

Research projects

FENAC has conducted and completed a number of projects in cooperation with different Universities and research centres in the United Kingdom.

Examples of these projects include the characterisation of nanoparticles in understanding the microbial toxicity of TiO₂ nanoparticles and carbon nanotubes, determining size and surface properties of nanoparticles in understanding their toxicity to fish, and distinguish between uptake of dissolved and nanoparticulate iron to marine invertebrates.

2012

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Impact of engineered nanoparticles on aquatic microorganisms and their processes

Prof. I. Colbeck; University of Essex (<http://www.essex.ac.uk/bs/staff/profile.aspx?ID=1115>)

This project investigates the effect of engineered nanoparticles (e.g. silver, titanium dioxide and carbon nanotubes) on the community structure and activity of aquatic microorganisms that biodegrade hydrocarbon pollutants and those responsible for nitrification. Analytical techniques will be used to measure rates of hydrocarbon-degradation and nitrification, while molecular techniques will identify and quantify shifts in the *in situ* microbial community structure in the presence of different nanoparticle species. TEM imaging will be used to investigate interactions between nanoparticles and bacterial cells. Results from this project will provide a major advance on how nanoparticles interact with key microbial processes in aquatic ecosystems.

Characterization of isotopically labelled ¹⁰⁹Ag nanoparticles

M. Rehkämper; Imperial College London (<http://www3.imperial.ac.uk/earthscienceandengineering/research/magic>)

Preliminary analytical studies conducted at Imperial College have shown that the technique of stable isotope labelling should enable extremely high sensitivities for the detection of Ag nanoparticles in bulk natural samples. Hence, we will prepare isotopically labelled ¹⁰⁹Ag nanoparticles for subsequent pilot studies that investigate toxicology and environmental fate. Here, we seek funds that will allow us to use the FENAC facilities to characterise such ¹⁰⁹Ag nanoparticles for key physico-chemical properties in various aqueous media that are relevant for toxicological and environmental studies. Additional analyses will be carried out for conventional Ag nanoparticles, to confirm that labelled and unlabelled Ag nanomaterials display identical behaviour.

Cellular handling of synthetic and natural (mineral dust- and volcanic ash-derived) iron-rich NPs and their influence on the growth of marine microalgae

Dr. E. Kadar; Plymouth Marine Laboratory (http://www.pml.ac.uk/about_us/the_pml_team.aspx)

It is well established that Fe availability controls phytoplankton productivity, community structure, and ecosystem functioning in vast regions of the global ocean (approx. 30%). While we have demonstrated in our previous project (FENAC/2010/05/006) that nanoparticles are derived from iron-rich mineral dusts/volcanic ash via cloud processing, the consequently improved bioavailability of the particles needs further in depth investigations. Here we propose culturing algae with distinct Fe handling strategies at the laboratory scale using nano-iron amended media. In doing so we will test the hypothesis that natural, Fe-rich NPs from cloud processed mineral dusts (CPMD) are more bioavailable than the bulk analogue. We will thus be able to make predictions on the larger scale implications of dust deposition on ocean productivity and potential effect on changing climate.

Characterisation of marine colloids and nano particles associated with metals

Dr. M. Gledhill; National Oceanography Centre Southampton (<http://www.southampton.ac.uk/oes/about/staff/martha.page?#background>)

Shelf sediments represent an important supply of iron to open ocean waters. The supply of iron from shelf sources involves long range transport of iron in seawater, which requires stabilisation of iron in order to prevent loss by scavenging. The physico – chemical form of iron within the water column is critical to stabilisation. We propose to investigate the nature of soluble, colloidal and particulate iron released from shelf sediments and thereby assess the importance of different iron fractions to offshore iron transport. We will use FENAC to characterise particulate and colloidal iron released from the shelf sediments.

