %, ii) the loss of fresh biomass did not exceed 20 %, and iii) the production of juveniles was higher than 30 individuals with a variability lower than 30 %.

For *in situ* tests, the procedure was the following one: PVC test chamber (20 cm high, diameter of 19.5 cm) with mosquito nets at top and bottom, were placed outside, in soil, and filled with soil up to 7.5 cm height, then with soil/OWP mixture to 7.5 cm height or with the organic horse dung in case of control assay. Soil/OWP mixtures were prepared five days before the test, at the mixing rate of 10 g/kg (DW/DW) and maintained at 70 % of their WHC. Each treatment was tested in four replicates. Eighteen earthworms were placed in each PVC chamber, under real climatic conditions but watered each two days to maintain the water content. After 28 days, chambers were removed from soil and earthworms and cocoons were counted. Living adult earthworms were weighted then frozen at −20 °C for later analysis. Cocoons were kept in a moistened Petri dish for 28 more days, then juveniles and cocoons were counted.

6.2. Lethality test

ISO 11268-1 [7] was performed to assess the acute lethality of earthworms. One litre glass jars (Le Parfait©) were filled with 600 g of soil or soil/OWP mixture. The soil was sampled in the non-amended Bellecombe plot, and its water content was adjusted to 70 % of the WHC (Fig. 3). Mixing rate was 10, 20, 30, 40, 50, 100, 120, 140, 160, 180 and 200 g/kg (DW/DW) for the sewage sludge; 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 g/kg (DW/DW) for manure and liquid

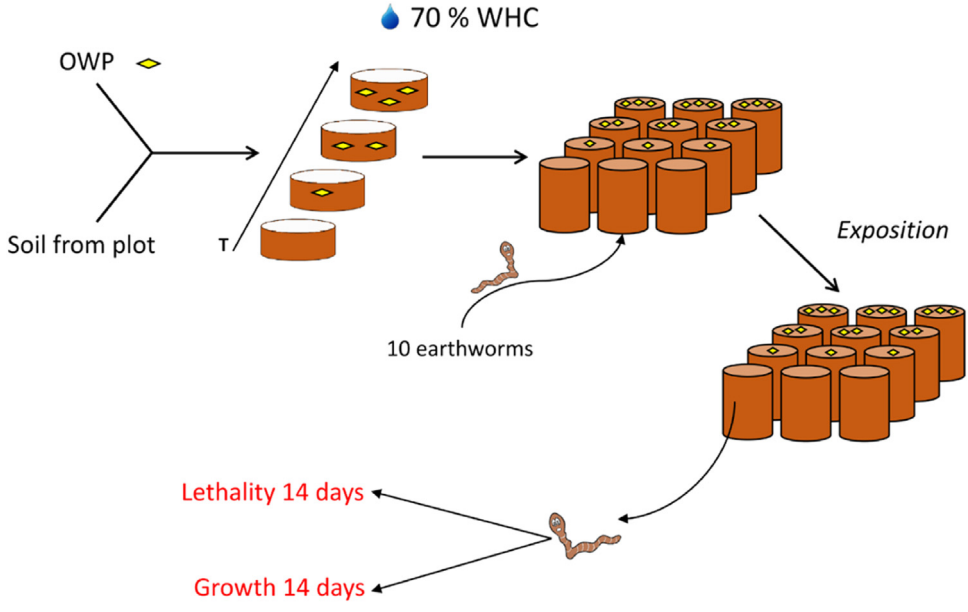

Fig. 3. Lethality test protocol ISO 11268-1 [7].

manure. Each treatment was run in triplicate. After five days of soil/OWP acclimatisation, ten earthworms were introduced in each jar, then the jars were closed with perforated plastic lids and placed in an air-conditioned room at 20 ± 2 °C and a photoperiod of 16 h day/8 h night. After fourteen days, living earthworms were counted and weighted. The test was valid if i) the mortality was less than 10 %, and ii) the loss of fresh biomass did not exceed 20 %.

6.3. Avoidance test

The ISO 17512-1 [10] avoidance test was performed with an artificial ISO soil, mixed with OWP at the following rate: 10, 20, 50 g/kg (DW/DW) for the sewage sludge and manure; 5, 10, 20 g/kg (DW/DW) for the liquid manure. Mixtures were prepared five days before the test, in four replicates for each treatment. The water content was adjusted to 70 % of WHC of the soil or the mixture soil/OWP. In a plastic container divided into six equal sections, 700 g DW of soil were placed, alternating control soil (without amendment) and soil/OWP mixture in the sections. After five days of soil/OWP acclimatisation, ten earthworms were placed in the empty central cavity (Fig. 4). Perforated lids were used to close the containers. Then the containers were placed in an air-conditioned room at 20 ± 2 °C. The photoperiod was 16 h day/ 8 h night. After 48 h, earthworms were counted in each section of the container, whose sections were previously partitioned using plates. The test was valid if the mortality was lower than 10 % in each treatment.

