Circular economy and pathogenic *Escherichia coli*: unfolding the paradigm of human exposure to *E. coli* infections, including STEC, through crops

18th Annual Workshop of the

National Reference Laboratories for *E. coli* in the EU

October 1st, 2024

ISS, Aula Nitti-Bovet

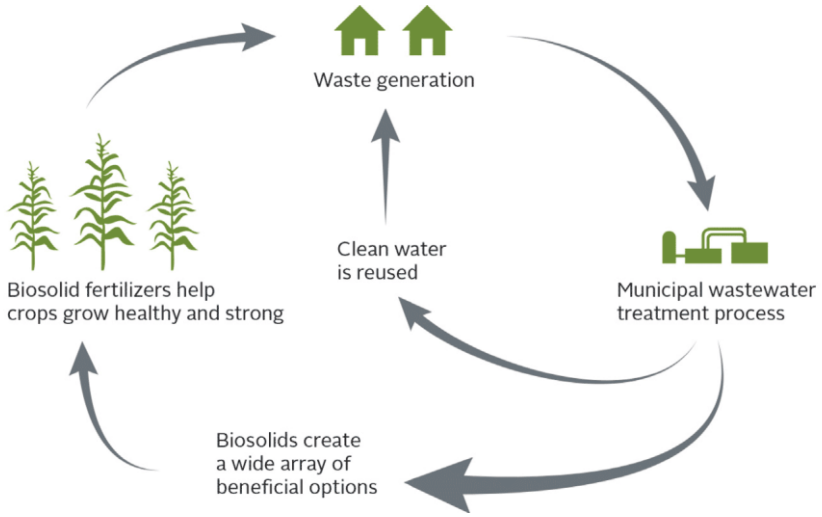
Giorgia Barbieri

DIPARTIMENTO
SICUREZZA ALIMENTARE, NUTRIZIONE
E SANITÀ PUBBLICA VETERINARIA

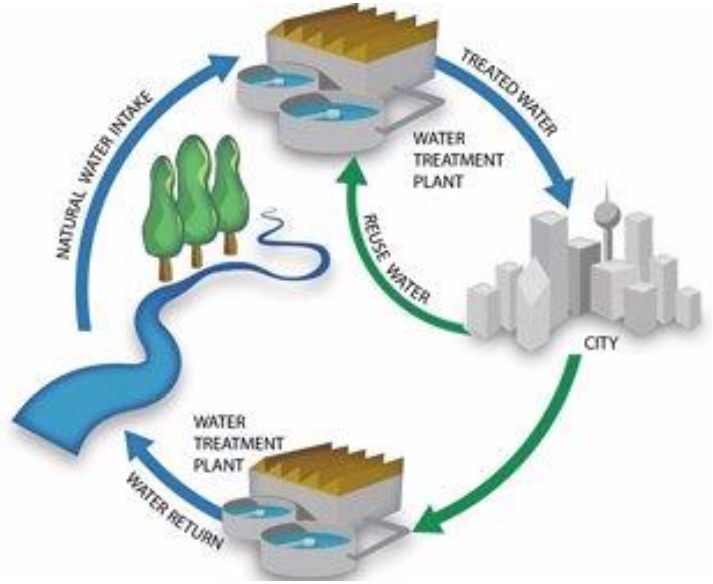
"Emerging food safety risks from microbial hazards deriving from anthropogenic pressures in agricultural settings"

RF-2019-12369714

Circular economy: waste and water management



https://www.synagro.com/community-outreach/the-facts-about-biosolids/



https://www.wateronline.com/doc/water-reuse-as-an-emerging-solution-0001

- **Sustainability:** reusing biological matrices to be reintegrated into the biosphere

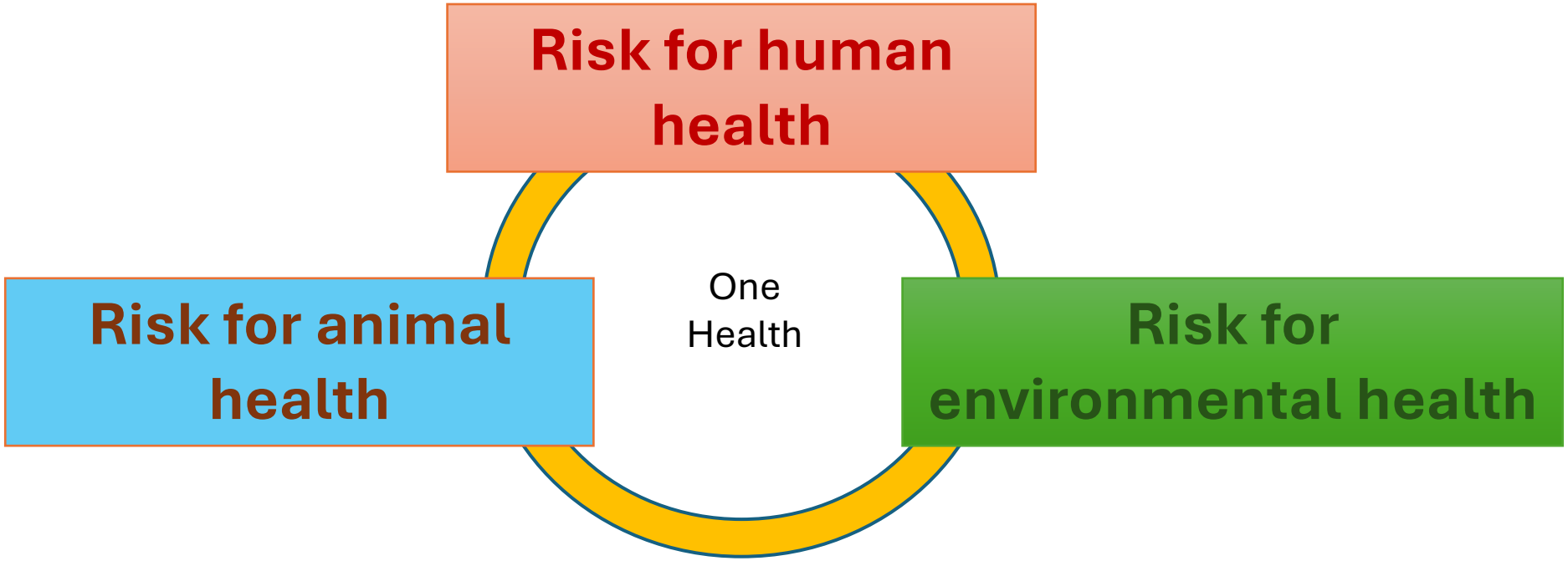
Recovery of nutrients and water

Risk assessment?

