Earthworm activity reduces bacterial pathogen loads in sewage sludge

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Introduction

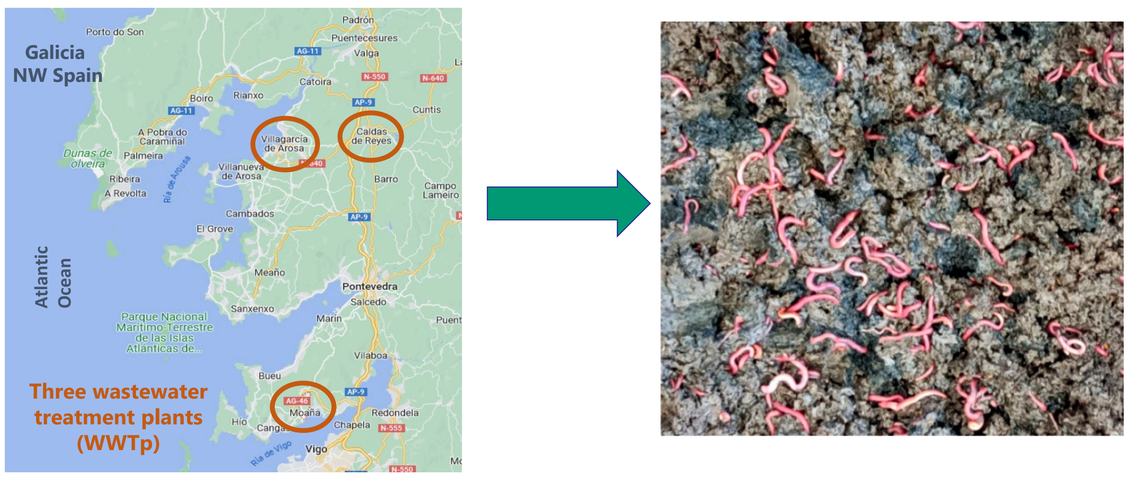
Generation of sewage sludge has increased greatly in recent years. The sludge is generally disposed of by application to soils; however, it often contains human bacterial pathogens, such as *Salmonella* spp., *Listeria monocytogenes* and pathogenic strains of *Escherichia coli*, which may represent a threat to human health.

Vermicomposting is a process in which earthworms and microorganisms enhance the decomposition of organic waste. Vermicomposting is known to reduce human pathogen loads in organic waste, despite being a mesophilic process, and in contrast to the sanitization promoted by thermophilic composting. This raises the question of how earthworms eliminate microbial pathogens.

We aimed to determine whether earthworms can reduce or eliminate the pathogenic bacterial load of sewage sludge, and whether this effect occurs during the active (cast) or maturation (vermicompost) stage of vermicomposting. We used qPCR to determine the presence of several pathogenic bacteria (*Escherichia coli* O157, *Listeria monocytogenes Salmonella* spp. and total *E. coli*) in three sewage sludges and the corresponding fresh casts and vermicompost, in order to assess the impact of earthworms on pathogen reduction and/or elimination.

Material and methods

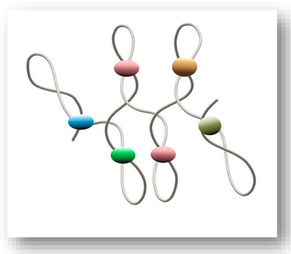
We set up **vermireactors** with sewage sludges from three WWTps:



During vermicomposting, we sampled the **resident earthworms**, to analyze their intestinal contents (**fresh casts**) and the **vermicompost**

Bacterial and pathogen loads evaluation:

DNA was extracted from *sewage sludge*, *earthworm casts* and *vermicompost*



The qPCR reaction was performed and all **five genes** were detected in *simplex assays*

	Target gene	Primer	Sequence 5′ → 3′	Reference
Total bacteria	16S	1055f	ATG GCT GTC GTC AGC T	(Ferris et al., 1996)
		1392r	ACG GGC GGT GTG TAC	
Escherichia coli	*ybbW*	401 F	TGA TTG GCA AAA TCT GGC CG	(Walker et al., 2017)
		611 R	GAA ATC GCC CAA ATC GCC AT	
E. coli O157	*rfbE*	rfbE-F	TCA ACA GTC TTG TAC AAG TCC AC	(Garrido-Maestu et al., 2020)
		rfbE-R	ACT GGC CTT GTT TCG ATG AG	
Listeria monocytogenes	*actA*	actA F	TTA AGA CTT GCT TTG CCA GAG AC	(Azinheiro, Kant, et al., 2020)
		actA R	GGT GGT GGA AAT TCG AAT GAG C	
Salmonella spp.	*fimA*	fimA F	CAC TAA ATC CGC CGA TCA AAC G	(Azinheiro, Carvalho, et al., 2020)
		fimA R	TTC AGG ACG ATG GAG AAA GGC	