Nanoparticles of Hydroxyapatite are Templated onto *Serratia* sp. and Form Particles with Increased Metal Remediation Capacity

Prof. L. Macaskie; University of Birmingham

Biomineral hydroxyapatite (Bio-HAP) produced by *Serratia* sp. could be a suitable material for the remediation of heavy metals from waters and as a nuclear waste storage material. Bio-HAP is up to 10 times more efficient at aqueous metal removal than commercially available hydroxyapatite [1]. However, Bio-HAP contains up to 50% organics which can degrade, releasing heavy metals back into the environment [2]. Once normalized for surface area, a significant relationship between Bio-HAP crystallite size and metal uptake has been observed. This proposal aims to understand the mechanism of Bio-HAP formation and growth so that this unique material can be synthesised in laboratories.

2011

Nanoparticles and their impact on the bioremediation of hydrocarbons in aquatic ecosystems

Ian Colbeck, University of Essex (<http://www.essex.ac.uk/bs/staff/profile.aspx?ID=1115>)

This project will investigate the effect of different nanoparticle species (e.g. silver (capped and uncapped), carbon nanotubes and titanium dioxide) on the community structure and activity of microorganisms that actively metabolise specific hydrocarbon pollutants in marine and freshwater environments. Molecular techniques will identify and quantify shifts in the *in situ* microbial community structure and activity in the presence of different nanoparticles, and this information will be correlated (via analytical methods) to the physical and chemical characteristics of the nanoparticles (e.g. size, species, surface properties etc) and rates of hydrocarbon-degradation. This will provide important information on how nanoparticles affect the microbial interactions involved in hydrocarbon-degradation in aquatic environments, and is a major advance on how nanoparticles interact with key microbial processes in marine/freshwater ecosystems.

The ecotoxicology of zinc oxide and silver nanoparticles with respect to aquatic sediment dwellers

V. Stone and T. Fernandes, formerly of Edinburgh Napier University (<http://www.napier.ac.uk/fh/ls/Staff/Pages/Staff.aspx>)

This study aims to investigate the effects of engineered ZnO and Ag nanoparticles in aquatic sediment dwellers. *Lumbriculus variegatus* is a fresh water oligochaete, found throughout North America and Europe. Due to their feeding behaviour, *Lumbriculus variegatus* make excellent test organisms for studying the bioaccumulation of contaminants. *Nereis diversicolor* is a marine polychaete worm found throughout Europe and the North American Atlantic coast which has been extensively used as a biological models it can tolerate a wide range of salinities and pH. Benthic-dwelling organisms are ideally suited for the assessment of any effects of nanomaterials given their propensity to settle when in aquatic environments. The effects of the selected nanoparticles will be assessed via a number of endpoints, e.g. behaviour, mortality, reproduction, histology and biochemical assays, with and without the addition of humic acid.

Advanced fish model systems for understanding biological effects of metal oxide nanomaterials

In this project we propose three integrated studies developing the use of fish models to assess the potential health effects of metal/metal oxide nanoparticles (NPs): 1.) exploring the sensitivity of zebra fish embryos to NPs via the application of whole mount *in situ* hybridisation with selected gene targets, 2.) assessing NPs effects at the level of the whole genome (using digital transcriptomics on tissues that accumulate NPs), and 3.) exploring a new fish model (*Xenotoca eiseni*) for studying maternal – offspring transfer of NPs. We require access to the FENAC facility to complete a comprehensive characterisation on the particles raw and in their exposure matrices and to assess tissue burdens in exposed animals.

Characterisation of natural nanoparticles in fumarolic gas/fluids from Ischia and Vulcano (southern Italy)

Dr. E. Kadar; Plymouth Marine Laboratory (http://www.pml.ac.uk/about_us/the_pml_team.aspx)

The fumaroles within the geothermal field on the islands of Ischia and Vulcano (southern Italy) have recently been in the spotlight providing natural laboratory conditions to study adaptations to ocean acidification i.e. valuable predictors of future ocean ecology owing to their elevated CO₂ flux. Moreover, steep pH gradients combined with metal enriched fluid emissions typical at hydrothermal vents (Kadar et al., 2005) are in fact conditions known to facilitate formation of iron rich nanoparticles (NPs) (Kennedy et al., 2004). The actual morphology, mineralogy and chemical properties of the NPs emitted in hydrothermal fluids are virtually unknown because analytical techniques for NP characterisation were not available until recently. We propose to investigate metal-rich and organic nanoparticles from various volcanic fluids and/or gas along a natural pH gradient (Hall-Spencer et al., 2008) as part of a larger scale horizon scanning study to search for and characterise new naturally occurring nanoparticles. This would give us clues to answer to the fundamental nanoscience question: are biological feedbacks to naturally present NPs inherently different from those in response to engineered NPs?