- **Spread of hazards** along the agri-food chain
- **Food safety non-compliance**

<u>Consequences on:</u>

- Production of vegetables
- Pasture for livestock
- Soil health



Food safety hazards in the circular biobased economy

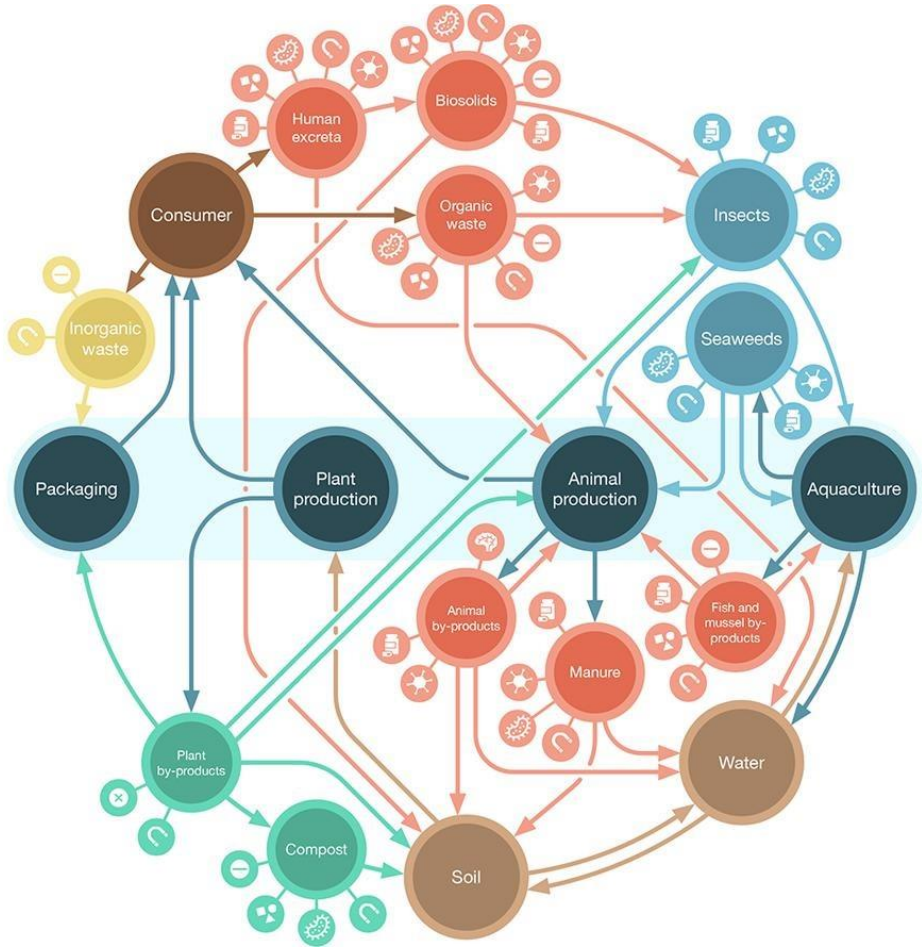
Potential (food) safety hazards in the circular biobased economy; showing the possible accumulation in the main production domains (plant, animal, aquaculture, packaging) and the secondary production systems through the reuse of (by-)products.

LEGEND

- Main production domains
- Secondary production systems
- Inorganic by-products
- Plant-based by-products
- Natural resources
- Consumer
- (By-)products containing animal proteins

SELECTED FOOD SAFETY HAZARDS

- Pathogenic bacteria
- Viruses
- Heavy metals
- Pharmaceuticals
- Dioxins and PCBs
- PFASs
- Mycotoxins
- Prions



https://doi.org/10.1016/j.foodres.2022.111505

Targeted treatments can be not fully effective in eliminating such hazards

efsa
European Food Safety Authority
EFSA Journal 2011;9(10):2390

SCIENTIFIC REPORT OF EFSA

Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Europe: Taking Stock[1]

European Food Safety Authority[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

Centers for Disease Control and Prevention
MMWR
Morbidity and Mortality Weekly Report
Weekly / Vol. 73 / No. 18
May 9, 2024

Shiga Toxin–Producing *Escherichia coli* O157:H7 Illness Outbreak Associated with Untreated, Pressurized, Municipal Irrigation Water — Utah, 2023

BreAnne Osborn, MPH[1]; Jennifer Hatfield[2]; William Lanier, DVM[1,3]; Jennifer Wagner, MS[1]; Kelly Oakeson, PhD[1]; Ravyn Casey[1]; Jacob Bullough[1]; Pallavi Kache, PhD[4,5]; Shanna Miko, DNP[5]; Jasen Kunz, MPH[5]; Grace Pederson, MPH[5]; Molly Leeper, MPH[5]; Nancy Strockbine, PhD[5]; Haley McKeel[5]; Jessica Hofstetter, PhD[5]; Alexis Roundtree[5]; Amy Kahler, MS[5]; Mia Mattioli, PhD[5]

Environmental Research 172 (2019) 630–636

Contents lists available at ScienceDirect

Environmental Research

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

Prevalence of Shiga-toxigenic and atypical enteropathogenic *Escherichia coli* in untreated surface water and reclaimed water in the Mid-Atlantic U.S

Joseph Haymaker[a,1], Manan Sharma[b,*,1], Salina Parveen[a], Fawzy Hashem[a], Eric B. May[a], Eric T. Handy[b], Chanelle White[a], Cheryl East[b], Rhodel Bradshaw[b], Shirley A. Micallef[d], Mary Theresa Callahan[d], Sarah Allard[c], Brienna Anderson[e], Shani Craighead[e], Samantha Gartley[e], Adam Vanore[e], Kalmia E. Kniel[e], Sultana Solaiman[d], Anthony Bui[c], Rianna Murray[c], Hillary A. Craddock[c], Prachi Kulkarni[c], Derek Foust[a], Rico Duncan[a], Maryam Taabodi[a], Amy R. Sapkota[c]

[a] University of Maryland Eastern Shore, Department of Agriculture and Resource Sciences, Princess Anne, MD, United States
[b] United States Department of Agriculture, Agricultural Research Service, Northeast Area, Beltsville Agricultural Research Center, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, United States
[c] Maryland Institute for Applied Environmental Health, University of Maryland School of Public Health, College Park, MD, United States
[d] Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD, United States
[e] University of Delaware, Department of Animal and Food Sciences, Newark, DE 19716, United States

Multistate outbreak of *Salmonella* Poona infections associated with imported cucumbers, 2015–2016

M. Laughlin[1], L. Bottichio[1], J. Weiss[2], J. Higa[4], E. McDonald[5], R. Sowadsky[7], D. Fejes[9], A. Saupe[10], G. Provo[12], S. Seelman[14], J. Concepción-Acevedo[1], L. Gieraltowski[1] and The Outbreak Investigation Team*

The legislative frame for the use of biosolids and irrigation waters in Europe…

- The **Directive 86/728/EEC regulating the use of biosolids in the EU**, to avoid harmful effects on soil, vegetation, animals and humans, has been **embedded by only a few Member States** and the implementation criteria lack harmonization (Switzerland prohibits agricultural reuse, France and the UK reused them for > 70%!).
- The recent **EU Regulation 2020/741 has implemented water-reuse practices** by establishing standards and requirements for reclaimed water agricultural use, but certain aspects have **not been comprehensively addressed**, especially in terms of **the microbiological criteria that need to be met** (identification of generic *Escherichia coli*, *Legionella* spp. and nematode eggs).
- Technical guidance by the Joint Research Centre (JRC) of the European Commission takes into account **STEC O157:H7 as the only reference *E. coli* pathotype.**
- The Commission Regulation (EU) 209/2013 prescribes the **absence of STEC of serogroups O157, O26, O103, O111, O145 and O104:H4 in 200 ml of spent irrigation water** used in the sprout production process

… and in Italy

- Although a new legislative decree is being worked on, the reference rule **in Italy** is still the Dlgs. 99/1992, which provides as **the only microbiological criterion** for compliance of biosolids the microbial load of ***Salmonella* spp**. (<103 MPN/g).
- **Sewage sludge management in Italy is regulated at the regional level**, with the only exception being the national legislation implementing the SSD on national level in Italy. This means that there is **a large variability between** the sewage sludge management practices in **the different Italian regions.**
- **As regards waters,** in Italy **the legislation in force is limited** to the following parameters:

Parametro	UdM	Classe I	Classe II	Classe III
Coliformi Totali	UFC/100 ml	< 5000	5000 - 12000	> 12000
Coliformi Fecali	UFC/100 ml	<1000	1000 - 12000	> 12000
Streptococchi Fecali	UFC/100 ml	<1000	1000 - 2000	> 2000

Source: Rapporto sulla qualità delle acque a uso irriguo 2022, ARPAE

Classe I: acque impiegabili senza limitazioni;

Classe II: acque da impiegare almeno 30 giorni prima della raccolta evitando il contatto con prodotti destinati ad essere consumati crudi dall'uomo;

Classe III: acque che devono essere preferibilmente distribuite con metodi che evitino il contatto con la vegetazione, Anche in questo caso gli interventi irrigui vanno sospesi almeno 30 giorni prima della raccolta.

