Earthworm activity reduces bacterial pathogen loads in sewage sludge

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Sewage sludge (sludge) generation is one of the current concerns in environmental policies due to the stepped increase in their production. Large amounts of sludge are simply disposed in soils, being only a minor fraction treated. The main problem with soil disposal of sludge is that usually carry a plethora of human pathogens from bacteria (*Escherichia coli*, *Salmonella* spp. or *Listeria monocytogenes*) to helminths (*Taenia saginata* or *Ascaris lumbricoides*), pathogens that quickly will contaminate soil and aquifers after rainfall events (European Communities, 2001).

Giving these concerns, current environmental policies of European Union regarding waste management (Council Directive 199/31/EC of 26 April 1999) encourage waste recycling over waste disposal on soils. Moreover, wastes and their recycled products must be free of contaminants, including pathogens, whenever they will be used in agriculture. Thus, to remove human pathogens from sludge, European Commission recommends heating the sludge at 55°C for 2 hours (at pH above 12) or 4 hours (thermophilic or composting processes) in its report Evaluation of sludge treatments for pathogen reduction (European Communities, 2001). In this way, the Commission Decision 2006/799/EC of 3 November 2006 and the Council Directive 2007/64/EC of 13 November 2007 stablish the hygienisation limits of absence of *Salmonella* spp. in 25 g of sample and *E. coli* abundance lesser than 1000 MPN g^{-1} to consider safe a waste or its recycled by-product.

Vermicomposting is a biooxidative process in which the joint action of detritivorous earthworms and microorganisms enhance the decomposition process, largely modifying the physical, chemical, and biological properties of organic wastes (Domínguez, 2004). Vermicomposting has two phases, an active one in which earthworms digest and egest the material (GAPs, gut-associated processes, Domínguez et al., 2010). In this phase, there is a marked modification of microbial communities of ingested materials in terms of biomass, diversity, and structure (Aira et al., 2022; Domínguez et al., 2021). The second phase is a maturation process, which starts when earthworms leave the substrate (Domínguez et al., 2010). This phase consists in the ageing process of the earthworm casts (Aira et al., 2019, i.e., cast-associated processes, CAPs) with its associated microbial succession (Kolbe et al., 2019, Domínguez et al., 2019). CAPs are strong enough to modify microbial community dynamics (i.e., microbial biomass and activity) in a similar way than earthworms were processing actively the substrate (Aira and Domínguez, 2011).

Opposed to traditional composting, which is a thermophilic process that eliminates pathogens with temperature, vermicomposting process also reduces the number of human pathogens despite being a mesophilic process (Eastman et al., 2001; Craig and Ankers, 2006, Aira et al., 2011). This raises the question of how earthworms may eliminate microbial pathogens. Recent studies have clearly shown that earthworm cast are mostly populated (>90%) with bacteria from earthworm origin, that is, bacteria that are not found in the uningested material (Domínguez et al., 2021; Aira et al. 2022). Thus, it is highly probable that any bacterial pathogen present should be removed after earthworm digestion. In this way, transit through the earthworm gut drastically reduced coliform counts (as much as 85%, Monroy et al., 2008). Since GAPs are dense-dependent processes (Aira et al., 2008), there should be a trade-off between waste dosage and earthworm density on pathogen elimination. According to our rationale vermicomposting also reduced coliform counts, but this effect depended on earthworm density (Monroy et al., 2009).

In this work we aimed to study whether earthworms can reduce or remove the bacterial pathogenic load of sludges, and whether this effect occurs during the active (GAPs) or maturation phase (CAPs) of vermicomposting. To do this, we analysed several bacterial pathogens (*Escherichia coli* O157, *E. coli*, *Listeria monocytogenes* and *Salmonella* spp.) of three sewage sludges and their corresponding fresh casts (GAPs) and vermicompost (CAPs) using qPCR to assess the impact of earthworms on the pathogen reduction and/or elimination.

References

Aira M, Domínguez J (2011) Earthworm effects without earthworms: inoculation of raw organic matter with worm-worked substrates alters microbial community functioning. PloS One 6(1): e16354.

Aira M, Sampedro L, Monroy L, Domínguez J (2008) Detritivorous earthworms directly modify the structure, thus altering the functioning of a microdecomposer food web. Soil Biology and Biochemistry 40: 2511-2516.

Aira M, Gómez-Brandón M, González-Porto P, Domínguez J (2011) Selective reduction of the pathogenic load of cow manure in an industrial-scale continuous-feeding vermireactor. Bioresource Technology 102: 9633-9637.
Aira M, Bybee S, Pérez-Losada M, Domínguez, J (2015) Feeding on microbiomes: effects of detritivory on the taxonomic and phylogenetic bacterial composition of animal manures. FEMS Microbiology Ecology 91(11):fiv117.
Aira M, Pérez-Losada M, Domínguez J (2019) Microbiome dynamics during cast ageing in the earthworm *Aporrectodea caliginosa*. Applied Soil Ecology 139: 56–63.
Aira M, Pérez-Losada M, Crandall KA, Domínguez J (2022) Host taxonomy determines the composition, structure, and diversity of the earthworm gut microbiome under homogenous feeding conditions. FEMS Microbiology Ecology 98(9):fiac093.
Craig L, Ankers S. (2006) Vermiculture produces EQ class A biosolids at wastewater plant. BioCycle 47:42–6.
Domínguez, J. 2004. State of the art and new perspectives on vermicomposting research. In Earthworm Ecology (ed. Edwards, C. A.) 401–424 (CRC Press).
Domínguez J, Aira M, Crandall KA, Pérez-Losada M (2021) Earthworms drastically change fungal and bacterial communities during vermicomposting of sewage sludge. Scientific Reports 11:15556.
Eastman BR, Kane PN, Edwards CA, Trytek L, Gunadi B, Stermer AL, Mobley JR (2001) The effectiveness of vermiculture in human pathogen reduction for USEPA biosolids stabilization. Compost Science and Utilization 9:38–49.
Evaluation of sludge treatments for pathogen reduction – final report. 2001. European Commission. Luxembourg: Office for Official Publications of the European Communities, 2001. ISBN 92-894-1734-X.
Monroy F, Aira M, Domínguez J (2008) Changes in density of nematodes, protozoa and total coliforms after transit through the gut of four epigeic earthworms (Oligochaeta). Applied Soil Ecology 39: 127-132.
Monroy F, Aira M, Domínguez J (2009) Reduction of total coliform numbers during vermicomposting is caused by short-term direct effects of earthworms on microorganisms and depends on the dose of application of pig slurry. Science of the Total Environment 407: 5411-5416.

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