Science for Environment Policy

Transformed nanoparticles in effluent can affect sediment-dwelling species

Silver nanoparticles present in the effluent from waste-water treatment plants could have toxic effects on aquatic organisms, new research suggests. The lab-based study tested the effects of nanoparticle-containing effluent on several crustacean and algae species. The researchers observed that epibenthic crustaceans (those living in or on sediments at the bottom of water bodies) were the most sensitive; notably, a 20–45% higher death rate was observed compared with those exposed to nanoparticle-free effluent.

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Silver and titanium dioxide nanoparticles are being increasingly used in various consumer products, from toothpaste and sunscreen to textiles and food packaging, and are, therefore, also finding their way into waste water. They are removed efficiently by waste-water treatment plants; removal rates of 95–99% are reported in previous research cited by this study[1]. However, the design and efficiency of the plant[2] affects the rate of removal, and silver and titanium dioxide nanoparticles have been detected in waste-water effluent.

This raises concerns for aquatic wildlife, as research has shown that these nanoparticles can have toxic effects. However, most previous studies have explored nanoparticles' effects at concentrations that are higher than those expected to be found in the environment. This study instead investigated their effects at concentrations considered to be more environmentally relevant. Importantly, the particles in this study had also been altered or 'transformed' by the waste-water treatment processes; evidence shows that transformed nanoparticles can have effects different from those of pristine nanoparticles.

The researchers created a small-scale waste-water treatment plant in their laboratory and combined it with ecotoxicological assays and analytical techniques. The plant simulated biological treatment and consisted of a non-aerated denitrifying reactor, an aerated nitrifying reactor and a settler.

The researchers fed the plant with artificial waste water containing silver nanoparticles at a concentration of 10 micrograms per litre (µg/L) and titanium dioxide nanoparticles at 100 µg/L for a period of five weeks. During these weeks, analytical and imaging techniques, including fractionation, (single particle) inductively coupled plasma mass spectrometry and electron microscopy, were used to study the behaviour and transformation of the nanoparticles.

The researchers applied the resulting effluent, containing transformed nanoparticles, to four different organisms: two marine species, *Skeletonema pseudocostatum* and *Tisbe battagliai* (an alga and a crustacean), and two freshwater species, *Raphidocelis subcapitata* and *Daphnia magna* (also an alga and a crustacean). This acknowledged the fact that effluent is released into both freshwater and marine environments in many countries. In addition, the effects on cultured gill cells from rainbow trout were studied.

Post-treatment, the effluent contained silver nanoparticles at concentrations of 0.1–0.22 µg/L and titanium aggregates (present in the fraction above 0.7 micrometres in size) at 0.9–24.2 µg/L, varying irregularly over the five weeks. The silver nanoparticles were associated with sulphur, copper and zinc.

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1. For example: Li *et al*. (2016). *Environ. Sci. Technol.*, *50* (12) : 6327–6333. DOI: 10.1021/acs.est.6b00694; Westerhoff, *et al*. (2011), *J. Environ. Monit.*,13, 1195-1203. DOI:10.1039/C1EM10017C.

2. Not all urban waste-water treatment plants (WWTPs) necessarily include the level(s) of treatment that would be effective at removing nano-particles, as their primary objective is the removal of organic and microbiological pollution, and/or nutrients, if needed. There is currently no obligation in the Urban Waste Water Treatment plants to remove nanoparticles, but a significant proportion may be retained (along with other pollutants) in the sewage sludge. Because of the pollutants it contains, the use of sewage sludge needs to be controlled carefully. It is out of the scope of the Urban Waste Water Treatment Directive (UWWTD), but subject to certain requirements under the Sewage Sludge Directive.

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The effluent had various effects. Notably, death rates of the marine crustacean *T. battagliai* were 20–45% higher than for those exposed to nanoparticle-free effluent. No effects on mortality were observed for the freshwater crustacean *D. magna.* The effluent also inhibited growth of the marine alga *S. pseudocostatum* by 20–40%, but increased growth in the freshwater alga *R. subcapitata* by 40%. This growth may be explained by increased cell aggregation, a defence mechanism which reduces the amount of exposed surface to foreign substances. Increased oxidative stress levels were observed in the cultured gill cells, resulting in increased permeability of the layer of cultured cells.

The researchers conclude that nanoparticle effects vary by species. Those that live or feed on or near the seafloor, like *T. battagliai,* may be most at risk because they come into more direct contact with nanoparticles that settle on the floor after attaching to suspended solids. Over 80% of the silver and titanium in the effluent was associated with suspended solids.

The study, therefore, also raises concerns regarding land-based species, which may be exposed to nanoparticles in sewage sludge when it is used as 'biosolids' to improve agricultural soil quality. This study also highlights the need for fusrther investigation into the potential hazards of transformed nanomaterials at realistic concentrations in more relevant exposure scenarios and complex matrices.