Removal of Radionuclides into Biogenic Hydroxyapatite: Implications for Nanoparticulate Biomineral Remediation Technology

Dr. J. Renshaw; University of Birmingham ([/staff/profiles/gees/renshaw-joanna.aspx](http://staff.profiles.gees.renshaw-joanna.aspx))

Detailed bio-synthesis and sorption experiments supported by FENAC showed that Biological Hydroxyapatite (Bio-HAp) is superior to commercial hydroxyapatite for the remediation of aqueous radionuclides. Studies showed that *Serratia* sp. produces nanoparticulate Bio-HAp with properties that increase metal uptake (i.e. smaller crystallite size of <40 nm and higher surface area of >70 m² g⁻¹). Gradual sintering will slowly change Bio-HAp properties and subsequently affect metal uptake. Evidence from this study will help determine the most stable and appropriate forms of Bio-HAp for nuclear waste remediation and will compliment synchrotron X-ray absorption spectroscopy time which aims to elucidate modes of metal incorporation .

Ecotoxicology of metal oxide nanoparticles in a sediment dwelling invertebrate

Prof. T. Galloway; University of Exeter (http://biosciences.exeter.ac.uk/staff/index.php?web_id=tamara_galloway)

This project will investigate the bioaccumulation and biological effects of zinc oxide nanoparticles (ZnO NPs) in a sediment dwelling invertebrate. ZnO NPs are widely used in sunscreens and cosmetics. The amphipod *Corophium volutator* (mud shrimp) is an OECD model species and constituent of the marine food web. Here, we will combine isotope tracing methods with high detection sensitivities, physico-chemical and bio-imaging techniques, to provide a comprehensive analysis of the effects to *C. volutator* of ZnO NPs, compared with ZnO in bulk and soluble forms. The study will offer insight into the normal physiological processes used to detoxify metal oxides *in vivo*.

Ecotoxicology of cerium oxide nanoparticles in aquatic invertebrates

Prof. T. Galloway; University of Exeter (http://biosciences.exeter.ac.uk/staff/index.php?web_id=tamara_galloway)

This project will investigate the sub lethal biological effects of cerium oxide nanoparticles (CeO₂ NPs) in an aquatic invertebrate species. CeO₂ NPs are widely used as fuel additives and polishing agents. The amphipod *Corophium volutator* (mud shrimp) is an OECD model species and constituent of the marine food web. Here, we will explore the uptake and biological effects of CeO₂ NPs in the gut and storage tissues, with a focus on oxidative damage. The results of this study will aid in determining the risks associated with environmentally relevant exposures to CeO₂ NPs.

2010

Nanoparticles and atherothrombosis: resolving the paradox

N. Mills, University of Edinburgh (<http://www.cvs.med.ed.ac.uk/users/nicholas-mills>)

Exposure to ambient ultrafine particles has been associated with adverse cardiovascular events. Combustion-derived nanoparticles are thought to be the main mediators of these effects. We will attempt to establish the properties of nanoparticles which are most likely to elicit a prothrombotic effect and determine whether pulmonary exposure to nanoparticles can lead to extrapulmonary distribution and contribute to cardiovascular disease. We will take a systematic approach and endeavour to relate nanoparticle toxicity to physicochemical structure, with the hope of developing a QSAR model, which would be applicable to many different types of nanoparticles, from many sources thus aiding in the design of safer nanoparticles for medical purposes and in establishing safe limits of exposure in occupational and environmental settings.

