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Influence of soil properties on the effect of silver nanomaterials on microbial activity in five soils

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ABSTRACT

We investigated the effects of silver nanomaterials (AgNMs) on five well-characterized soils with distinct physicochemical properties using two standardized test systems. The carbon transformation test (OECD 217) showed minimal sensitivity whereas the ammonia oxidizing bacteria test (ISO 15685) showed extreme sensitivity over 28 days of exposure. AgNM toxicity was compared with the physicochemical properties of the soils, revealing that toxicity declined with increasing clay content and increasing pH. AgNM toxicity did not appear to be affected by the organic carbon content of the soil. Our results showed that AgNM toxicity cannot be attributed to any single soil property but depends on the same parameters that determine the toxicity of conventional chemicals. Recommendations in the test guidelines for soil ecotoxicity studies are therefore applicable to AgNMs as well as conventional chemicals.

1. Introduction

Silver nanomaterials (AgNMs) have potent antimicrobial properties (Morones et al., 2005) making them suitable for diverse applications such as the manufacture of plastics, textiles, healthcare products, coatings and electrical appliances. The demand for such materials is rising, and this increases the risk of AgNM contamination entering the environment. Many studies have confirmed the release of AgNM particles from textiles (Benn and Westerhoff, 2008; Geranio et al., 2009) and coatings (Kaegi et al., 2010). Estimates of environmental exposure (based on material flow analysis during the life cycle of various nanomaterials) suggest that aquatic organisms may be harmed by AgNMs present in the effluent of sewage-treatment plants (Nowack et al., 2012). However, there is also evidence that AgNMs affect the terrestrial environment, e.g. through the use of sewage sludge as agricultural fertilizer (Schlich et al., 2013b).

The antimicrobial effect of AgNMs is persistent. AgNMs that have been passed through a model sewage treatment plant, applied to soil via the sewage sludge and tested after 100–140 days, remain as toxic towards microorganisms as freshly-prepared AgNMs applied to soil and tested after 28 days (Schlich et al., 2013b). AgNMs also affect the reproduction of earthworms (Heckmann et al., 2011; Schlich et al., 2013a; Shoults-Wilson et al., 2011a, 2011b; Tsyusko et al., 2012), as well as the biomass content by substrate-induced respiration and the enzyme activity of soils (Hänsch and Emmerling, 2010; Shin et al., 2012). These studies used pristine AgNMs and provided important information for risk characterization. However, the detection, fate and effects of AgNMs in soils and other complex media remain a challenging area of research (von der Kammer et al., 2012).

The physicochemical characteristics of soil (e.g. pH, ionic composition, grain size, organic matter content, temperature, solar radiation exposure and hydrostatic pressure) influence the chemical form, mobility, bioavailability and thus the toxicity of pollutants. The evaluation of toxicity is complex because the impact of pollutants on soil biota is determined by a combination of physicochemical soil characteristics, the chemical form of the pollutant and the physiological status of the biota (Babich et al., 1980).

Fate studies have identified factors that influence the transport of AgNMs in soils (Aiken et al., 2011; Akaighe et al., 2011; Coutris et al., 2012; El Badawy et al., 2013; Sagee et al., 2012). The effect of soil type (Shoults-Wilson et al., 2011b) and ion content in the soil pore water (Schlich et al., 2013a) have been considered in earthworm (*Eisenia andrei*) reproduction tests, but the soil parameters that affect the toxicity of AgNMs towards soil microorganisms are poorly understood and the role played by soil properties has not been investigated. Soil microorganisms are the agents of most important soil processes, including the transformation of organic matter and nutrients (Powlson et al., 2001). It is difficult to predict the bioavailability of AgNMs in natural soils because of the

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Table 1
Physicochemical properties of reference soils.

Parameter	RefeSol 01A[a]	RefeSol 02A[a]	RefeSol 04A[a]	RefeSol 03G[b]	RefeSol 05G[b]
Soil type	Dystric cambisol	Stagnic luvisol	Gleyic podsol	Eutric cambisol	Gleyic fluvisol
Properties	Loamy sand, medium acid, very light humic	Silt loam, sub-acid, light humic	Loamy sand, medium acid, medium humic	Silt loam, medium acid, medium humic	Silt loam, strongly acid, strongly humic
Sand [%]	71	2	85	21	22
Silt [%]	24	84	1	52	62
Clay [%]	5	15	4	27	16
pH ($CaCl_2$)	5.30	6.57	5.03	5.98	4.59
Corg [%]	0.93	1.30	2.91	3.85	3.08
CEC_{eff} [mmolc/kg]	37.9	133.2	85.7	135.8	116.1
WHC_{max} [mL/kg]	264	419	346	768	584

C_{org}: organic carbon; CEC: cation exchange capacity; WHC_{max}: maximum water-holding capacity.
[a] Arable land.
[b] Grassland.

interactions between different biological processes, physicochemical properties and environmental conditions (Cornelis et al., 2014).

We investigated the impact of different soil properties on the toxicity of NM-300K, an AgNM from the OECD Sponsorship Programme. We focused on microorganisms because they are affected most severely by the antimicrobial properties of AgNMs. Following OECD Guideline 217 (2000) and ISO Guideline 15685 (2012), we investigated the effects of AgNM on substrate-induced respiration and ammonia oxidizing bacteria over 28 days with five reference soils varying in pH, organic carbon content and grain size distribution. These soils are suitable for testing the influence of substances on the habitat function of soils, i.e. bioavailability and effects on organisms (Kördel, 2007). We also distinguished between the effects of particles and released ions by testing silver nitrate under the same conditions.

