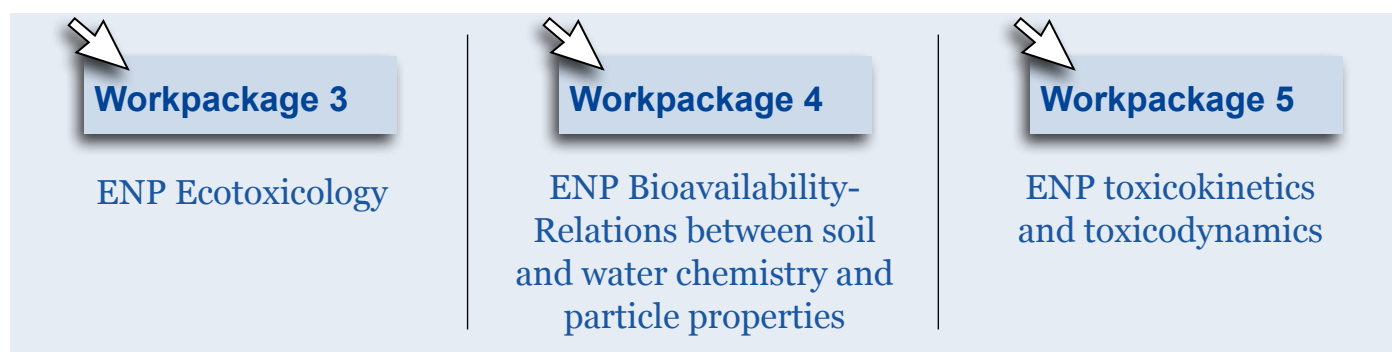


Component 2 Ecotoxicology and Bioavailability

The work is focused on assessing the ecotoxicity of selected engineered nanoparticles (ENPs), including toxicokinetic and toxicodynamic aspects.

Studies and methodological development contribute to:



[Visit the NanoFATE Workpackage descriptions](#)

Many questions within WP 3, 4, 5 are related, and data from all three WPs are needed to gain understanding of the ecotoxicology and bioavailability of Zinc Oxide (ZnO) and Silver (Ag) nanoparticles.

Meet the Master, PhD and Post-Doc scientists conducting research for NanoFATE Component 2

Filipa Calh�a	Fate and Toxicity of ENPs: Subcellular Partitioning of ENPs in Aquatic and Terrestrial Organisms as a Tool to Assess Potential Trophic Transfer
Maria Diez Ortiz	Nano-Ecotoxicity
Maja Halling	Effects of ENPs on Microbial Communities
Laura Heggelund	Nano-Ecotoxicology: Soil Exposure of Earthworms to ZnO Nanoparticles
Pauline Kool	Ecotoxicity of ZnO and Ag Nanoparticles to <i>Folsomia candida</i> in Relation to Bioavailability in Soil
Marianne Matzke	Aquatic Ecotoxicology: Effects of Metallic/Metalloxic Nanoparticles on Single Species (Algae and Bacteria) and on Microbial Communities
Fabianne Ribeiro	Chemical Flow in an Aquatic Trophic Chain
Ilenia Saggese	Biological effects of metal-based and metallic ENPs in the marine bivalve <i>Mytilus galloprovincialis</i>
Paula Tourinho	Effects of Zinc Oxide and Silver ENPs on the Terrestrial Isopod <i>Porcellionides pruinosus</i>
William Tyne	The Effects of Nanoparticles on <i>C. elegans</i>

Filipa Calhã

UAVR

Fate and Toxicity of Engineered Nanoparticles: Subcellular Partitioning of ENPs in Aquatic and Terrestrial Organisms as a Tool to Assess Potential Trophic Transfer



Filipa Calhã, PhD, is a Post-Doctoral Fellow. She has collaborated in projects on Ecotoxicology and Environmental Risk Assessment and she has worked on trophic transfer in soil invertebrates.

Filipa, from Portugal, is working on subcellular partitioning of ENPs in aquatic and terrestrial organisms as a tool to assess potential trophic transfer.