6.4. Plant germination test

Germination tests performed on *Medicago sativa, Sinapis alba* and *Cucurbita pepo* (Table 3, column 2) were carried out using transparent plastic plates Phytoxkit© (ISO 18763 [11]) to compare the number of seeds germinated and the length of young roots between different matrices exposure (soil/OWP mixture or non-contaminated control soil). The batches of seeds were purchased

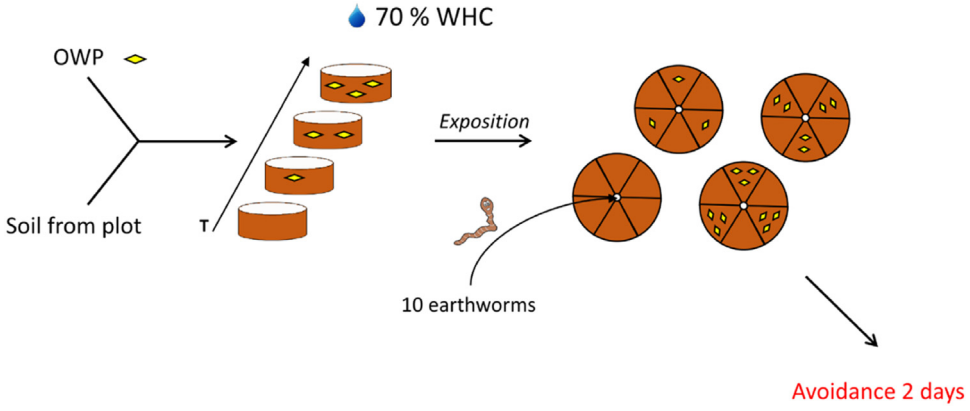

Fig. 4. Avoidance test protocol ISO 17512-1 [10].

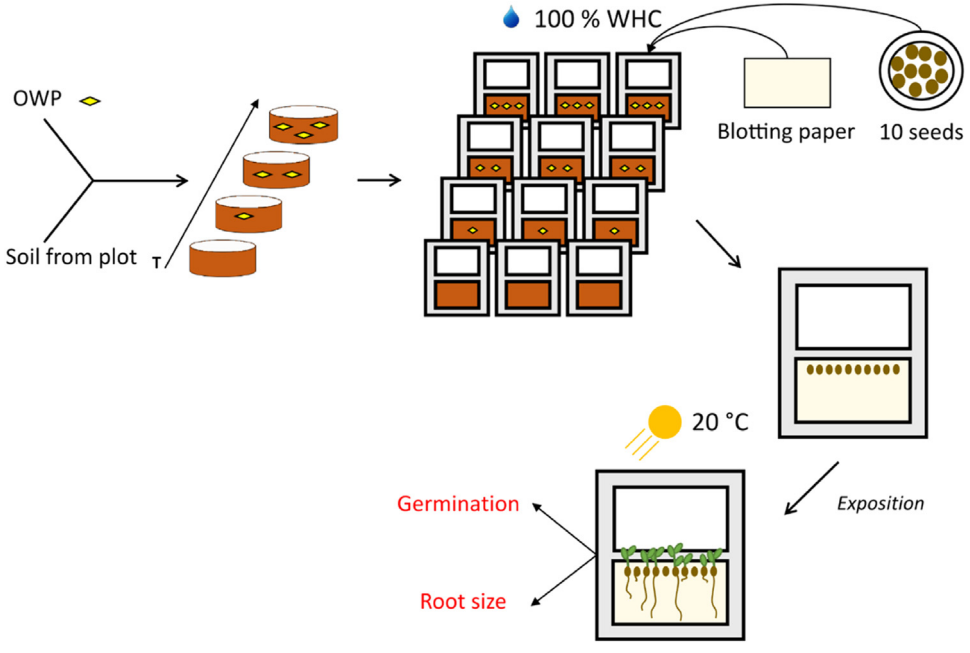

Fig. 5. Plant germination test ISO 18763 [11].

from Gamm Vert©. Tested matrices are either sandy soil mixed with OWP at the following rate: 0, 10, 20, 50, 100, 200, 500 g/kg (DW/DW) or non-amended soil from Bellecombe plot and OWP mixed at the following rate: 0, 10, 20, 50, 100, 200 g/kg (DW/DW). Three replicates were used for each plant species and each mixture rate (Fig. 5). After five days of soil/OWP acclimatisation, ten seeds, placed at equal distance of 1 cm, were placed on a blotting paper covering the solid matrices saturated to its WHC. Plastic plates were then placed vertically in an air-conditioned room at 20 ± 2 °C. Photoperiod was set to 16 h day/8 h night. When the stopping criteria was reached (roots growth at least 1 cm from the edge of the plate), the lengths were measured using a digital photo of each plate, processed with ImageJ©. Validity criteria of the test were i)