2. Materials and methods

2.1. Materials

2.1.1. Test soil

The experiments were carried out using five different reference soils (RefeSol) whose physicochemical properties are listed in Table 1. RefeSol soils were selected as reference soils by the German Federal Environment Agency (Umweltbundesamt UBA) and they match the properties stated in various OECD terrestrial ecotoxicological guidelines (e.g. tests with plants and soil microflora). The soils were sampled in the field and stored in high-grade stainless steel basins with drainage and ground contact at the Fraunhofer IME in Schmallenberg. Red clover was sown in all the stored soils and no pesticides were used. Appropriate amounts of soil were sampled 1–4 weeks before the test. If the soil was too wet for sieving it was dried at room temperature to 20–30% of the maximum water holding capacity (WHC_{max}) with periodic turning to avoid surface drying. If the tests did not start immediately after sieving, the soil was stored in the dark at 4 °C under aerobic conditions (ISO Guideline, 18512, 2007).

2.1.2. AgNMs

NM-300K was used as required by the OECD Sponsorship Programme (Organisation for Economic Co-operation and Development, 2007). This is a colloidal silver dispersion with a nominal silver content of 10% (w/w) and a particle size of ~15 nm with a narrow size distribution (99%). A second particle size of 5 nm, which is much less abundant (1%), was identified by TEM. The particles are dispersed in mixture of a stabilizing agents (NM-300K

Table 2
Actual data of the substrate-induced respiration in mg O_2/(kg dw * h) for the five different soils.

AgNM	**Soil**		**Control ± SD**	**Dispersant ± SD**	**0.56 mg/kg ± SD**	**1.67 mg/kg ± SD**	**5.0 mg/kg ± SD**
	01A	d1	3.5 ± 0.5	3.7 ± 0.5	3.2 ± 0.0	3.2 ± 0.0	3.2 ± 0.0
		d28	2.4 ± 0.0	2.4 ± 0.0	2.1 ± 0.5	2.1 ± 0.5	1.1 ± 0.6
	02A	d1	5.6 ± 0.7	5.8 ± 0.4	5.6 ± 0.7	5.8 ± 0.4	5.2 ± 0.5
		d28	4.0 ± 0.0	3.8 ± 0.4	4.0 ± 0.7	4.2 ± 0.4	4.4 ± 0.5
	04A	d1	3.8 ± 0.4	3.6 ± 0.5	3.6 ± 0.5	4.0 ± 0.0	4.6 ± 0.4
		d28	3.4 ± 0.4	3.8 ± 0.4	3.6 ± 0.5	3.2 ± 0.0	3.4 ± 0.4
	Soil		**Control ± SD**	**Dispersant ± SD**	**1.67 mg/kg ± SD**	**5.0 mg/kg ± SD**	**15.0 mg/kg ± SD**
	03G	d1	20.8 ± 0.7	18.8 ± 1.5	2.6 ± 0.4	19.8 ± 0.8	19.6 ± 0.8
		d28	16.4 ± 0.5	16.4 ± 0.8	15.8 ± 1.0	15.0 ± 1.5	13.8 ± 1.2
	05G	d1	9.0 ± 0.4	9.0 ± 0.4	8.6 ± 0.8	8.4 ± 0.5	8.4 ± 0.5
		d28	7.0 ± 0.4	6.4 ± 0.0	6.4 ± 0.0	5.3 ± 0.5	4.4 ± 0.5
$AgNO_3$	**Soil**		**Control ± SD**	**0.19 mg/kg ± SD**	**0.56 mg/kg ± SD**	**1.67 mg/kg ± SD**	**5.0 mg/kg ± SD**
	01A	d1	4.1 ± 0.4	3.9 ± 0.8	4.4 ± 0.4	3.9 ± 0.0	3.6 ± 0.5
		d28	4.6 ± 0.0	4.6 ± 0.1	4.6 ± 0.0	4.6 ± 0.0	3.1 ± 0.0
	02A	d1	4.4 ± 0.4	4.4 ± 0.4	4.1 ± 0.9	4.6 ± 0.0	2.8 ± 1.9
		d28	4.1 ± 0.4	3.4 ± 0.5	3.6 ± 0.5	3.1 ± 0.0	2.6 ± 0.5
	04A	d1	3.6 ± 0.5	3.6 ± 0.5	3.4 ± 0.5	3.6 ± 0.5	3.1 ± 0.0
		d28	3.1 ± 0.0	2.6 ± 0.5	3.1 ± 0.8	2.3 ± 0.0	2.3 ± 0.0
	Soil		**Control ± SD**	**0.19 mg/kg ± SD**	**1.67 mg/kg ± SD**	**5.0 mg/kg ± SD**	**15.0 mg/kg ± SD**
	03G	d1	18.5 ± 0.8	18.8 ± 0.9	18.3 ± 0.4	17.8 ± 0.0	13.6 ± 0.5
		d28	15.1 ± 1.1	15.0 ± 1.2	15.0 ± 1.2	13.4 ± 0.6	9.2 ± 0.1
	05G	d1	8.1 ± 0.1	8.1 ± 0.0	7.7 ± 0.7	7.3 ± 0.7	5.8 ± 0.0
		d28	9.0 ± 0.5	8.8 ± 0.5	8.0 ± 0.5	6.9 ± 0.0	5.4 ± 0.0

SD: Standard deviation.