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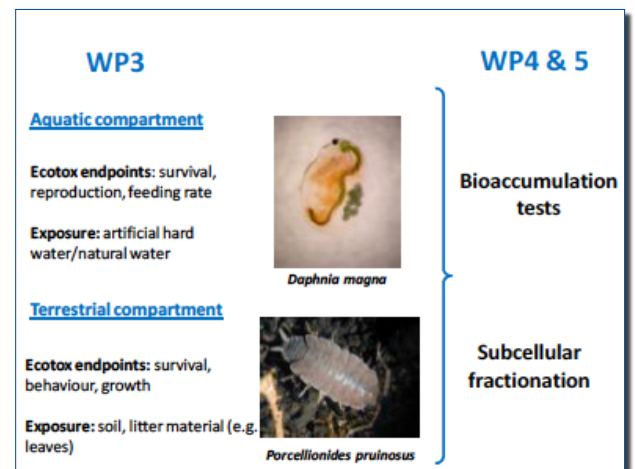
I will seek to understand the variability in the assimilation and fate of ENPs and their corresponding material. My work tests the hypothesis that different ENPs deployed in food, water or soil will influence the manner which these ENPs are detoxified, stored in cells and distributed at subcellular level in isopods, thereby influencing trophic transfer.

In a first phase I selected the following pure ENPs: Nano ZnO 30 nm; Nano ZnO 80-100 nm; ZnO 200 nm; Nano Ag 3-8 nm; Nano Ag 50 nm; AgNO₃. My experimental design varies exposure media or conditions: pH, temperature, or ageing (in soil and in water) or OM (in soil).

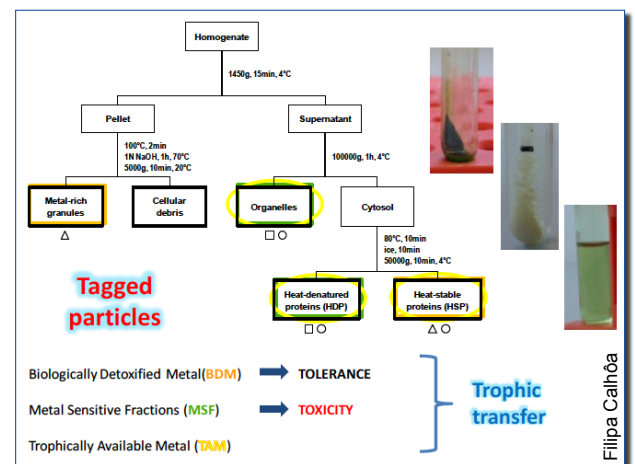
The internal metal concentration strategies of different species are complex and variable and depend on different accumulation strategies. My observations will rely on the subcellular fractionation method. This has been successfully applied in several studies of dietary accumulation of metals, with the purpose of explaining the variability observed in metal accumulation across the different species and food chains.

I will be carrying out an ecotoxicological study of ENPs under environmentally realistic conditions. Combined exposures will be performed to unravel the interactions of ENPs with other environmentally important factors:

- exposure to ZnO particles combined with UV radiation
- mixture effects of Ag and ZnO ENPs
- mixture effects of ENPs and their correspondent material (e.g. Ag ENP mixed with AgNO₃, and ZnO ENP mixed with ZnO/ZnCl₂)
- ENPs from waste water treatment plants.



Subcellular fractionation method



Maria Diez Ortiz

Nano-Ecotoxicity

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Maria Diez Ortiz, PhD, is a Post-Doctoral Fellow. In NanoFATE she will determine the toxicity and kinetics of ZnO and Ag nanoparticles to nematodes and earthworms in relation to bioavailability in soil. From Spain, Maria obtained her PhD in Environmental Science at the Soil Science Department, University of Granada. Maria has been working for 3 years as a Post-Doc at the Vrije Universiteit of Amsterdam on the toxicokinetics of trace elements in soil invertebrates.