Preliminary evidence

Evidence of microbiological hazards in soil improvers has recently been identified, requiring a more thorough assessment of the health risks associated with their use in agricultural environments.

Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Pathogenic *Escherichia coli* and enteric viruses in biosolids and related top soil improvers in Italy

R. Tozzoli, I. Di Bartolo, F. Gigliucci, G. Brambilla, M. Monini, E. Vignolo, A. Caprioli and S. Morabito

Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare, Istituto Superiore di Sanità, Rome, Italy

Environmental Research 155 (2017) 108–115

Contents lists available at ScienceDirect

Environmental Research

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

Comparative analysis of metagenomes of Italian top soil improvers

Federica Gigliucci[a,b,*], Gianfranco Brambilla[a], Rosangela Tozzoli[a], Valeria Michelacci[a], Stefano Morabito[a]

[a] Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità, Viale Regina Elena, 299 00161 Rome, Italy
[b] Department of Sciences, University Roma,Tre, Viale Marconi, 446, 00146 Rome, Italy

Aim:
Identification of microbiological hazards in re-used water and top-soil improvers

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ENVIRONMENT & HEALTH

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pubs.acs.org/EnvHealth

Perspective

Cross-Cutting Approach for the Characterization of Microbial Emerging Hazards in Agriculture Settings from Circular Economy-Driven Wastewater Streams

Giorgia Barbieri, Federica Gigliucci,* Gianfranco Brambilla, and Stefano Morabito

Lab workflow

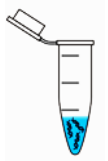
Convenience sampling scheme

- 31 top-soil improvers samples
- 15 irrigation water samples

Target genes:

-**STEC:** *stx1, stx2, stx2f*
-**EPEC:** *eae*
-**EAEC:** *aggr, aaiC*
-**ETEC:** *lt, stp, sth*
-**EIEC:** *ipaH*
-***Salmonella* spp.**: *invA*

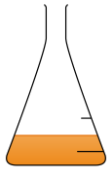
1



DNA extraction from raw samples

Real Time PCR screening

2



BPW
37°C
for 24h

DNA extraction from enriched samples

Real Time PCR screening

3

Sub-isolation on selective and differential media

- DNA extraction
- Real Time PCR screening
- Possible isolation in purity and genomic sequencing

Types of top-soil improvers and treatment

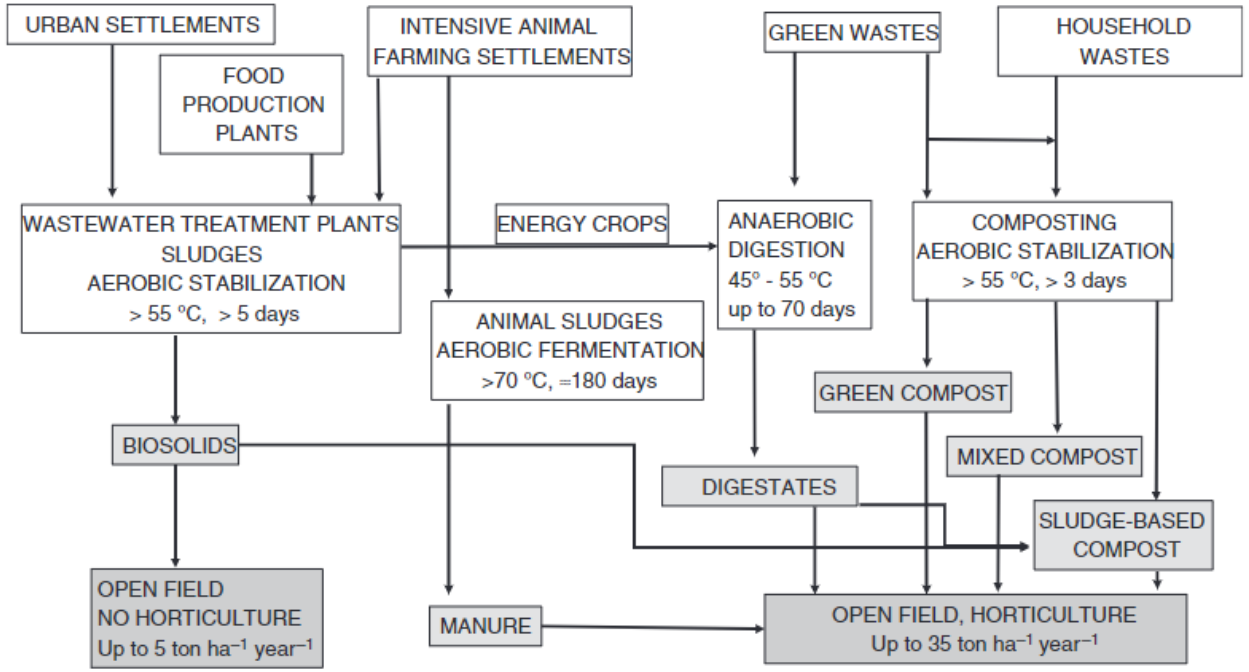
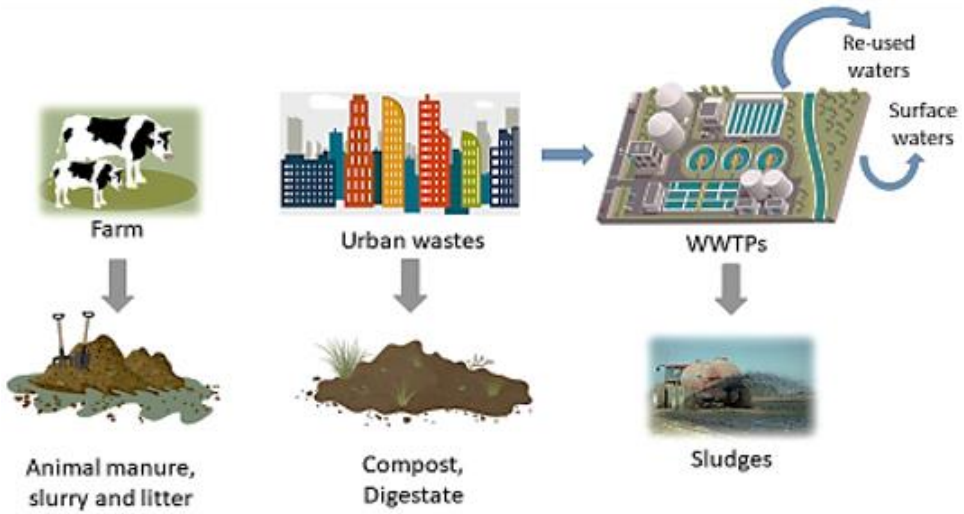


Figure 1 Schematic diagram of the production of biosolids, digestates and composts intended for agriculture soil dressing starting from different sources: urban, food industry and intensive animal farming sludges from wastewater treatment plants, green and household wastes.

