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Estuarine sediment hydrocarbon-degrading microbial communities demonstrate resilience to nanosilver

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ABSTRACT

Little is currently known about the potential impact of silver nanoparticles (AgNPs) on estuarine microbial communities. The Colne estuary, UK, is susceptible to oil pollution through boat traffic, and there is the potential for AgNP exposure via effluent discharged from a sewage treatment works located in close proximity. This study examined the effects of uncapped AgNPs (uAgNPs), capped AgNPs (cAgNPs) and dissolved Ag_2SO_4, on hydrocarbon-degrading microbial communities in estuarine sediments. The uAgNPs, cAgNPs and Ag_2SO_4 (up to 50 mg L^{-1}) had no significant impact on hydrocarbon biodegradation (80–92% hydrocarbons were biodegraded by day 7 in all samples). Although total and active cell counts in oil-amended sediments were unaffected by silver exposure; total cell counts in non-oiled sediments decreased from 1.66 to 0.84 × 10^7 g^{-1} dry weight sediment (dws) with 50 mg L^{-1} cAgNPs and from 1.66 to 0.66 × 10^7 g^{-1} dws with 0.5 mg L^{-1} Ag_2SO_4 by day 14. All silver-exposed sediments also underwent significant shifts in bacterial community structure, and one DGGE band corresponding to a member of Bacteroidetes was more prominent in non-oiled microcosms exposed to 50 mg L^{-1} Ag_2SO_4 compared to non-silver controls. In conclusion, AgNPs do not appear to affect microbial hydrocarbon-degradation but do impact on bacterial community diversity, which may have potential implications for other important microbial-mediated processes in estuaries.

Introduction

Between 470 thousand and 8.3 million tonnes of petroleum hydrocarbons are released into aquatic environments globally each year (National Research Council, 2003). Although almost half of this is released naturally from oil seeps, the remainder comes from incidental deposition and represents a significant source of pollution (National Research Council, 2003). For example, between 2000 and 2013, 43 large marine oil spills (>700 tonnes) and 167 medium sized marine oil spills (7–700 tonnes) were reported (ITOPF, 2013). Estuarine systems are particularly susceptible to anthropogenic hydrocarbon contamination, with concentrations of polyaromatic hydrocarbons (PAHs) shown to exceed 100 mg kg^{-1} sediment in one UK location at Milford Haven (Woodhead et al., 1999). Of particular concern is the accumulation of low molecular weight PAHs such as naphthalenes, which have been found at concentrations of up to 2.4 mg kg^{-1} dry weight sediment in the Tyne estuary (Woodhead et al., 1999), and which are acutely toxic to aquatic invertebrates at concentrations as low as 8 μg L^{-1} (Sanborn and Malins, 1977). In addition, many high molecular weight PAHs such as chrysene, which has been found at concentrations of up to 6.94 mg kg^{-1} dry weight sediment at Milford Haven (Woodhead et al., 1999), are classed as carcinogens and can cause chronic toxic effects in fish and invertebrates (Barron et al., 2003). Fortunately, there are over 175 known bacterial genera that are able to utilize a range of different crude oil compounds as a source of carbon and energy, thus petroleum hydrocarbons that contaminate aquatic environments are commonly biodegraded by *in situ* microbial communities (Prince et al., 2010). For example, the stimulation of indigenous oil-degrading bacteria and their corresponding

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biodegradation genes were notably observed following the Deepwater Horizon event in 2010 (Hazen et al., 2010; Lu et al., 2011).

Recent advances in nanotechnology have led to an increase in the concentration of engineered nanoparticles reaching aquatic systems, where they pose a direct risk to *in situ* microbial communities and their processes (Moore, 2006; Nowack and Bucheli, 2007; Klaine et al., 2008; Baker et al., 2013). Ionic silver and silver nanoparticles (AgNPs) are used as a broad spectrum antimicrobial agent in consumer products including medical equipment, clothing, pharmaceuticals, food storage containers, children's toys, cosmetics, optical devices and household appliances (Kim et al., 2007; PEN, 2014). The release of AgNPs from many of these products through general wear and tear results in entry of AgNPs into aquatic systems where they have the potential to detrimentally impact microbial hydrocarbon-degraders and rates of petroleum biodegradation. Furthermore, exposure to multiple stressors such as crude oil and AgNPs may have a synergistic detrimental effect on communities. For example, Sargian and Mas (2007) observed a synergistic toxic effect of crude oil and UVB radiation on microplankton communities. The impact of AgNPs on aquatic microbial communities and specifically hydrocarbon-degraders is currently poorly understood (Oberdörster et al., 2005; Moore, 2006; Nowack and Bucheli, 2007; Klaine et al., 2008).

The present study focused on the Colne estuary, UK, as a model system from which to measure the effect of AgNPs on estuarine microbial communities, due to its susceptibility to oil pollution (Coulon et al., 2012) and the presence of a large wastewater treatment works which is a potential release site for AgNPs (Fig. 1). The overall aim of the study was to determine the effects of two types of AgNPs: uncapped (uAgNPs) and capped (cAgNPs) on the community structure and activity of hydrocarbon-degrading microbial communities in estuarine sediments. The cAgNP were coated with methoxypolyethylene-glycol (mPEG), a capping agent which is commonly used to stabilize AgNPs and aid dispersion (Christian et al., 2008; Crane et al., 2008). A silver salt (Ag_2SO_4) was also used as a source of silver ions for comparison.