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The aim of my work (May 2011-May 2013) is to determine the relative contribution of the different metal forms (single NPs, free metal ions and NP aggregates) to the total ecotoxicity caused by exposure to NPs in soil. The exposed soil organisms I will study are earthworms (*Eisenia andrei*) and nematodes (*Caenorhabditis elegans*). The compounds to be tested are:

- ZnO Nanoparticles, non nano ZnO and ZnCl₂ (supplied by NanoSun, different surfaces, tagged particles)
- Ag NP, Ag NO₃ (8 nm supplied by NanoFATE partner Amepox, 50nm by NanoTrade)

My first task is the assessment of a reliable set of terrestrial ecotoxicity endpoints of ZnO and Ag NPs in soil invertebrates. Subtasks will include:

- Characterization of source materials. I'll look at particle size distribution, zeta-potential and specific surface area.
- Determination of NP fate and speciation in soils. I'll detect NP in bulk soil and soil pore water using image/analytic techniques, and observe total Zn and Ag concentration using filtration and ultrafiltration.
- Examination of NP toxicity to soil invertebrates, using OECD and ISO protocols to look at survival, reproduction and body mass changes after a fixed time period.
- Investigation of NP toxicokinetics and toxicodynamics. I'll run bioaccumulation tests including an uptake and an elimination phase.



Task 2 is the evaluation of ZnO and Ag NP ecotoxicity in environmentally realistic settings. I aim to unravel the effect of ageing in NP toxicity (using freshly spiked soils and soils aged 3 months to one year), and identify the influence of a variety of soil properties on the fate, bioavailability and toxicity of nanoparticles.

This work will help address issues like:

- How crucial NP properties (size distribution, surface chemistry, shape, optical properties) influence toxicity
- How NP-NP and environmental interactions affect the bioavailability of NPs and how different NP forms (free metal ion, aggregates or single NP) affect exposure/uptake
- The specific mechanisms of NP toxicity when compared to the bulk chemical or free metal ion
- Whether existing risk assessment approaches designed for standard chemicals are fit for use with NPs
- Modifications needed to use existing frameworks and policies for the risk assessments of NPs

NanoFATE partners from each component will aid with sectioning and preparation of tissue samples for imaging, with performing electron microscopy, with characterizing particles and identifying their behavior and fate in soils.

Maja Halling

Effects of Engineered Nanoparticles on Microbial Communities

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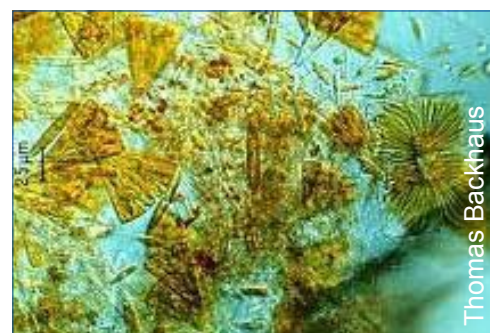


Maja Halling, from Sweden, is performing PhD research on effects of engineered nanoparticles on microbial communities. Maja will implement ecotoxicological hazard studies, applying the SWIFT periphyton test. Maja Halling graduated from University of Gothenburg with a BSc in Environmental Science and a MSc in Zoophysiology.



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I am investigating the effect of engineered nanoparticles on algae and bacteria, and on communities of these. I will perform the Swift periphyton test, and proteomics as an application of the test. Attached microbial communities are also called periphyton or biofilm – or slime, in common parlance! They consist of primary producers (algae) and degraders (bacteria and fungi). A realistic scenario for their exposure to nanoparticles is via wastewater effluents.



The SWIFT periphyton test integrates effects over different trophic levels, including direct effects on individual species and indirect effects caused by changes in species composition. Four phases are involved:



Picture: Henrik Johansson

1. Colonization

- Collection of microbial communities over 7-10 days

2. Toxicant exposure

- 72-96 h exposure
- Filtered river water with added nutrients
- Community succession

3. Detection of effects

- Function and composition of community
- Biomass or gross activity
- Bray Curtis index
- Multi-dimensional scaling.

4. Quantification of effects

Within NanoFATE, my work contributes to:

- Developing the testing procedures, and modifying existing assays for ENP testing for microbial ecotoxicity
- Determining the toxic effect of ENP on natural microbial communities of algae and bacteria
- Finding NOEC and LOEC values for biofilm communities
- Proteomic advances.

Partners will support me with analytical measurements (substance content, size determination before and after exposure, particle characterization in natural waters) and TEM imaging, as well as inputs to the proteomics work.

Laura Heggelund

CEH (NERC)

Nano-Ecotoxicology: Soil Exposure of Earthworms to ZnO Nanoparticles



Laura Heggelund, from Denmark, is working on toxicity of ZnO nanoparticles to earthworms in natural soils and potential associated pH effects.