Tozzoli R, Di Bartolo I, Gigliucci F, Brambilla G, Monini M, Vignolo E, Caprioli A, Morabito S. Pathogenic Escherichia coli and enteric viruses in biosolids and related top soil improvers in Italy. J Appl Microbiol. 2017 Jan;122(1):239-247. doi: 10.1111/jam.13308.

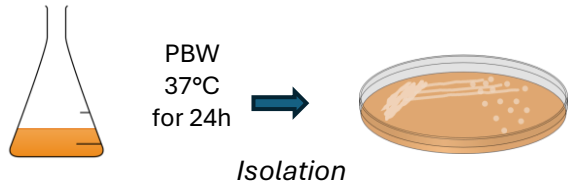
- **Sludges** from wastewater treatment plants (n=7) and from food plant (n=1) ;
- **Mixed compost** from urban organic and green wastes (n = 8);
- **Digestates**, accounting for different contributions from household, green and animal wastes (n=6);
- **Animal waste** (bovine manure, pig and bovine slurry and poultry litter) (n=9).



Results from top-soil improvers samples

Biosolids																							
Category	Samples	STEC						EPEC		EAEC				EIEC		ETEC						*Salmonella*	
		stx1		*stx2*		*stx2f*		*eae*		*aggR*		*aaiC*		*ipaH*		*lt*		*stp*		*sth*		*invA*	
		pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post
AW	Poultry litter	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AW	Pig slurry	-	-	+	+	-	-	-	+*	-	-	-	-	-	-	-	+	-	+*	-	-	-	-
AW	Pig slurry	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AW	Bovine manure	-	+	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-
AW	Bovine manure	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
AW	Manure+compost	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
AW	Organic farm manure	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
AW	Bovine slurry	-	+*	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
AW	Poultry litter	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
SL-WWTP	WWTP sludge	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SL-WWTP (DOPPIO)	WWTP sludge	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
SL-WWTP	WWTP sludge	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SL-WWTP	WWTP sludge	+	+	+	+	-	-	+	+	-	+	+	+	-	-	+	+	+	-	+	-	-	-
SL-WWTP	WWTP sludge	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SL-WWTP	WWTP sludge	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SL-WWTP	WWTP sludge	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MCO	Compost	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MCO	Compost	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MCO	Compost	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
MCO	Compost	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
MCO	Compost	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
MCO	Compost	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-
MCO	Compost	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
MCO	Compost	+	-	+	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
DIG	Digestate	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DIG	Digestate	-	-	+	+	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
DIG	Digestate	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
DIG	Digestate	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-
DIG	Digestate	-	-	+	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
DIG	Digestate	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
SL-FP	Food plant sludge	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1. Detection of virulence genes associated with the different *E. coli* pathogroups and *Salmonella* spp. from DNA extracted directly from biosolids samples (pre-enrichment) and after enrichment in non-selective medium (post-enrichment).
Green boxes = no signal; red boxes= positive signal; **+*** = isolated strain.



- **Hybrid strain STEC-ETEC** (*stx1a*+, *sta*+) from bovine slurry
- **ETEC** (*stp*+) ed **EPEC** (*eae*+) from pig slurry
- All possessing AMR genes

Water sampling points and sources

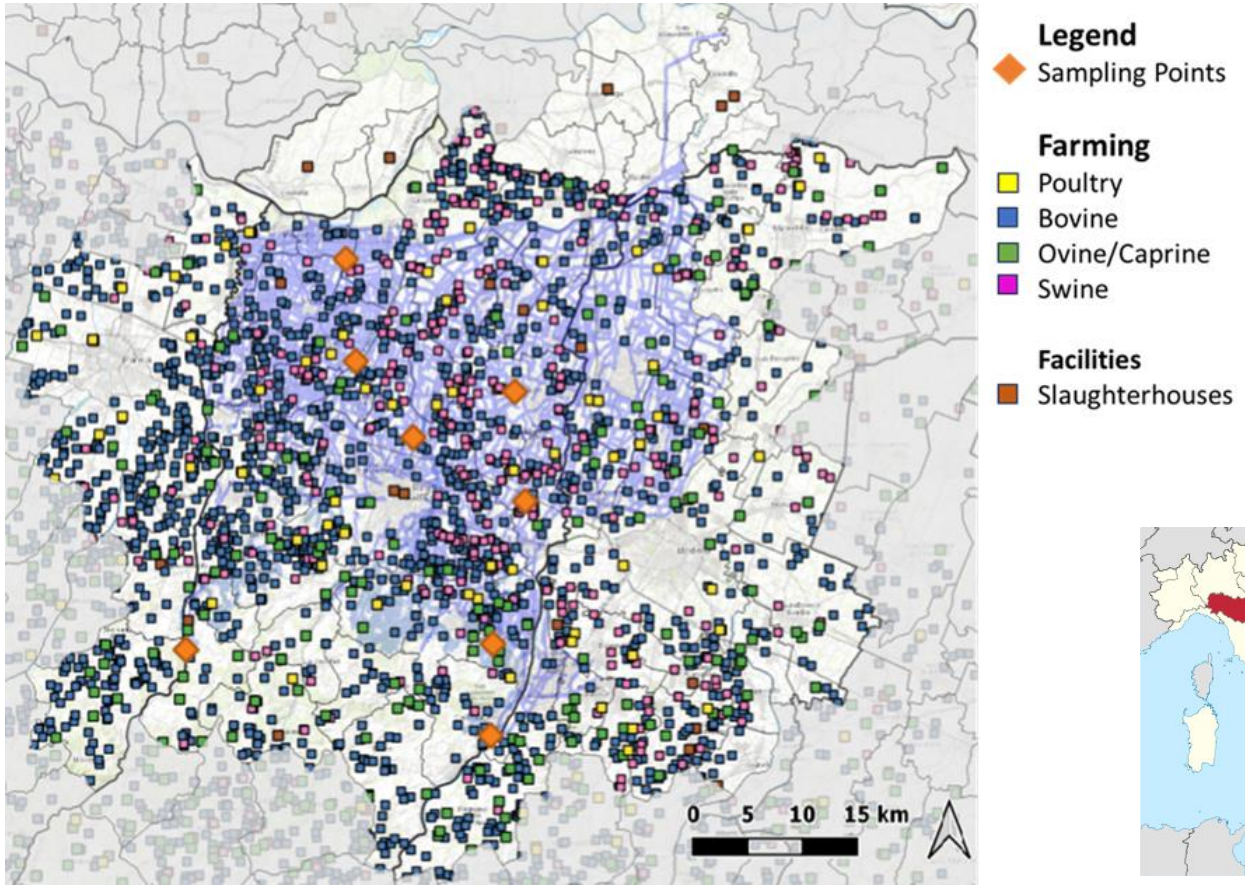
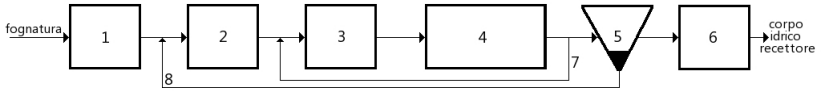


Figure 2. Sampling points of surface and reclaimed water in the river Po water body, in continuity with farming and abattoir's activities. Modified figure supplied by Servizio Epidemiologico of IZS Lombardia and Emilia Romagna.