Nanoparticles and their impact on the bioremediation of hydrocarbons in aquatic ecosystems

Ian Colbeck, University of Essex (<http://www.essex.ac.uk/bs/staff/profile.aspx?ID=1115>)

The main aim of the project is to investigate the effects nanoparticles (carbon nanotubes, silver nanoparticles and titanium oxide nanoparticles) on the breakdown of crude-oil hydrocarbons by marine and freshwater hydrocarbonoclastic microbial communities. Further work will later focus on the impact of nanoparticles on other integral processes carried out by indigenous marine microbial populations, such as methane oxidation, nitrification and denitrification. Whilst the microbiological techniques are readily available within the Department a wide range of physical and analytical detection methods are also required to understand how structure and chemistry can influence the environmental function of nanoparticles.

Lung surfactant polymer interactions with nanoparticles

M. Kendall, University of Birmingham (<http://www.pcmd.ac.uk/profiles.php?id=mkendall1&tab=full>)

Clark and Kendall proposed a novel hypothesis that lung surfactant polymers have a role in inflammation of the lung resulting from nanoparticle (NP) exposure. One completed NERC project (Kendall et al 2009) and a MRC project have begun to elucidate the protective role of lung polymers in the toxicology of nanoparticles (Kendall 2009, Submitted to *Nanotoxicology*). Here we propose a NP characterisation project to support these important studies. The project will characterise NP surfaces and aggregation behaviour in interaction with the molecules of interest. The project is a unique collaboration of cross-faculty members and a fledgling PhD project.

Nanoparticle characterisation in floodplain and wetland environments

D. Lapworth, British Geological Survey (<http://www.bgs.ac.uk/staff/profiles/4537.html>)

Groundwater is an important natural resource, providing drinking water and sustaining rivers and wetland ecosystems. Natural nanoparticles, such as mineral particles and complex organic molecules are important vectors for contaminants. Their formation can enhance the mobility of otherwise immobile contaminants, providing a pathway for groundwater and river water pollution, and are important in terms of biogeochemical cycling and bioavailability. This study will characterise the evolution of natural nanoparticles and particle-contaminant associations during flooding and recession cycles at two contrasting sites in the Thames river basin. One site is in the Oxford floodplain, with a history of anthropogenic pollution, the second site is situated in the Boxford wetland area on the River Lambourn.

Nano-crystalline Hydroxyapatite Bio-mineral for the Treatment of Nuclear Wastes

Bacterially produced hydroxyapatite (Bio-HA) is superior to its inorganic equivalents in the sorption and immobilization of toxic radioelements. It has the potential to play a significant role in remediation of existing contaminated sites and future radionuclide release. Building on detailed bio-synthesis and sorption experiments we propose to characterise the materials using the FENAC. Thus providing information that can be related to the sorption behaviour of Sr, Co and U and their modes of incorporation. We will examine Bio-HA material prepared by different bio-syntheses to determine the most stable and appropriate forms of Bio-HA for nuclear waste cleanup.

Characterisation and assessment of bioavailability of cloud-processed dust

E. Kadar, Plymouth Marine Laboratory (http://www.pml.ac.uk/about_us/the_pml_team.aspx)

Consensus is that globally transported mineral dusts contribute to ocean fertilisation (Jickells et al., 2001). Whilst mainly free iron is known to enhance primary production, our recent research indicates that nano-sized iron particles can readily penetrate biological membranes (Kadar et al., 2010). We propose to investigate iron-rich nanoparticles derived from Saharan dust and their influence on phytoplankton productivity. We propose a suite of laboratory experiments using Fe rich Saharan dust (samples already available) exposed to the photochemical processes likely to occur during cloud formation. Sequential extractions and high resolution microscopic techniques will be used to demonstrate changes in morphology, mineralogy and chemical properties of cloud processed mineral dusts. Subsequently, bioavailability of "cloud-mediated" nanoparticles to a well described phytoplankton species (*Emiliana huxleyi*) will be investigated; changes in biochemical and physiological function of the microalgae will be assessed using flow cytometry, microscopy and fluorimetry (Readman et al., 2004) while batch cultures are obtained on growth media fortified with nano-Fe versus conventionally used EDTA-Fe. The proposed study could offer opportunities to tackle global Fe-deficiency concerns.