She graduated from the University of Copenhagen with a BSc in Natural Resources, Environmental Science. As part of her Master's degree in Environmental Chemistry and Health she has conducted experiments at the Center for Ecology and Hydrology comparing the reproductive toxicity of dissolved zinc, non nano scale zinc oxide, and nano zinc oxide.

Her Master's thesis will focus on potential differences in the toxic effects of the three zinc types associated with soil pH.



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My work in NanoFATE aims to compare the toxic effects of dissolved, non nano scale and nano zinc in order to assess whether the current risk assessment for zinc can be applied to nanoparticulate zinc. My focus is soil exposure of earthworms (*Eisenia fetida*) and I will also try to uncover potential effects of soil pH on toxicity of the nano particles.

My terrestrial exposure studies of earthworms involve an initial characterization of nano particles in test media:

- Particle size distribution: Dynamic Light Scattering
- Zeta-potential: Electrophoretic light scattering
- TEM: transmission electron microscopi

I'll perform pH adjustments of soil (CaCO₃ amendment), and look at NP fate and speciation in soils (detection in bulk soil and soil pore water by AAS). Determining the toxicity of NPs to soil earthworms will then rely on 4-week exposure study (weight, reproduction and survival; OECD 2004).

This work should help to reply to the following NanoFATE questions:

1. How do crucial NP properties (e.g. size distribution and zeta potential) influence their toxicity?
2. How does soil pH affect these properties and are there associated toxic effects?
3. Does the existing risk assessment for standard chemicals apply to NPs?

My research will benefit from the help of NanoFATE Component 1 colleagues at Oxford for particle characterization and electron microscopy, and VU Amsterdam colleagues for particle detection in bulk soil and pore water.

Pauline Kool

Ecotoxicity of ZnO and Ag Nanoparticles to Folsomia candida in Relation to Bioavailability in Soil



Pauline Kool is conducting PhD research on the ecotoxicity of ZnO and Ag nanoparticles to *Folsomia candida* in relation to bioavailability in soil, at VU University Amsterdam, The Netherlands. Pauline will determine the toxicity of different nanoparticles to springtails in relation to bioavailability in soil. Pauline obtained her MSc degree in environmental toxicology at the University of Utrecht (The Netherlands) and was employed by TNO (Quality of Life, The Netherlands) for two years, working on the registration of industrial chemicals under REACH.



I'm looking at the effects of ZnO and Ag nanoparticles on the soil organism *Folsomia candida*. This collembolan species lives in top soil layers and is exposed to metals in the soil mainly via its cuticle and ventral tube. Therefore, it is especially important to establish the Zn and Ag concentration not only in the soil but also in the soil pore water.

To determine toxicity animals are exposed to metal nanoparticles in soil in the laboratory and effects on survival, reproduction and growth are studied. Metal concentrations will be determined in soil and soil pore water using flame atomic absorption spectrometry (AAS) and internal body concentration will be analyzed using graphite AAS. I would like to apply RAMAN microscopy in collaboration with Oxford University to visualize nanoparticles in my test animals.

Currently (Summer 2011), an experiment of one year is taking place in our laboratory, assessing the effects of ageing on the bioavailability of ZnO-NP in soil. Soils were spiked with three zinc forms (ZnO-NP, non nano ZnO and ZnCl₂) and incubated at 20°C in glass jars. Sampling after three, six and twelve months was performed to analyse the pore water for dissolved zinc and for exposure of *F. candida*. We found that zinc concentrations in pore water increase with time, with peak at intermediate concentrations (400-800 mg Zn/kg). Data on survival and reproduction of *F. candida* are under assessment at the moment.

My experimental work with *Folsomia candida* will help illuminate:

- The toxicity of ZnO and Ag nanoparticles
- Bioavailability of metal nanoparticles and free metal ions in soil, and soil properties (e.g. pH, organic matter) for input to the NanoFATE hazard assessment
- Bioaccumulation: Uptake and elimination rates of ZnO and Ag nanoparticles will be determined to gain insight into (WP5). Many questions within WP 3, 4, 5 are related and data from all three WPs are needed to answer the questions on the ecotoxicology and bioavailability of ZnO and Ag nanoparticles.