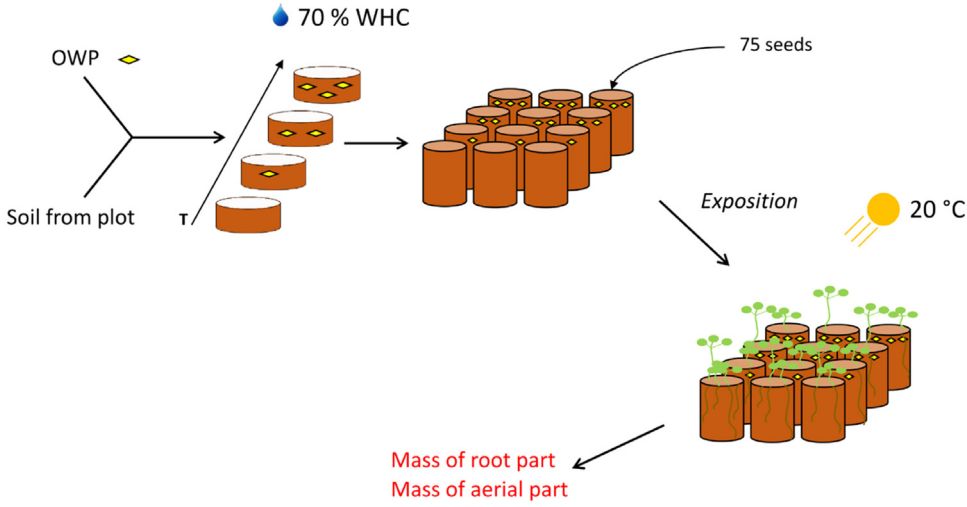

Fig. 6. Plant germination and growth test protocol ISO 11269-2 [9].

germination rate more than 70 % for the negative control, and ii) root growth reached a length of at least 30 mm.

6.5. Plant growth test: chronic phytotoxicity

The ISO 11269-2 [9] procedure was adapted to characterise the impact of OWP on plant emergence and growth and performed on *Medicago sativa* and *Sinapis alba*. Terracotta pots (14 cm diameter and 13.5 cm high), covered with aluminium foil, were filled with mixed soil from plot and OWP up to 2 cm below the edge of the pots and the water content was adjusted to 70 % of their WHC (Fig. 6).

Mixing OWP rates were 0, 10, 20 g /kg (DW/DW) and each treatment was performed in triplicate. Pots were placed in a controlled climatic chamber at 20 ± 2 °C and demineralized water in a dish plate was placed below the pots three time a week to water the pots by capillarity. Photoperiod was 16 h day/ 8 h night.

After five days of soil/OWP mixture acclimatisation, 75 seeds were placed on top and then covered with a 1 cm layer of the same mixture. After 28 days for *M. sativa* and 21 days for *S. alpa*, aerial and root parts were cut, counted and measured using a digital photo processed with ImageJ©. Then, cleaned by successive ultrasound baths, aerial and root parts were weighed (wet and dry mass).

7. Ecotoxicological Tests on Exposed Soil

Supplementary ecotoxicological tests (Fig. 7) were performed on earthworms (*Eisenia fetida*) to characterise the impact of soil exposed to several land spreading campaigns of Bellecombe sludge and liquid manure. The soil between 0 and 20 cm depth was sampled on 15 December 2022 from lysimeter Ma (five land spreadings of liquid manure), Mb (four land spreadings of liquid manure), Sa (five land spreadings of Bellecombe sludge), Sb (four land spreading campaigns) and Ra (no land spreading, reference lysimeter). The ecological test performed was a reproduction test based on three cycles of exposition of earthworms, each lasting 28 days before changing medium. The same soil medium was used for the three cycles. Each cycle cor-