Emilia Romagna region

Reused IN → Water entering the plant
Reused OUT→ Refined water from the treatment plant
Surface waters→ Irrigation water from canals and rivers



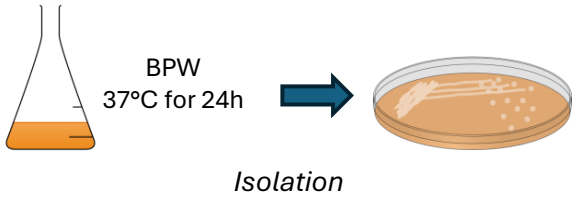
IMPIANTO PER IL TRATTAMENTO DELLE ACQUE REFLUE
1: filtrazione, disoleazione, desabbiatura (trattamento primario)
2: vasca anossica di rilascio del fosforo
3: vasca anossica di denitrificazione
4: vasca di aerazione
5: sedimentatore
6: disinfezione e abbattimento inquinanti particolari
7: ricircolo miscela aerata nitrificata
8: liquame e fango di ricircolo

Sampling Time (2022)	Cumulative Precipitation (mm/month)
May	42
June	19
August	138,4

Results from irrigation water samples

Irrigation water																							
Sampling Time	Sampling points	STEC						EPEC		EAEC				EHEC		ETEC						*Salmonella*	
		stx1		*stx2*		*stx2f*		*eae*		*aggR*		*aaiC*		*ipaH*		*lt*		*stp*		*sth*		*invA*	
		pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post
First - May 2022	Reused IN	-	+	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-
	Reused OUT	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
	River	-	-	+	+	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	+
	Canal	-	-	-	-	-	-	+	+	-	+	+	+	-	-	-	-	-	+	-	-	-	+*
	River	-	+*	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+*
	Canal	-	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Second - June 2022	Reused IN	-	-	-	+	-	+	-	-	-	+	-	+	-	+	-	+	-	-	-	-	-	-
	Reused OUT	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Canal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	River	-	+	-	-	-	+	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-
	Canal	-	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Third - August 2022	Reused IN	-	+	-	+	-	+	-	+	-	+	+	+	-	+	-	+	-	+	-	+	-	+*
	Reused OUT	-	-	-	-	-	-	+	+	+	+	-	+	-	-	+	+	-	+	-	-	-	-
	River	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	River	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2. Detection of virulence genes associated with the different *E. coli* pathogroups and *Salmonella* spp. from DNA extracted directly from irrigation water samples (pre-enrichment) and after enrichment in non-selective medium (post-enrichment).
Light blue boxes = no signal; dark blue boxes= positive signal; **+*** = isolated strain.

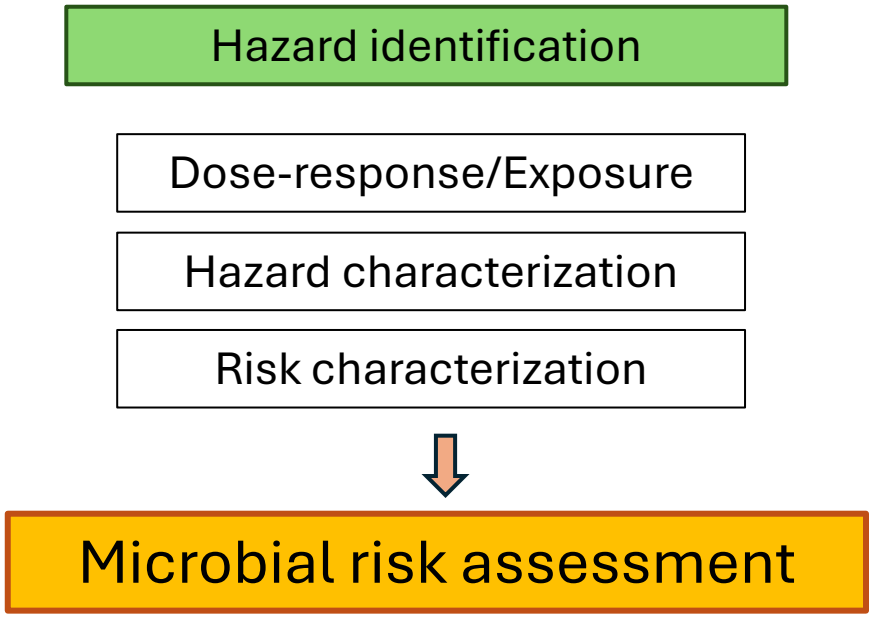


- **STEC** (*stx1*d+) with AMR genes (*bla*(AMP) cassette) from a river sample
- ***Salmonella* Veneziana** from the same river sample and from a canal
- ***Salmonella* Taksony** from a sample of water entering the WWTP

Take home messages

- **Viable pathogenic bacteria** potentially able to cause disease in humans
- Viable pathogenic *E. coli*, including STEC, and *Salmonella* spp. in samples of irrigation waters and TSI **collected in the last step of their production process**, immediately before their application on cultivated soils
- Stabilization **treatments may not be completely effective** in abating the microbiological hazards
- Risk that these **hazards may be introduced into the agro-industry** and expose consumers to infections

Workflow in the risk assessment



Circular economy and agro-industrial chain: problem or chance?

The use of purified water and soil improvers from circular economy circuits in agriculture is a practice with high added value.

It is necessary:

1. Estimate the problem of spread of these hazards directly in the field with **monitoring sampling**
2. Verify the **transfer rate of pathogenic and antibiotic-resistant microorganisms to crops** to combine information from consumption data and estimate exposure
3. Develop **treatment practices for soil improvers and reclaimed water** to be applied before use in the field to mitigate health impacts while ensuring food availability from the agro-industrial chain

Acknowledgments

Thanks for your attention!