Marianne Matzke

Aquatic Ecotoxicology: Effects of Metallic/Metalloxic Nanoparticles on Single Species (Algae and Bacteria) and on Microbial Communities

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Marianne Matzke is a Post-Doctoral Fellow who gained her PhD in 2008 at the University of Bremen (Center for Environmental Research and sustainable Technologies) contributing to a prospective hazard assessment of ionic liquids for terrestrial systems. Since 2008 she has been investigating the ecotoxicological hazards of silver nanoparticles and titanium dioxide in aquatic and terrestrial systems as well as coordinating a graduate school dealing with effects of engineered nanoparticles for man and the environment (Nano-ToxCom, University of Bremen). Marianne started work at the University of Gothenburg in April 2010.



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In NanoFATE, I carry out an analysis of the effects of silver and zinc nanoparticles on freshwater algae, cyanobacteria, bacteria and aquatic microbial communities. I conduct standard ecotoxicological tests with selected single species (green algae, bacteria and cyanobacteria) as well as aquatic microbial communities.

APPLIED METHODS Single Species Assays

Algae & Cyanobacteria according to OECD Guideline 201, modified

Green algae

Chlamydomonas reinhardtii
Pseudokirchneriella subcapitata

Cyanobacteria

Synechococcus leopoldensis
<http://microbewiki.kenyon.edu>

Diatoms

Selection rationale:

- Diverse in terms of the morphology - different sensitivities?
- *Pseudokirchneriella* & *Synechococcus* were selected according to OECD 201
- *Cyclotella* as a substitute for *Navicula* – planktonic diatom, brackish
- *Chlamydomonas* standard lab species, data available & fully sequenced

Marianne Matzke

Additionally the study undertakes investigation of potential modes of toxic action by e.g using fluorescence stains applying flow cytometry.

ROS & Vitality-via flowcytometry by applying several fluorescence dyes

FDA = Fluorescein diacetate

CC(=O)Oc1ccc2c(c1)oc3c2c(=O)oc4ccccc34

indicator for cell vitality indicating membrane damage

HE = Dihydroethidium

CN1C=CC=C2C(=C1)N(C)C=C2

both are intracellular indicators for ROS

DCF = 2',7'-Dichlorofluorescein

CC(=O)Oc1ccc2c(c1)oc3c2c(=O)oc4cc(Cl)c(Cl)cc43

Marianne Matzke

Using the SWIFT Periphyton assay to characterize the algal community, I'll identify shift in types of algal pigments via HPLC (indicating changes in the community structure) and change in quantity (giving information on the biomass). The bacterial community will be characterized using BIOLOG plates. A change in the pattern of ability to metabolize various carbon sources indicates a change in the community structure.

Marianne Matzke / UGOT

At the end of 18 months, we have quantities of data and it's time to reflect about what they tell us. We are facing and refining NanoFATE questions like these:

- *Are there nanospecific effects? That is, are the nanoparticles more toxic than the “bulk” form?*
- *Are there differences in sensitivity between the different species? Which is the most sensitive for AgNP hazards? Can we identify possible explanations for mode of toxic action?*
- *Which modifications of the standard test systems are necessary for the detection of nano-related hazards?*
- *What is the influence of the test medium on the bioavailability of the metal ions/nanoparticles?*
- *What are the effects of the particles on aquatic microbial communities? What does comparison to the standard ecotoxicological single species test systems tell us?*

The answers have broad implications for future study of ENP ecotoxicology and bioavailability.

Fabianne Ribeiro

Chemical Flow in an Aquatic Trophic Chain



*Fabianne Ribeiro is a PhD student at the University of Aveiro, working with biological effects of silver nanoparticles in three different aquatic species. She obtained her Master degree at the University of Aveiro in 2009, evaluating effects of combined exposures of chemical and natural stressors on *Daphnia magna*.*



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During NanoFATE I will be doing the ecotoxicological assessment of potential risk of silver nanoparticles to non-target organisms, like the water flea *Daphnia magna*.

This involves exposure of *Daphnia magna* to silver nanoparticles to assess their toxicity and the bioaccumulation. In conducting this exposure I will follow the OCDE guidelines for testing with chemicals – and adapt them for testing with nanoparticles.