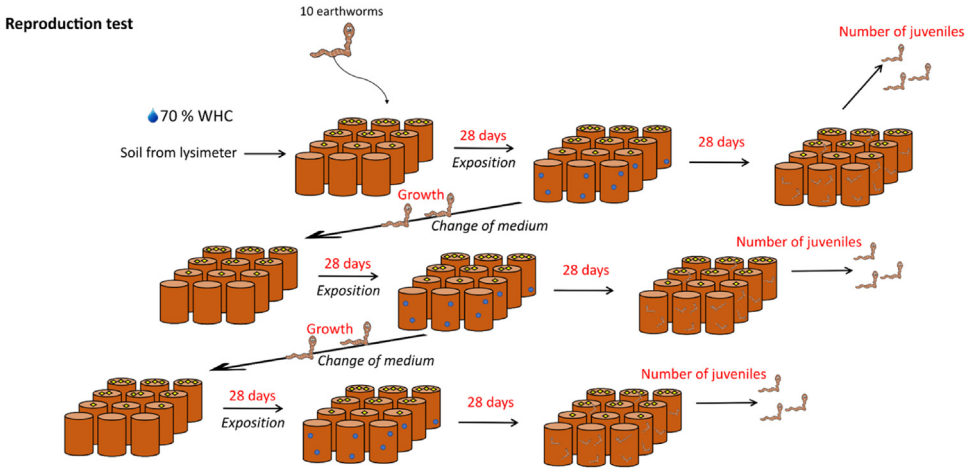

Fig. 7. Protocol of reproduction test on exposed soil.

responded to one reproduction test performed to characterise the impact of OWP exposition (Table 3).

More information on the bioassays is available in Roques [14] and Roques *et al.* [15,16].

8. Analyses of Pharmaceuticals, Biocides and Metals

A list of 33 pharmaceuticals and biocides to be monitored in RISMEAU has been established and a new HP-LC–MS/MS analytical protocol has been developed by Guironnet [6]. The robustness of this new analytical method was ensured by systematic blanks, quality controls and field blanks (MilliQ water, mineral water, real samples) integrated into each batch of analyses. Furthermore, to compensate for matrix effects and recoveries, each sample was spiked prior to sample preparation with stable isotopes of the compounds of interest. Between sampling and analysis, samples were stored at −20 °C. All samples were extracted and analysed in duplicates.

For the analyses of metallic trace elements, precision, analytical and procedural accuracies were controlled by replicate measurements performed on certified materials to check digestion and analyses. Repeated measurements (triplicates) on the same samples were achieved to test repeatability.

Limitations

- Most samples in the *in situ* lysimeters were collected from Oct. 2020 to Nov. 2022: this two-year long experiment has not allowed to detect significant effects or impacts of sludge and manure spreading. However, this is a too short duration to estimate possible long-term effects and impacts of sludge and manure spreading over decades.
- The experiment lasted only two years with very contrasted meteorological conditions: the spring summer 2021 was moderately rainy while the spring summer 2022 was extremely dry (see details in Etienne [4]). Under such dry conditions in 2022, the possible transfer of pharmaceuticals and biocides molecules through the lysimeters was very low and general conclusions cannot be easily drawn.
- Even though the lysimeters have been installed is a grassland area closed by a fence where spreading is forbidden since 2012 (it is used for a drinking water pumping station), some

molecules of pharmaceuticals and biocides were detected in the local reference soil without spreading and also possibly in the drinking water. This implies that the molecules found in the lysimeters soil and leachate cannot be entirely attributed to the sludge and manure we spread during the experiments and that some previous and/or cross contamination may exist.
- Even if the lysimeters have been built carefully and as homogeneously as possible, some dedicated investigations have shown a very significant heterogeneity in both infiltration capacity and concentrations of pharmaceuticals and biocides in the soil, which means that the measured concentrations in samples of lysimeters soil are not necessarily representative of the entire 2m x 2 m surface of the lysimeters (see details in Etienne [4]).

Ethics Statement

The authors have read and follow the ethical requirements for publication in Data in Brief and confirming that the current work does not involve human subjects, animal experiments, or any data collected from social media platforms.

CRediT Author Statement

Jean-Philippe Bedell: Conceptualization, Methodology, Reviewing, Supervision, Investigation, Funding acquisition, **Pierre Benoit:** Conceptualization, Methodology, Investigation, Reviewing, Supervision, **Jean-Luc Bertrand-Krajewski:** Conceptualization, Methodology, Reviewing and editing, Supervision, Funding acquisition, **Elodie Brelot:** RISMEAU project management, Funding acquisition, **Christophe Dagot:** Investigation, Funding acquisition, **Noémie Etienne:** Original draft preparation, Writing and editing, Conceptualization, Methodology, Data curation, Investigation, **Margaux Gaschet:** Investigation, **Alexandre Guironnet:** Investigation, **Isabelle Lamy:** Investigation, Funding acquisition, **Sylvie Nélieu:** Investigation, **Dominique Patureau:** Conceptualization, Methodology, Investigation, Supervision, Reviewing, **Olivier Roques:** Conceptualization, Methodology, Investigation, **Laure Wiest:** Investigation, Reviewing, Funding acquisition.

Data Availability

Rismeau data set (Original data) (Zenodo).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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