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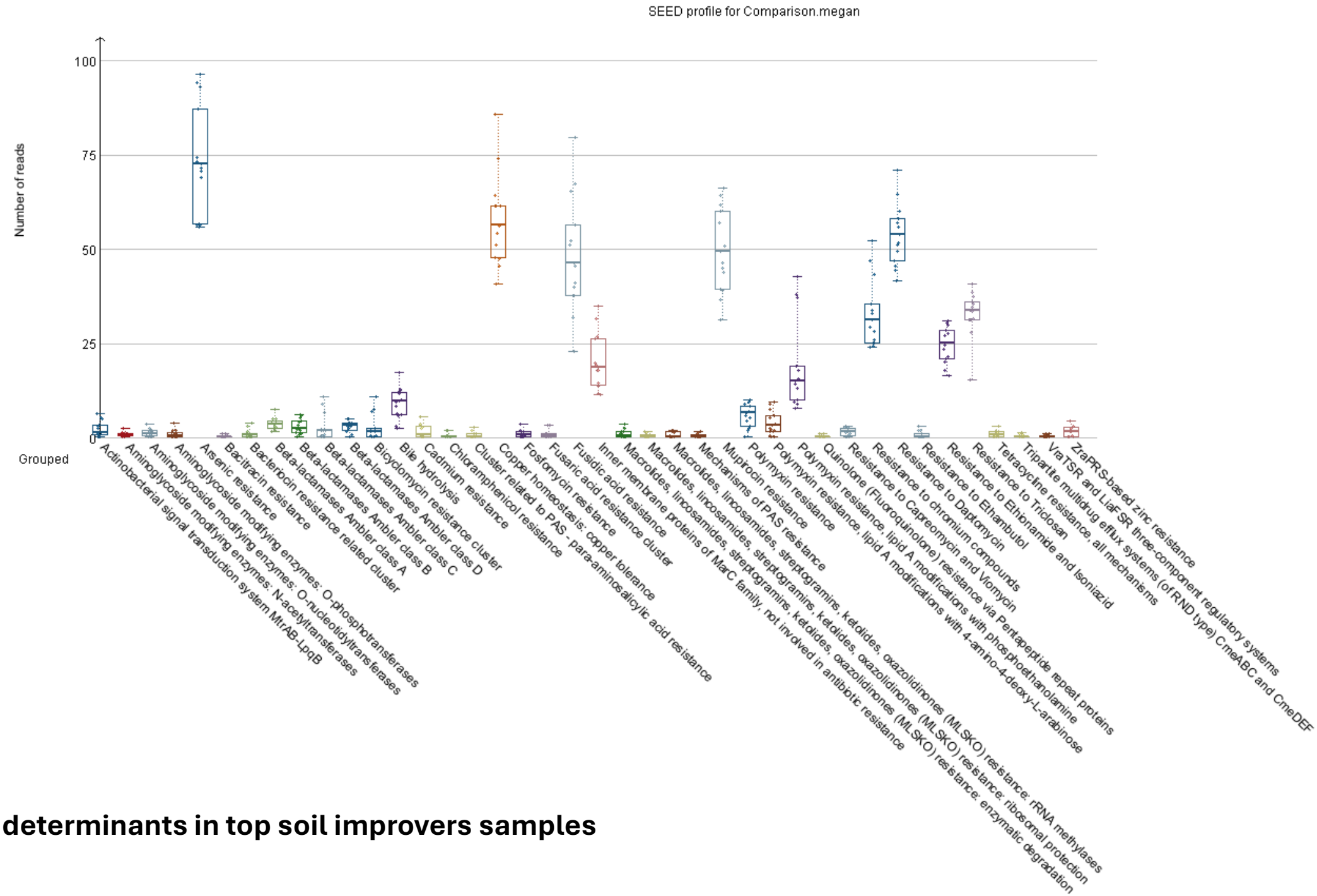
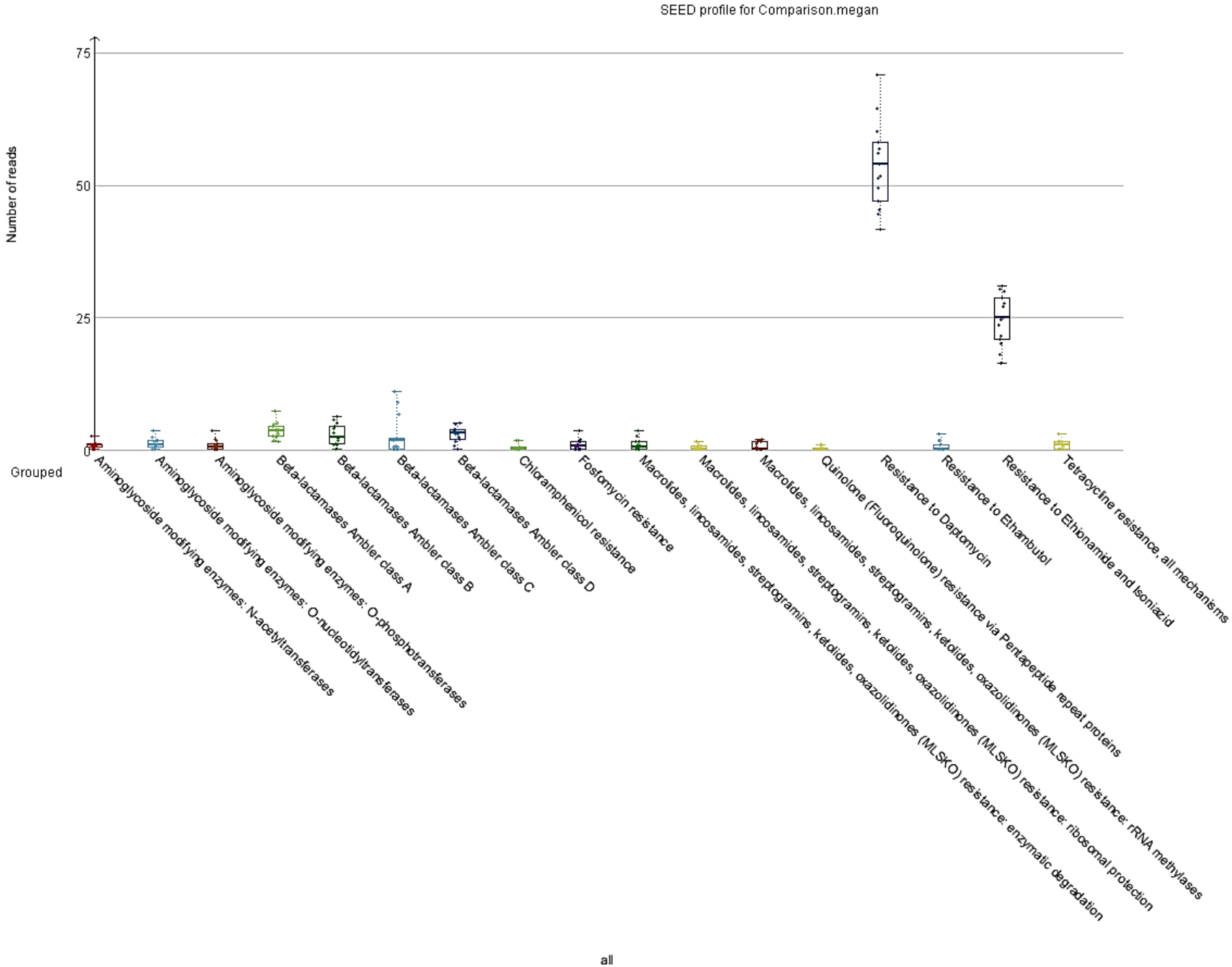
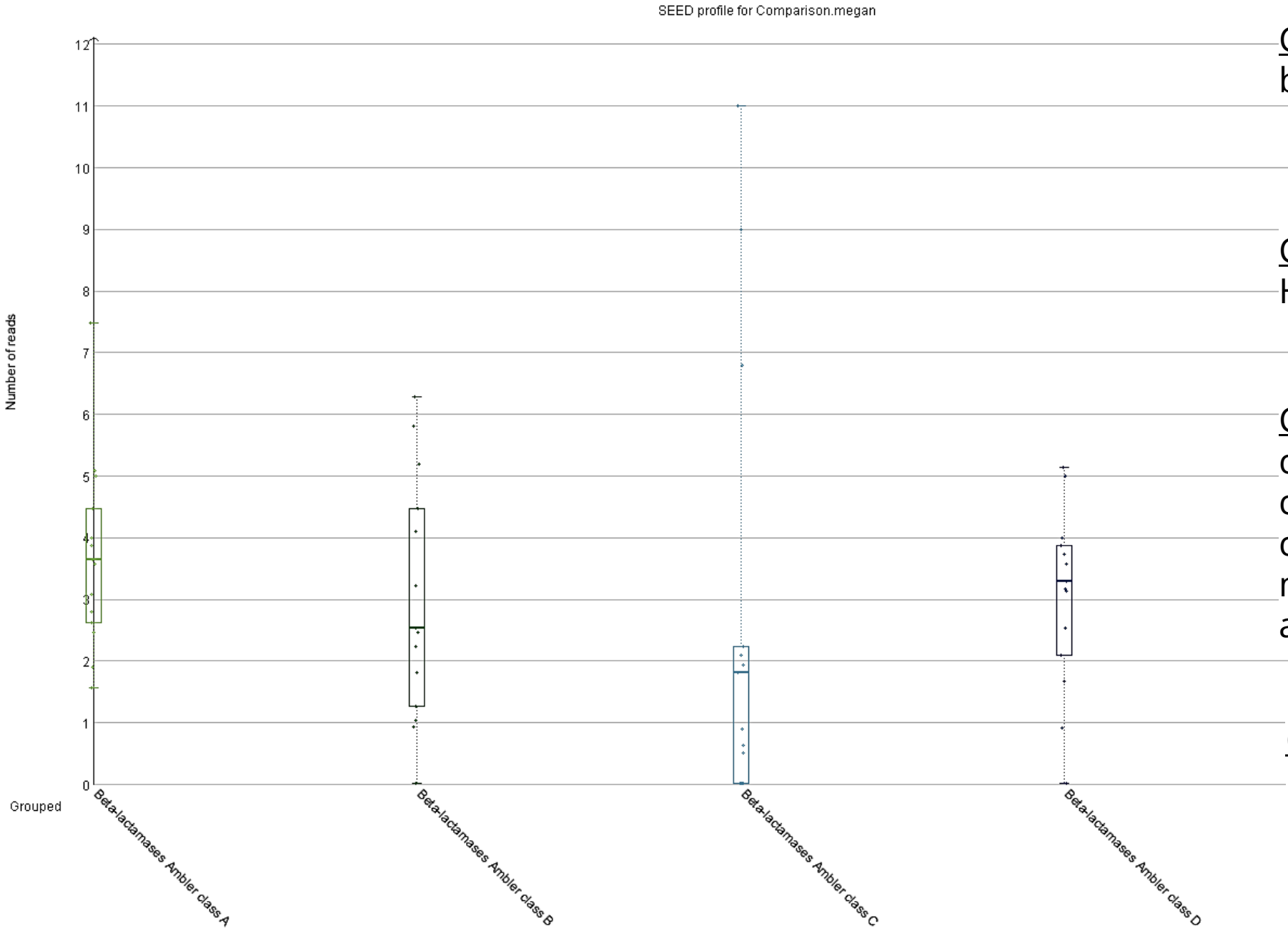
Spread of genetic determinants of antimicrobial resistance (AMR) in biosolids and the environment