The bioaccumulation tests analysing *Daphnia magna* tissues will rely in part on TEM imaging and analytic expertise from NanoFATE colleagues (Component 1) to track the distribution of NP inside organisms. I will also receive partner input on the behavior of nanoparticles in the *Daphnia* test media.



Within NanoFATE, my work will help answer the following questions:

- *Are nanoparticles more toxic to aquatic organisms than the same material in non-nano size?*
- *Can NP be more accumulated by organisms due to their small size and specific properties?*
- *Which are the biological compartments in which NP will mostly end up? Is there an affinity to a certain organelle or tissue?*

Ilenia Saggese

UNIPMN

*Biological effects of metal-based and metallic ENPs in the marine bivalve *Mytilus galloprovincialis**



Ilenia Saggese, from Italy, is performing a PhD study of ecotoxicology, toxicokinetics and toxicodynamics of ENPs in seawater organisms at UNIPMN. Ilenia will determine the toxicity of different nanoparticles in marine mussels in relation to bioavailability in seawater.

Ilenia has good experience in ecotoxicological studies with both soil and aquatic organisms using ultrastructural and cytochemical approaches.



My research topic involves the exposure of mussels to ENPs to identify the acute and chronic toxicity range. I will set up facilities for the exposure of the marine organism *Mytilus spp* in artificial seawater. Experimental exposures will allow me to look into bioaccumulation and localization of ENPs in biological tissues of mussels, as well as study the sublethal effects of ENPs in mussel tissues. I will test the influence of water physico-chemical parameters on ENP toxicity in mussels.

Methodology includes the development of facilities for the production of large quantities of artificial seawater (about 2000L). Controlled exposures will be carried out in semi-static condition, with chemicals added daily along with water renewal from freshly prepared stock water-suspension.

This research will help answer NanoFATE questions as follows:

- *What is the acute and chronic toxicity range in marine filter feeding organisms (*Mytilus galloprovincialis* Lam)?*
- *What is the main driver of ENP toxicity for marine sea water organisms among physico-chemical parameters (such as temperature, pH, organic matter content)?*
- *What is the main uptake route for ENPs in marine mussel?*
- *What is the role of oxidative stress in ENP toxicity?*
- *What are the sublethal effects of ENPs in mussel tissues?*
- *What are the differences with core metals? Does a “nanoparticle” effect per se exist?*

I will obtain input from Component 1 partners on characterization and fate of nanoparticles in seawater, and TEM-EDX analysis on mussel tissues.

Paula Tourinho

UAVR

Effects of Zinc Oxide and Silver Engineered Nanoparticles on the Terrestrial Isopod Porcellionides pruinosus



Paula Tourinho, from Brazil, is performing PhD research on the effects of nanoparticles on woodlice at University of Aveiro, Portugal. Paula will undertake isopod experiments, performing standardized ecotoxicity testing and observing mortality, avoidance behavior and feeding inhibition as endpoints. She obtained her Bachelor degree in Oceanology at Federal University of Rio Grande.



To evaluate the toxicity of nanoparticles, terrestrial isopods (or woodlice) will be exposed to contaminated soil and food. The isopods will be exposed to different concentrations of ZnO and Ag nanoparticles in soil and food (partners from NanoFATE Component 1 will aid in analyzing the soil and food). I will assess toxic effects through an acute test, as well as tests of avoidance behavior or feeding inhibition. For bioaccumulation tests, the isopods will be exposed to a single non toxic concentration, and then their levels of zinc and silver will be measured. I will look at biomarkers too.

NanoFATE partners from Component 1 will help me to understand the nanoparticles' behavior, such as their dissolution and aggregation processes. I will also get input on designing experiments using concentrations of nanoparticles predicted to reach the environment.

My work will answer the following questions:

- *What concentrations of nanoparticles affect a soil organism?*
- *How and where in the isopods' body are the nanoparticles accumulated?*
- *What mechanisms are involved in the detoxification of nanoparticles?*

William Tyne

The effects of nanoparticles on C. elegans

CEH (NERC)



Post-Doctoral Fellow Bill Tyne has worked for CEH since 2000. He undertook a PhD part time in the phylogenetic analysis of baculovirus chitinase and baculovirus-like chitinase (completed in 2009). His main skills lie in the field of molecular biology and biotechnology. He joined the ecotoxicology group at CEH Wallingford in 2010 and is currently working on the toxicity of nanoparticles in nematodes as part of the NanoFATE project.