Conclusions

Earthworms eliminated or significantly reduced the pathogenic bacterial loads in the sewage sludges. The removal of pathogens, in this case *E. coli* and *L. monocytogenes*, seems to be a consequence of the transit through the earthworm gut. Elimination of bacterial pathogens did not depend on the pathogen load in the sewage sludge.

Results

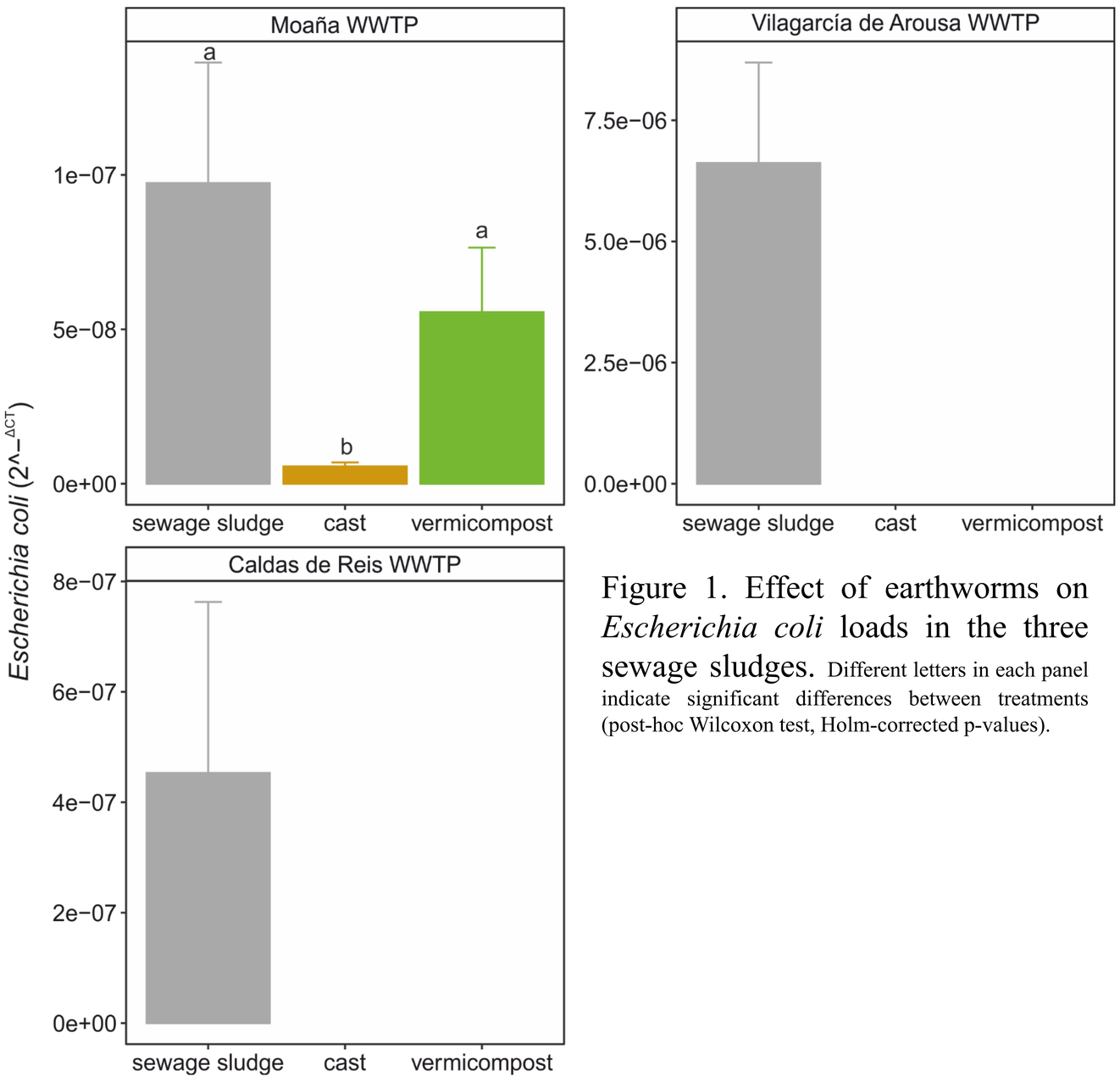


Figure 1. Effect of earthworms on *Escherichia coli* loads in the three sewage sludges. Different letters in each panel indicate significant differences between treatments (post-hoc Wilcoxon test, Holm-corrected p-values).