Ecotoxicology test protocols for zinc oxide and cerium oxide nanoparticles

T. Galloway, University of Exeter (http://biosciences.exeter.ac.uk/staff/index.php?web_id=tamara_galloway)

Nanotechnologies promise significant societal benefits, yet the ecotoxicology of manufactured nanoparticles released into the environment is not well understood. Our work is investigating the fate, behaviour and sublethal effects of OECD-selected and commercially important zinc oxide and cerium oxide nanoparticles to aquatic organisms. To fully interpret our results, we require more detailed knowledge of their physico-chemical behaviour in our biological systems. We request detailed characterisation of both zinc oxide and cerium oxide nanoparticles in different water systems to be able to proceed with more specific and intelligent toxicological studies.

Addressing biological uptake and maternal transfer capabilities of selected metal oxide nanoparticles

C. Tyler, University of Exeter (http://biosciences.exeter.ac.uk/staff/index.php?web_id=charles_tyler)

The effects of exposure pathways on responses to manufactured nanoparticles (MNPs) in aquatic organisms are largely unknown. We will apply 3 exposure scenarios to assess uptake, bio distribution and biological effects of selected metal oxide nanoparticles in fish. The exposures will include dosing CeO₂ NPs in combination with natural organic matter via the water, microinjection of various metal oxide NPs to zebra fish embryos, and dietary exposure of silver NPs to female guppies to investigate maternal transfer. We require access to the FENAC facility to provide comprehensive characterisation data on the particles in their raw state and in their exposure matrices, and to assess tissue burdens in exposed animals.

2009

Assessment of TiO₂ nanoparticle toxicity to aquatic and sediment dwellers

Prof. V. Stone and Prof. T. Fernandes, Edinburgh Napier University (<http://www.napier.ac.uk/fh/ISS/LS/Staff/Pages/Staff.aspx>)

This study aims to investigate the potential toxicity of different TiO₂ particles, varying in size, composition (crystal form) and coatings in a variety of freshwater species (*Pseudokirchneriella subcapitata*, *Daphnia magna* and *Lumbriculus variegatus*). The study allows comparisons between different types of organisms of different habitats in terms of their sensitivity to such particles. In order to understand the nature of the dose of the particles used, their potential for uptake into the organisms and their toxicity, a variety of physicochemical characteristics will be assessed.

Comparative study of the bioavailability of engineered vs naturally occurring Fe-nanoparticles

J.R. Readman, Plymouth Marine Laboratory (http://www.pml.ac.uk/about_us/the_pml_team/staff_directory/james_readman.aspx)

Recent increases in production of novel food additives in the form of Fe nano-particles bring attention to health risks that may be associated with overdose and/or failure of the iron-regulation mechanism. Since the human body has not evolved a mechanism to clear excess iron, disorders of iron balance, such as iron overload and iron deficiency, are among the most common diseases. Understanding the multiple factors that influence nanoparticle toxicity is essential from both human and environmental health perspectives not only regarding nutrition, but also due to its growing diagnostic and therapeutic use. We propose simple *in vitro* systems to assess putative nanoparticle versus solubilised Fe related toxicity indicators that may be used as standard bioassays and applied to other nanoparticles.

Assessment of the Utility of Primary Trout Hepatocyte Culture in Screening Nanoparticles for Potential Toxic Effects

Prof. C. Tyler, University of Exeter (http://biosciences.exeter.ac.uk/staff/index.php?web_id=charles_tyler)

With evidence that some engineered nanoparticles (ENPs) can induce biological harm and high likelihood for significant discharges into the aquatic environment, there is an urgent need to develop appropriate screens and tests for environmental risk assessment. High throughput *in vitro* screens are urgently needed. We have used rainbow trout hepatocyte primary cell cultures to test for biological effects of a range of metal oxide ENPs, including TiO₂, ZnO, CeO₂ and Ag nano- and bulk. We have the biological analyses completed, but require detailed characterizations of the ENPs for a complete interpretation of our results.