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I'm working with the nematode *C. elegans* because it is a standard lab organism, well characterised and easy to manipulate soil organism. I'll be performing toxicity trials (fecundity at different exposures) paying attention to effects of pH and of organic matter content. A "long term" exposure program is a possible with *C. elegans* as it has a short life cycle. Such a program may allow us to identify the development of resistant traits through successive generations.

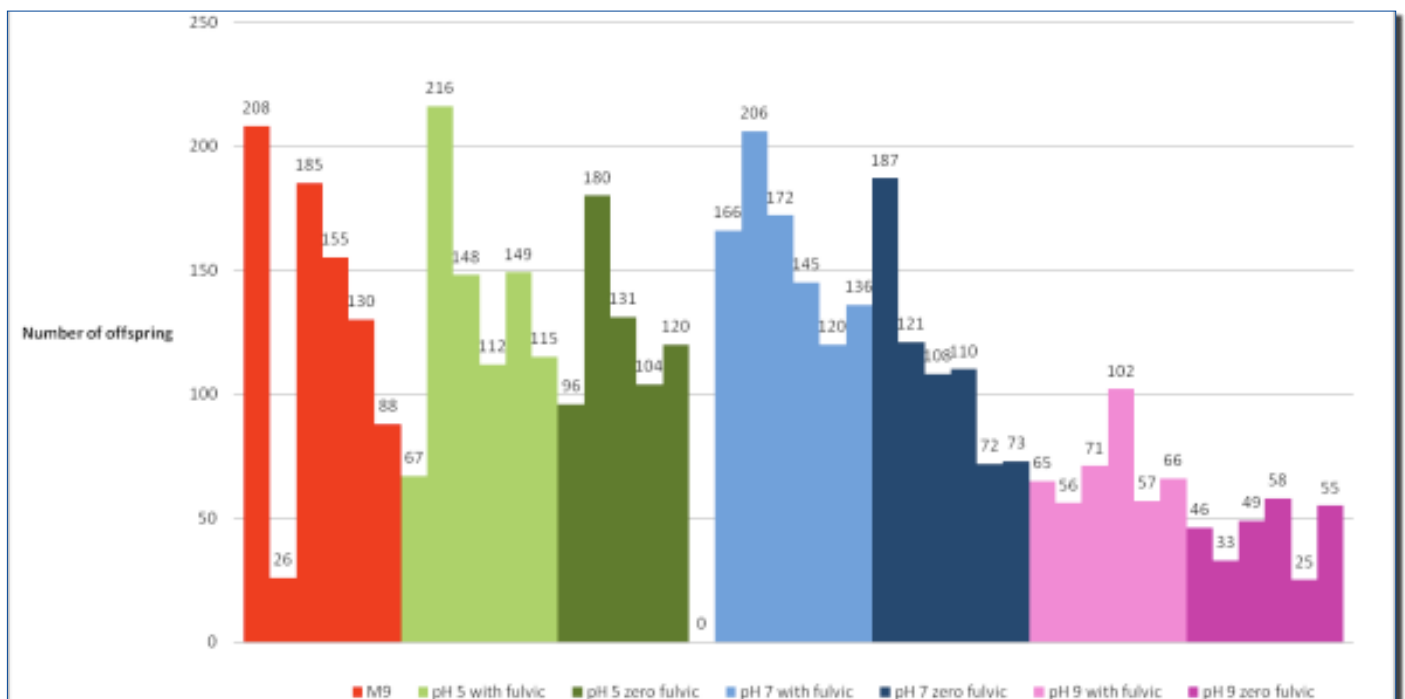
Skills in Raman microscopy and molecular biology will be called into play.

An early step is the creation of synthetic soil pore water. Why create a new medium?