Materials and methods

Silver nanoparticles

The uncapped silver nanoparticles (uAgNPs) were purchased as a dry powder (American Elements). A stock suspension (1 g L^{-1}) was prepared in sterile ultra-high purity (UHP) water and dispersed by sonication (75 Watts, 40 kHz) in an ultrasonic water bath (Decon F5 minor, Decon Ultrasonics Ltd) for 30 min prior to use. The capped silver nanoparticles (cAgNPs, capped with methoxypolyethylene glycol) were provided as a well-dispersed 0.9 g L^{-1} suspension of mPEG-coated AgNPs by Dr Paul Christian (University of Manchester, UK). Both nanoparticles have been thoroughly characterized previously (Beddow et al., 2014). The cAgNPs were found to be prominently present as single particles with an average diameter of 17–40 nm (depending on characterization technique) and a zeta potential of −37 mV when dispersed in UHP water, while the uAgNPs tended to form aggregates with an average diameter of 118–188 nm and a zeta potential of −32 mV (Beddow et al., 2014). A

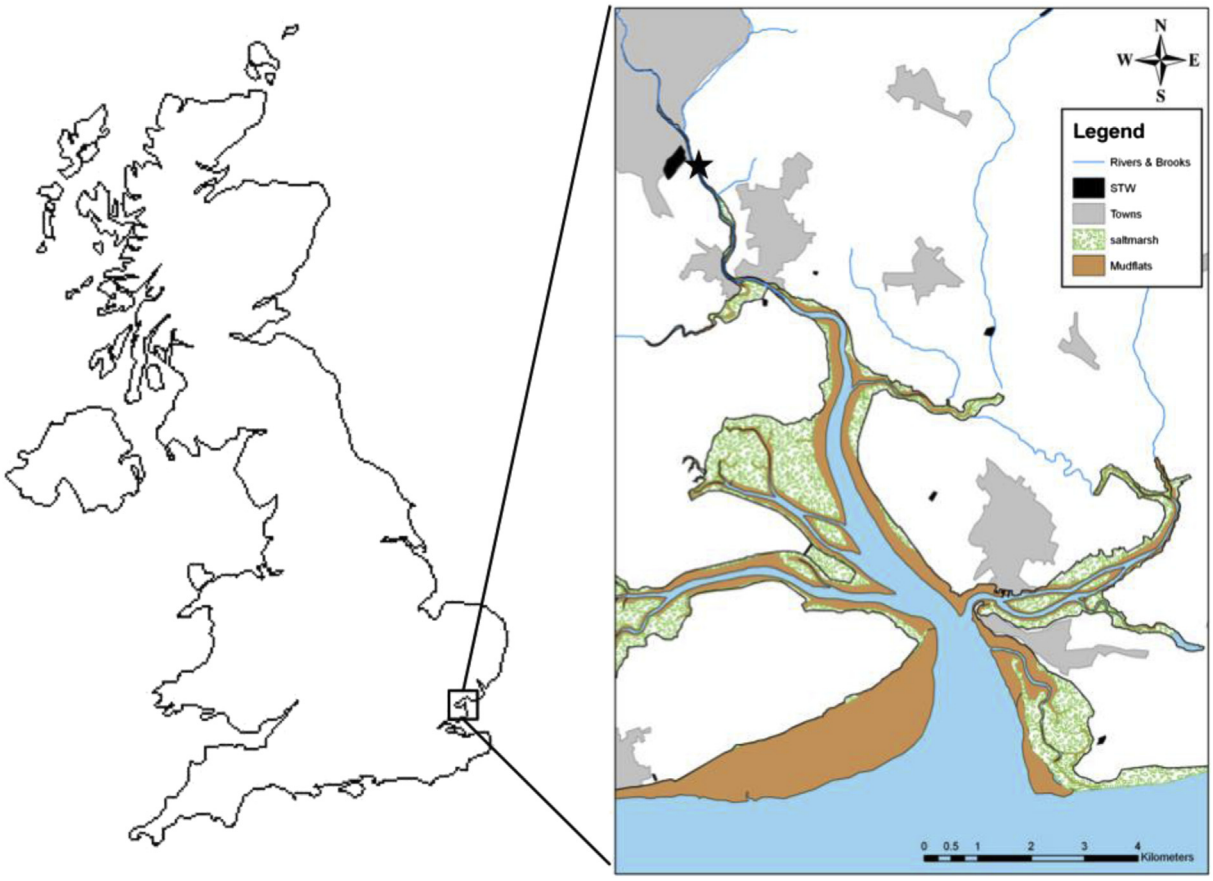

Fig. 1. Map of the UK with an enlarged box showing the Colne estuary. The sampling site (the Hythe, marked with a star) is located directly downstream of a large sewage treatment works (STW). Map provided by Dr Steve McMellor, University of Essex, UK.