# Cellular responses of *Eisenia fetida* coelomocytes exposed to wastewater treatment plant-transformed isotopically enriched nanomaterials

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## INTRODUCTION

Most nanomaterials (NMs) used in commercial applications enter wastewater streams and reach wastewater treatment plants (WWTP), where the vast majority is concentrated in sewage sludge and applied on agricultural land. By the time NMs enter soils, they have undergone various transformation processes, which results in particles with altered properties, bioavailability and toxicity which can differ from the original pristine NMs. The environmental hazard of environmentally relevant chemical forms of NMs is critical knowledge for risk assessment.

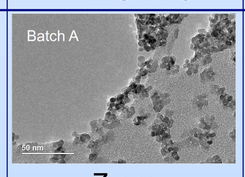
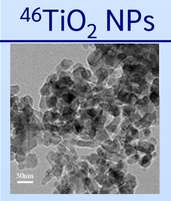
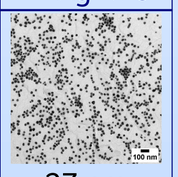
**Aim**: to assess the potential environmental hazard of WWTP-transformed NMs in environmentally relevant exposure conditions.

## APPROACH

### Isotopically enriched nanomaterials

- Diagnostic isotope ratio of samples determined by mass spectrometry
- Very sensitive detection
- Tracing at low levels at complex matrices

**Table 1.** Overview of the isotopically enriched $^{68}ZnO$, $^{46}TiO_2$ and $^{109}Ag$ NPs used in the study. The NPs were analysed by SEM, TEM, DLS and ICP-MS (isotope ratio).

| | $^{68}ZnO$ NPs | $^{46}TiO_2$ NPs | $^{109}Ag$ NPs |
|---|---|---|---|
| TEM |   |   |   |
| | 7 nm | 26-60 nm | 27 nm |
| DLS | 19 nm | µm scale | 28 nm |

### Pilot wastewater treatment plant

- Spiking to a pilot WWTP receiving municipal wastewater (Fig. 1)

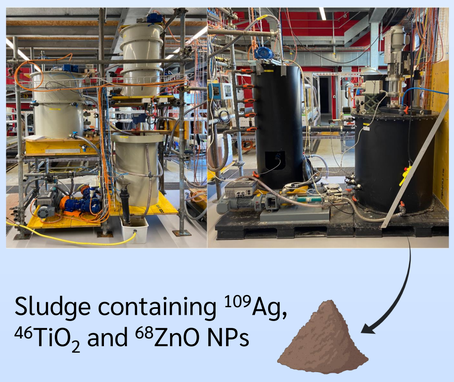



**Figure 1.** Pilot WWTP comprising of a conventinal activated sludge system (CAS) and an anaerobic digester unit. $^{109}Ag$ NPs were spiked into the CAS, $^{46}TiO_2$ and $^{68}ZnO$ NPs were directly spiked into the digester unit. Sludge for the exposure experiments was collected from the digester unit.

### Exposure in soil microcosms and effects

- Exposure to soil amended with sludge derived from the WWTP spiked with $^{109}Ag$, $^{46}TiO_2$ and $^{68}ZnO$ NPs
- Coelomocytes (primary immune cells) as model to study effects

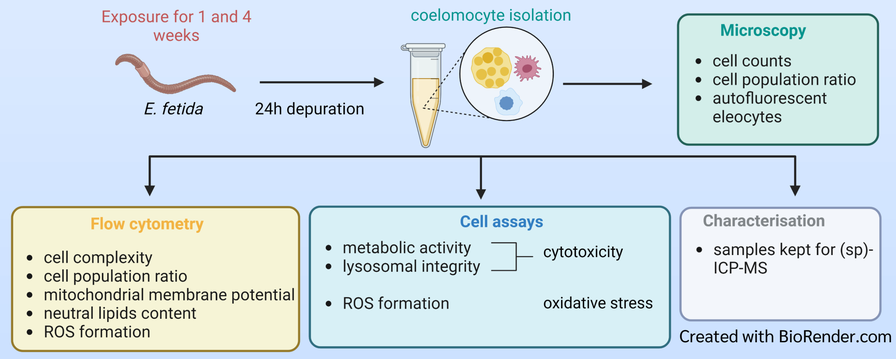



**Figure 2.** The earthworms were exposed for 1 and 4 weeks in soil amended with digested sludge derived from the pilot WWTP spiked with isotopically enriched $^{68}ZnO$, $^{46}TiO_2$ and $^{109}Ag$ NPs, applied at 20t sludge/ha (6.7 mg dw sludge/g dw soil). At the end of the exposure the earthworms were depurated, the coelomocytes were isolated and effects at a cellular level were assessed.