AMR determinants in top soil improvers samples



Clinically relevant AMR determinants

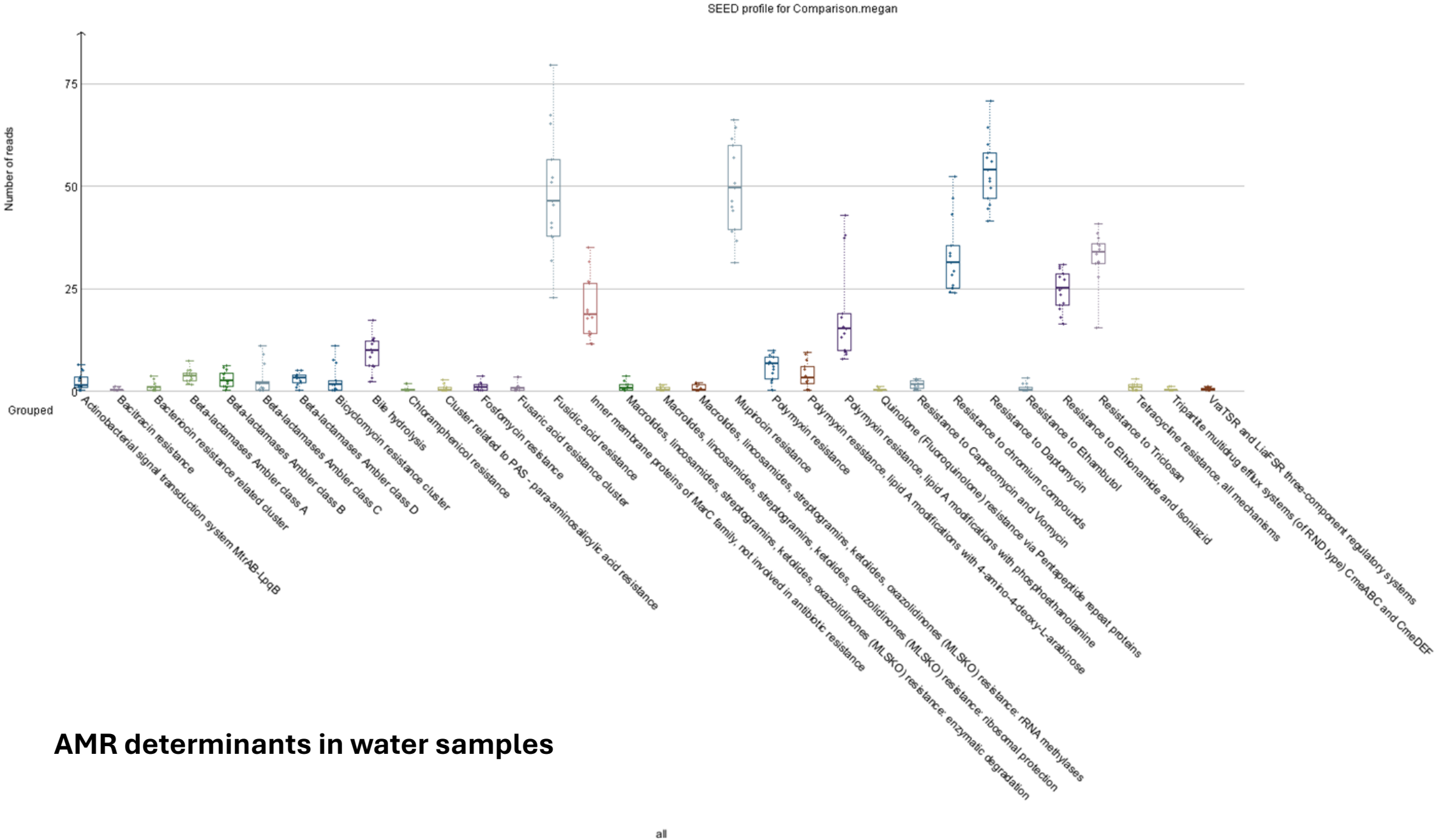


Class A: Extended-spectrum beta-lactamases, ESBLs.

Class B: metal-beta-lactamase. Hydrolyse carbapenems.

Class C: hydrolysis of most cephalosporins (except cefepime), cefamycin (e.g. cefocitin, cefotetan), monobattam (e.g. aztreonam) and penicillins.

