European Commission

Science for Environment Policy

Antibiotic resistance in struvite fertiliser from waste water could enter the food chain

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The application to crops of struvite (magnesium ammonium phosphate) recovered from waste water may cause antibiotic resistance genes (ARGs) present in this fertiliser to enter the food chain. Chinese researchers who conducted this study on *Brassica* plants suggest that ARGs in struvite pass from the soil into the roots of the plant, and from the roots to the leaves, via the bacterial community already present. The results of this research highlight the need for struvite production methods and agricultural practices that minimise the risk of antibiotic-resistance transmission from struvite to humans or animals via the environment.

Source: Chen, Q., An, X., Zhu, Y., Su, J., Gillings, M., Ye, Z. and Cui, L. (2017). Application of Struvite Alters the Antibiotic Resistome in Soil, Rhizosphere, and Phyllosphere. *Environmental Science & Technology*, 51(14): 8149-8157. DOI: 10.1021/acs.est.7b01420

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Phosphorous is a limited resource on Earth and is needed for the growth of plants — recovering it from waste water, however, helps to solve the shortage problem, and can reduce eutrophication of surface water resulting from waste-water pollution discharges, because the phosphorus and nitrogen in the waste water are recycled. Struvite is considered in many countries as a sustainable, economically important alternative form of organic fertiliser**.** Struvite has been commercialised worldwide and sells for US$198–1 885 (€161–1 536) per tonne in Australia and Japan. Its use as a fertiliser is likely to increase in the near future; however, it is not yet authorised everywhere in Europe and a robust understanding of the potential risks of using it as a fertiliser in agriculture is needed.

Antibiotic resistance is a global public health concern. Prior research[1,2] has shown that organic fertilisers — from sewage sludge and animal manure — may increase environmental antibiotic resistance due to the presence of antibiotic resistant bacteria, antibiotics and ARGs. These ARGs may enter the food chain via contaminated crops and potentially transfer horizontally into animal or human pathogens — increasing the risk that pathogens will acquire resistance to therapeutic antibiotics. Other studies found that the application of organic fertiliser to crops of tomato and carrot introduces ARGs into them. This study using *Brassica* plants (plants in the mustard family, many of which are commonly used for food) shows that struvite, similarly, increases environmental antibiotic resistance. ARGs present in the fertiliser were also found in the soil and in the leafy parts of the plants.

An EU organic farming committee has recommended — pending regulatory approval — the use of struvite in organic farming, as it meets environmental objectives, but only when there is no health risk from organic pollutants or pathogens[3].

The researchers used struvite from the waste water of a piggery, and soil from a vegetable field — not fertilised with struvite or any other organic fertiliser in the previous three years. Four different treatments were prepared, in triplicate: plastic pots were filled with 3 kilograms of soil with or without struvite and planted or not planted with *Brassica* plants. In the planted pots, a rhizo-bag (a nylon cloth, containing 500 grams of soil) was used; this allows smaller molecular-weight substances but not roots to penetrate it, thereby separating the soil immediately around the plant roots (in which the chemistry and microbiology is particularly influenced by a plant's growth, respiration, and nutrient exchange) from the bulk soil around it. Samples were taken after 60 days. Four sample types were taken — above-ground plant parts, root soil (from within the rhizo-bag), planted bulk soil and unplanted bulk soil. A library database of RNA was then used as a reference for picking operational taxonomic units (OTUs) — a unit of microbial diversity — to identify the bacterial community structure and composition of the samples.

Continued on next page.

1. Marti, R., Scott, A., Tien, Y.C., *et al.* (2013) *Applied & Environmental Microbiology,* 79(18): 5701–9

2. Rahube, T.O., Marti, R., Scott, A., *et al.* (2014) *Applied & Environmental Microbiology,* 80 (22): 6898–907

3. https://ec.europa.eu/agriculture/organic/sites/orgfarming/files/docs/body/final-report-egtop-on-fertilizers-2_en.pdf

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Antibiotic resistance in struvite fertiliser from waste water could enter the food chain *(continued)*

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The concentration of antibiotics in struvite was measured using liquid chromatography-MS/MS, a technique used to separate, identify, and quantify each component in a mixture. The abundance of ARGs and mobile genetic elements (MGEs) was then established by extracting DNA from soil and vegetables, and thereafter performing high-throughput polymerase chain reaction from root and leaf DNA samples to identify the sequences related to ARGs. Selected ARGs were sequenced and sequences obtained compared with an existing database of OTUs.

This study identified ARGs and MGE marker genes common to the struvite, soil, roots and the above-ground parts of the *Brassica* plants. Thirty genes found in the struvite were also found in the above-ground parts of the plant but not in the original soil. These genes included 29 ARGs conferring resistance to one or many of the most commonly used antibiotics in humans and animals, such as sulphonamide and tetracycline. Consumption of these contaminated leafy vegetables may provide a route for these ARGs to transfer to pathogenic bacteria, the researchers say, conferring antibiotic resistance, which poses a risk to animal or human health. In addition, similar bacterial communities were found amongst the roots and above-ground parts of the plants, with 303 shared OTUs. The presence of shared bacteria and genes suggests that bacteria may move and/or transfer between the roots and leaves.

The findings of this study may be relevant in the context of the ongoing revision of the EU Fertilisers Regulation[4,5], as it is considered to envisage a delegation to the Commission to define under what circumstances struvite can be used as a component material for CE marked fertilisers[6]. They also suggest that the risk of antibiotic resistance genes in struvite fertiliser transferring via crops into pathogenic bacteria warrants closer examination. Further research is also required into improved methods of struvite production and its use in agriculture in order to minimise the risk of antibiotic resistance development.

4. http://www.europarl.europa.eu/legislative-train/theme-new-boost-for-jobs-growth-and-investment/file-review-of-the-fertilising-products-regulation

5. http://www.phosphorusplatform.eu/images/download/ESPP-eNews-004-October-2016.pdf (Summary of Commission proposal: *SCOPE Newsletter* n° 120 and www.phosphorusplatform.eu/regulatory)

6. http://ec.europa.eu/environment/natres/pdf/phosphorus/SWD(2014)263%20final.pdf