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Contamination of organic raw materials and recycled organic fertilizers with antibiotics

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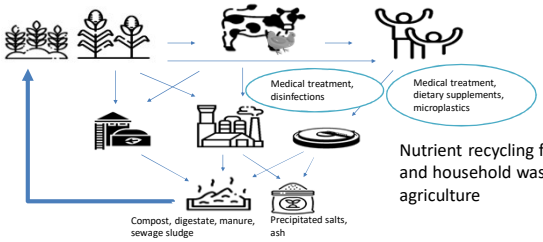
Background

Rising energy prices, resource scarcity and other factors have recently led to a significant increase in mineral fertilizer prices. In 2015 the EU has drawn up an action plan to transform the economy in a sustainable way. And numerous projects have been launched to produce fertilizer from various types of nutrient rich side streams in order to secure sufficient food supplies for the population.

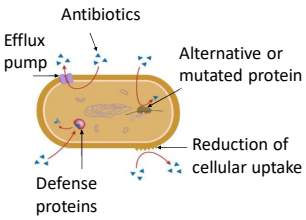


Nutrient recycling from animal and household wastes to agriculture

Problem

Due to their widespread use in the treatment of bacterial infections in humans and animals antibiotics are commonly found in wastewater and wastes from animal husbandry and the meat industries. This leads to the emergence and spread of antibiotic resistant bacteria which is of growing concern worldwide. Different forms of resistance can emerge and especially when bacteria carry genes for different mechanisms, multi-resistant bacteria can develop causing serious problems in hospitals.

Possible types of antibiotic resistance:
Bacteria have developed different strategies to escape the effects of antibiotics. The information for this is often found on plasmids, small circular DNA molecules, or other mobile genetic elements that can be exchanged between bacteria.

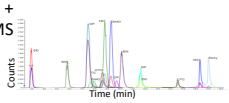


Methods

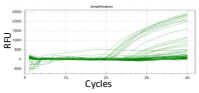
Analysis of 14 antibiotics out of 3 classes in organic fertilizers + and 3 resistance genes and a class 1 integron in fertilizers and soils

1. Drying and grinding
2. Extraction with citric acid buffer + acidified methanol
3. Extraction of lipids
4. Solid phase extraction
5. Identification + quantification with LC-MS

Typical LC-MS chromatogram



1. Releasing nucleic acids (DNA)
2. Separation of DNA from cell debris
3. Clean-up with a silica matrix
4. Desalting with ethanol
5. Quantification with real-time PCR (qPCR)



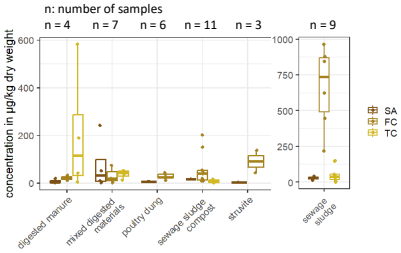
Typical quantitative qPCR amplification curves

Origin of samples

Various fertilizers from local producers in Lower Saxony were analyzed for antibiotics. A field trial with corn was fertilized with seven organic fertilizers according to their nutrient content with four repetitions. One day after fertilization, 7 days, 6 weeks and 5 months later, topsoil (0-15cm, silty loam) was sampled and examined for resistance genes together with the fertilizers.

Results

Antibiotic contents in 40 organic fertilizers (left) and in fertilizers used in the field trial (right): Sum of 3 sulfonamides (SA), 6 tetracyclines (TC) and 5 fluoroquinolones (FC)



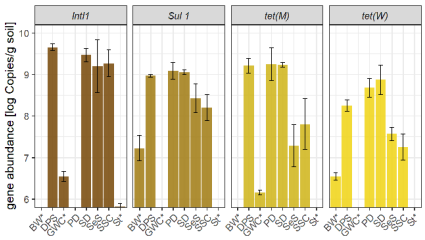
Amount of antibiotics in various organic fertilizers in µg/kg dry weight (DW)

Organic fertilizer	acronym	∑ SA	∑ FC	∑ TC
		µg/kg dry weight		
Biowaste*	BW*	-	-	-
Digestated pig slurry	DPS	-	<LOQ	3251
Green waste compost*	GWC*	-	-	-
Poultry dung	PD	6	25	<LOQ
Sewage sludge	SeS	<LOQ	447	108
Separated digestate	SD	<LOQ	<LOQ	455
Sewage sludge compost	SSC	-	9	27
Straw*	St*	na	na	na

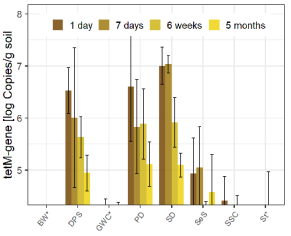
<LOQ: under the limit of quantification
- not detected, na: not analyzed

Antibiotic contamination of fertilizers used in the field trial (sum of sulfonamides, fluoroquinolones and tetracyclines)

Analysis of genes in eight fertilizers (left) and the gene *tet(M)* against tetracycline in soil at four times after fertilizer application (right)
*Fertilizers that do not originate from animals or sewage sludge



Abundance of class 1 integron (*intI1*) and resistance genes (*sul1, tet(M), tet(W)*) in fertilizers used in the field trial



Resistance gene *tet(M)* in top soil (0-15cm) at different times after applying organic fertilizer

Only a small selection of commonly used antibiotics in human and veterinary treatment is shown, but the results demonstrate that antibiotics are present in all fertilizers derived from animals or humans. Treatment also has a major influence. Composting of sewage sludge significantly reduced the antibiotic load and even less residues are found in struvite ($MgNH_4PO_4 \times 6H_2O$), a salt that can be precipitated during wastewater treatment.

Resistance genes can be found at least in low abundances in nearly all fertilizers. But while no antibiotics could be detected in the soil of the field experiment, increased abundances of resistance genes as a result of fertilizing the soils with manures of animal origin or sewage sludge were observed. In the further course of the year, the amount decreased significantly.

Conclusion

The experiments show that fresh fertilizers are contaminated with antibiotics and contain resistance genes. No antibiotics could be detected in the soil prior to fertilizer application and resistance genes decreased significantly over the season. The contribution of fertilizer application in agriculture to the occurrence of multi-resistant bacteria and the fate of resistance genes or mobile genetic elements in soil over time will be further investigated. In the ongoing project, other novel fertilizers from nutrient-rich waste streams such as meat and bone meal will be investigated and the impact of antibiotics on resistance levels will be considered.