- We did not detect the pathogenic strain *E. coli* O157.
- Earthworms significantly reduced the *E. coli* load in all sewage sludges, although the effect was variable. Earthworms completely removed *E. coli* from the sewage sludge from Caldas and Vilagarcía WWTPs.
- We only detected the presence of *L. monocytogenes* in one WWTP, and earthworms eliminated it from the sewage sludge.
- We did not detect *Salmonella* spp. in any of the sewage sludges.

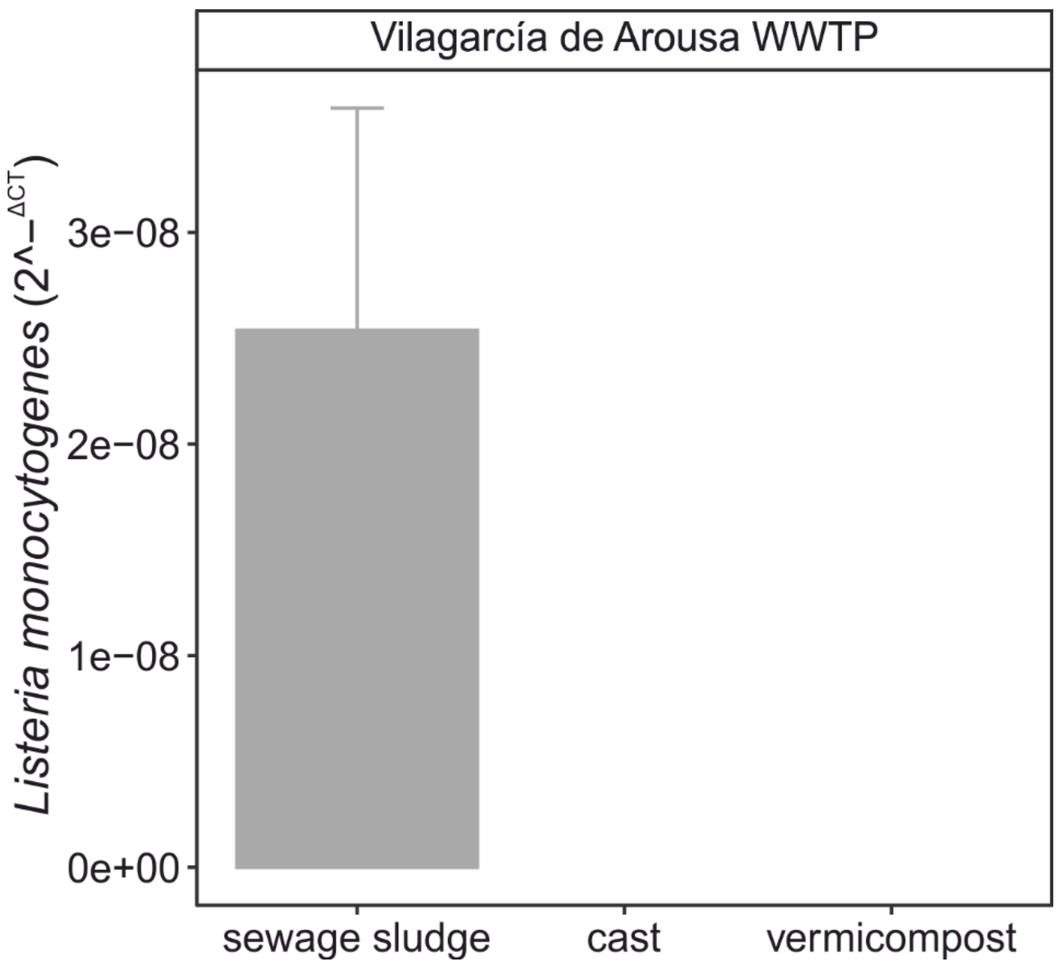


Figure 2. Effect of earthworms on *Listeria monocytogenes* load in the sewage sludge from the Vilagarcía de Arousa WWTP

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