## RESULTS

### Effects of sludge-amended soil

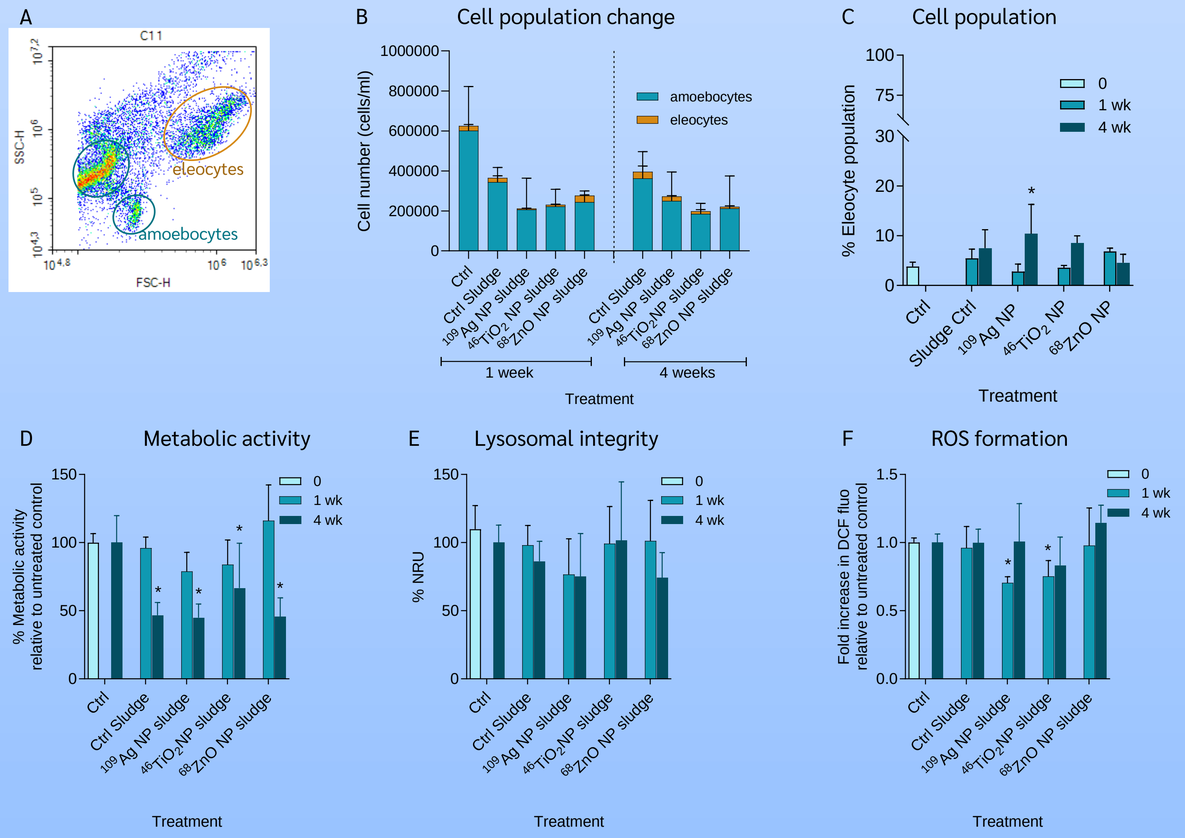



**Figure 3.** (A) Representative density plot (FSC-H size versus SSC-H cell complexity) of coelomocytes isolated from earthworms incubated in control soil. Effects of soil amended with sludge containing isotopically enriched $^{68}ZnO$, $^{46}TiO_2$ and $^{109}Ag$ NPs on (B, C) cell population composition, (D) metabolic activity, (E) lysosomal integrity and (F) ROS formation of coelomocytes isolated from earthworms exposed to 6.7 mg dw sludge/g dw soil (20 t/ha) for 1 and 4 weeks.