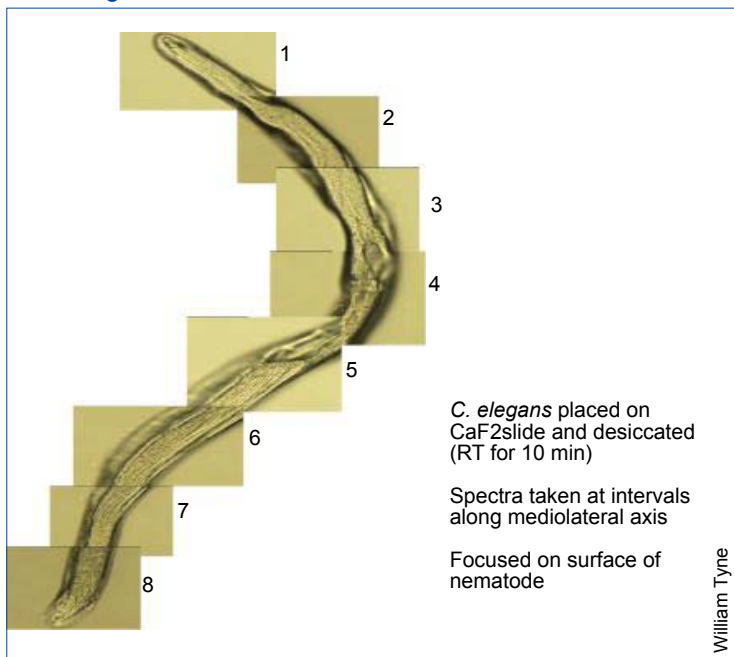
- The OP-50 food source will not grow on silver impregnated agar
- The phosphate components of M9 cause precipitation when silver is added
- The SSPW is more "realistic"
- pH and organic matter content can be manipulated

Synthetic Porewater allows characterisation of particle behaviour at different pHs and at different organic matter concentrations. The particle behaviour can then be linked back to effects on the organism.

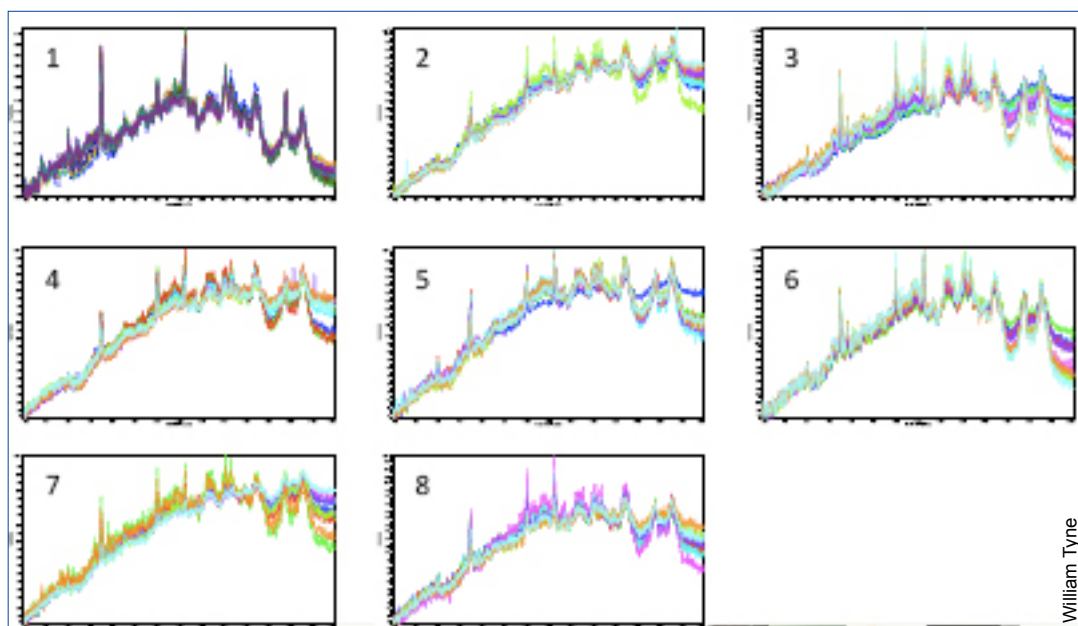
The most interesting thing I can do here is share some images from my work:



Scanning the nematode



Differences down the length of the nematode+



As for where I fit into NanoFATE:

- Development of standard protocol for Nanoparticle toxicity trials
- Toxicity trials using ENPs at different pH values and different organic matter contents
- Raman studies provide evidence of particle uptake and effects on the organism
- Knock out mutants and transcriptomics studies will provide a more detailed analysis of the mechanisms of particle toxicity.