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Nanoplastics may reduce efficacy of constructed wetlands for water treatment

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Water bodies absorb the nitrogen released by human activity and must, therefore, be protected against nutrient overloading (or eutrophication), which can cause significant environmental damage. Constructed wetlands (CWs) are widely used as an eco-friendly treatment method for this; however, the efficacy of CWs may be affected by the presence of emerging contaminants in wastewater. This study explores how nano-sized particles of polystyrene plastic (nanoplastics) affect nitrogen removal (denitrification[1]) in CWs.

CWs are a key example of a nature-based solution (NBS), an initiative that builds upon and draws from nature to remedy a societal or environmental challenge. Such solutions are a priority for the EU, and are recommended as part of several major EU policies including the European Green Deal, EU Biodiversity Strategy for 2030 and the EU Adaptation Strategy for climate. CWs show potential in treating agricultural wastewater and runoff, as microbes and plants within the system can take up or metabolise nitrogen (and other nutrients) to purify water.

In recent years, nanoplastics (plastic particles of under 100 nanometres in size, used in industry or created via fragmentation of larger plastics) have increasingly made their way into sewerage and waste-water treatment systems via consumer use, direct release or runoff. While nanoplastics are known to be present in raw wastewater — at reported quantities of up to 1 189 micrograms per litre[2] — their effect on treatment has not been thoroughly characterised. It is also thought that existing treatment methods (e.g. mechanical stirring and pumping) can fragment larger plastics down to the nanoscale, thereby further increasing the amount of nanoplastic within wastewater.

A recent study has explored the potential effects of nanoplastics on CWs. The researchers added polystyrene nanoplastics to synthetic wastewater at concentrations of 0 (control), 10 and 1 000 micrograms per litre to simulate different predicted concentrations. Each nanoparticle measured 60–70 nanometres in size. They then prepared three different treatment groups, each comprising three CW 'microcosms' filled with gravel up to a height of 40 cm, planted with cattails (*Typha*

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latifolia), and fed with synthetic wastewater via a pipe. The experiment ran for 180 days, with water samples from each group collected at three stages for analysis of nutrient levels, denitrification rate, microorganism uptake of polystyrene, microbial activity and plant physiology. Stage 1 ran from day 1 to day 45; stage 2 from day 45 to 160; and stage 3 from day 160 to 180.

The analysis revealed that denitrification was significantly inhibited after prolonged exposure to polystyrene nanoplastics, with the efficiency of total nitrogen removal decreasing by up to 40.6%.

The nanoplastics also penetrated and adhered to the cell membranes of wetland microbes, destroying membrane integrity and prompting overproduction (and therefore unbalanced levels) of reactive oxygen. Microorganisms in the two polystyrene treatment groups displayed inhibited *in vivo* enzyme activity (with activity levels being up to 2.7-fold lower) and 'electron transport system' activity (ETSA) (denitrification relies upon the effective transport and use of electrons [3], assessed via ETSA) compared with the control. The nanoplastics also appeared to affect the composition of the microbial community: the relative abundance of proteobacteria — the phylum (a taxonomic division of living organisms) that contains most nitrogen-removing microbes — was lower at the end of the 180-day period in the polystyrene treatment groups than in the control.

Plant activity was also affected. The roots of wetland plants can enhance nitrification by transferring oxygen to the surrounding soil, and promote denitrification by distributing organic carbon throughout root systems. Exposure to polystyrene nanoplastics inhibited root activity, which in turn reduced the amount of nitrogen taken up by plants (although it should be noted that this effect could also be due to other factors — for example, decreased production of NO_3, which plants may prefer). Furthermore, plant leaves showed increased levels of antioxidant enzyme activity, which in turn triggered a stress response that reduced photosynthetic ability and prevented oxygen and organic matter from being efficiently transferred through the plant system (as needed for nitrification and denitrification).

The findings highlight the importance of protecting the ecological microbial communities found in CWs and imply that, while both appear to be affected, microbes may be more sensitive to chronic nanoplastic exposure than wetland plant species. Overall, the researchers suggest that the presence of nanoplastics in aquatic environments may compromise the functioning of the nitrogen cycle – an impact which should be taken into consideration in assessments.

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1. Both nitrification and denitrification are important processes in water treatment; in nitrification, ammonium (NH_4^+) is converted to nitrate (NO_3^-). This is then converted, via denitrification, into molecular nitrogen (N_2), which can then safely be released into the atmosphere. Incomplete nitrification or denitrification can result in the emission of the potent greenhouse gas nitrous oxide (N_2O), the third-largest contributor to anthropogenic climate warming and nitrates in waters, leading to eutrophication.

2. Simon, M., van Alst, N., and Vollertsen, J. (2018) Quantification of microplastic mass and removal rates at wastewater treatment plants applying Focal Plane Array (FPA)-based Fourier Transform Infrared (FT-IR) imaging. *Water Res*. 142: 1–9.

3. **Oxidation** is the loss of electrons, and **reduction** is the gain of electrons. For oxidised forms of nitrogen (nitrates and nitrites) to be 'reduced' via denitrification, electrons must be sourced and transported from elsewhere for use – typically an 'electron donor' such as organic matter.