Class D: hydrolysis of mainly narrow-spectrum penicillins.



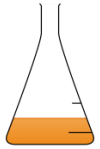
AMR determinants in water samples

Results of isolation of AMR+ strains in TSI

Sample	Daptomicin	Species	Source	Use
2747_D1	2ug/ml	*E. fecium*	Poultry litter	**Horticulture**
2747_D2	2ug/ml	*E. fecium*	Poultry litter	**Horticulture**
2747_D3	2ug/ml	*E. fecium*	Poultry litter	**Horticulture**
2746_D1	4ug/ml	Sequencing	Mixed Compost	**Horticulture**
ISS2_D1	8ug/ml - 4ug /ml	*Bacillus licheniformis*	Manure + compost	**Horticulture, on market**

Sample	Mupirocin	Species	Source	Use
2751_M1	64 ug/ml	*Bacillus*	Food plant sludge	**Open field**
2751_M2	512 ug/ml	*Clostridium amylolyticum*	Food plant sludge	**Open field**

Sample	Bacitracin	Species	Source	Use
ISS_3_B1	256 ug/ml	Sequencing	Mixed compost	**Horticulture, on market**
ISS_6_B1	Sequencing	Sequencing	Mixed compost	**Mixed uses, on market**



Enrichment
in PWB
37°C for 24h

Isolation on selective and differential media in presence of antibiotics (Reference MIC)

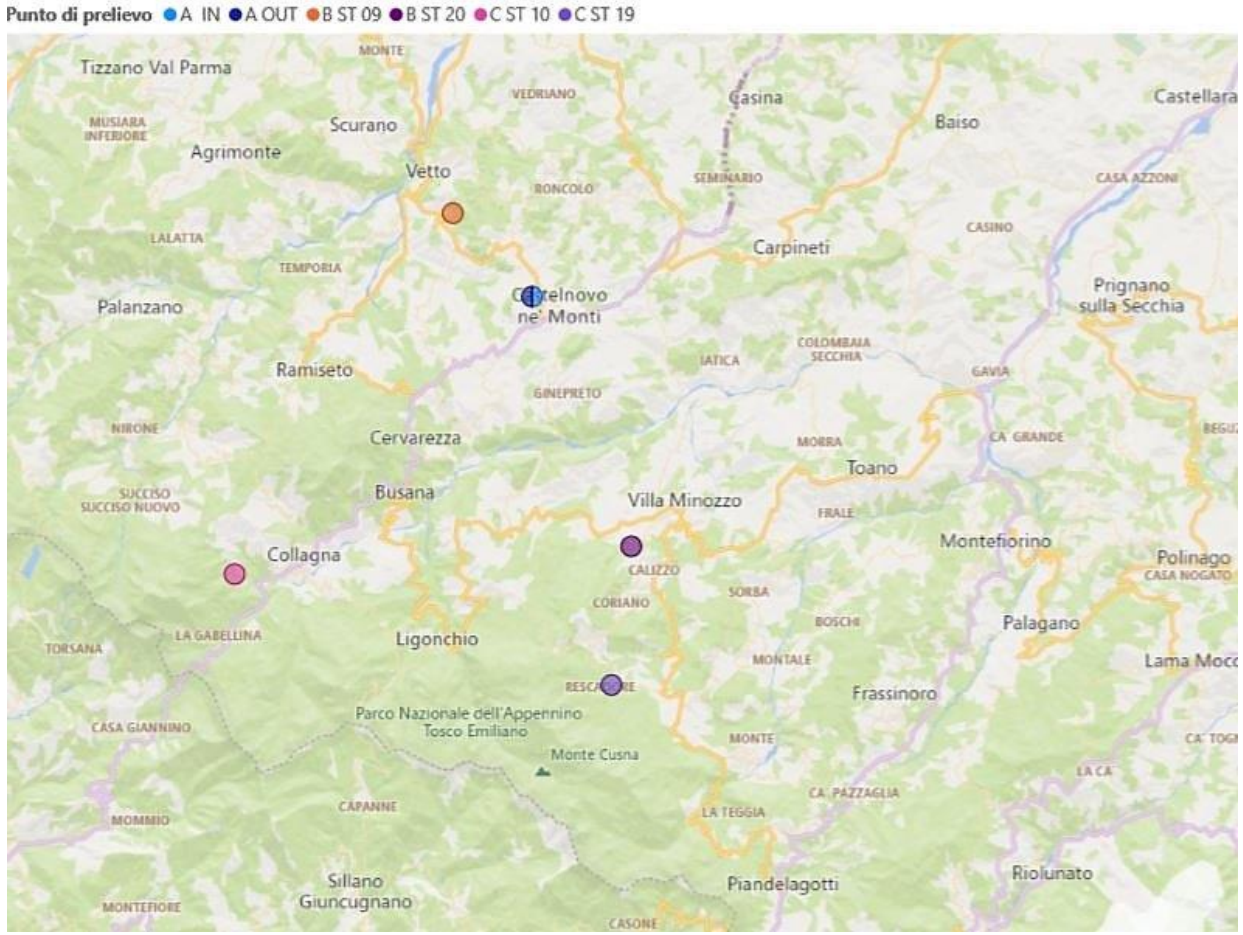
Sample	Fosfomicin	Species	Source	Use
2755_F1_G+	128 ng/ul	*Lysinibacillus boronitolerans* (93%)	WWTP sludge	**Open field**
2755_F2_G+	16 ng/ul	*Lysinibacillus capsici* (90%)	WWTP sludge	**Open field**
2755_F3_G-	128 ng/ul	*Lysinibacillus boronitolerans*	WWTP sludge	**Open field**
2755_F4_G-	16 ng/ul	*Paenibacillus azoreducens* (86%)	WWTP sludge	**Open field**

Sample	Tetraciclin	Species	Source	Use
2746_T1	12,5 ug/ml	*B. paranthracis*	Mixed Compost	**Horticulture**
2746_T2	25 ug/ml	Sequencing	Mixed Compost	**Horticulture**
2747_T1	12,5 ug/ml	Sequencing	Poultry litter	**Horticulture**
2755_T1	100 ug/ml	*Bacillus cereus*	WWTP sludge	**Open field**
ISS_2_T1	50 ug/ml	*Paenibacillus*	Manure + compost	**Horticulture, on market**

Co-occurrence of AMR determinance and heavy metal resistances

Take home messages

- Irrigation waters and top-soil improvers carries viable pathogenic and antibiotic-resistant microorganisms
- ARG related to molecules use in human medicine clinical practice are transferred to resident microorganisms, configuring the environmental microbiota as a reservoir of ARGs
- Selective pressure from heavy metal pollution of soils can act as a stabilizer for ARG’s reservoir



A IN → Water entering the plant
A OUT→ Refined water from the treatment plant
B→ Irrigation water from canals
C→ Irrigation water from rivers

WWTP specifications	
Area occupied by the plant	~ 300 thousand m²
Population equivalent	169.846
Sewerage system served	Unitary
Average daily flow rate	52.919 mc/day
Average hourly capacity	2.205 mc/h
Type of treatment	Tertiary treatment based upon multilayer rapid filtration and combined treatment with hydrogen peroxide and UV rays
Full course of treatment	24h
Water transit speed for treatment	12 m/h
Addition of H_2O_2	3 mg/L; contact time = 7 min
Equivalent UV dose	45 mJ/cm²