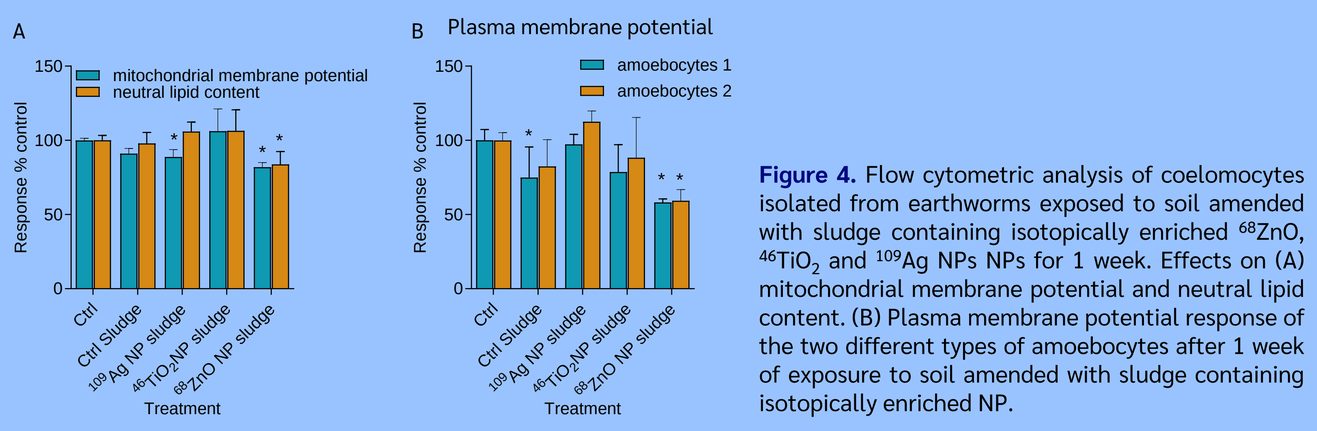



**Figure 4.** Flow cytometric analysis of coelomocytes isolated from earthworms exposed to soil amended with sludge containing isotopically enriched $^{68}ZnO$, $^{46}TiO_2$ and $^{109}Ag$ NPs NPs for 1 week. Effects on (A) mitochondrial membrane potential and neutral lipid content. (B) Plasma membrane potential response of the two different types of amoebocytes after 1 week of exposure to soil amended with sludge containing isotopically enriched NP.

### Analysis of sludge and sludge-amended soils

**Table 2.** Analysis by ICP-MS of the different types of sludge and soil amended with the sludge from the pilot WWTP spiked with $^{68}ZnO$, $^{46}TiO_2$ and $^{109}Ag$ NPs.

| Matrix | Type | $^{68}ZnO$ NPs | $^{46}TiO_2$ NPs | $^{109}Ag$ NPs |
|---|---|---|---|---|
| Sludge | Enriched | 90.8 ± 0.4 mg/kg | 32.7 ± 1.4 mg/kg | 19.9 ± 0.1 mg/kg |
| | Natural | 626 ± 5 mg/kg | 946 ± 7 mg/kg | 6.8 ± 0.2 mg/kg |
| Soil | Enriched | 0.55 ± 0.03 mg/kg | 0.22 mg/kg | 126 ± 4 µg/kg |
| | Natural | 57 ± 5 mg/kg | 3 ± 0.3 g/kg | 72 ± 9 µg/kg |

## HIGHLIGHTS

Under **environmentally relevant exposure conditions** – low concentrations and relevant NP chemical forms:

- **$TiO_2$ NP** did not induce adverse effects at a cellular level in earthworms
- **Ag NPs** led to cell population alteration and affected mitochondrial membrane potential
- **ZnO NPs** induced a signficant derease in mitochondrial and plasma membrane potentials.
- Amoebocytes sensitive cell type to **ZnO NP** sludge exposure

## ONGOING WORK

- Cellular uptake by single particle ICP-MS
- Immune function-associated effects
- Role of the different cell types in the observed effects

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