Precious Metals Consortium

Soil ecotoxicity and dissolution of a marketed nanosilver product - a direct comparison with ionic silver

***J Mertens**, K Arijs, E Smolders, D Leverett, K Oorts*

Introduction

- **Nanomaterials**:
  - engineered for specific phys-chem and biological characteristics
  - reactivity and behaviour:
    - dependent on properties (like specific surface area)
    - **might differ** from bulk form

- **EU-REACH**:
  - register substances put on EU market if >1 t/y
  - REACH registration dossier Ag metal **includes nanosilver**

Substance Evaluation

- **Substance Evaluation** by NL (started in 2014)
  → concern related to **nanoAg**
  → request for **additional data**: comparison nanoAg vs Ag salt
  - **TIER1**:
    - ecotox testing nanoAg vs ionic Ag:
      - toxicity to algae (OECD 201)
      - long-term toxicity to aquatic invertebrates (OECD 211)
      - toxicity to soil microorganisms (OECD 216) in 3 different soils
    - proper **characterisation** nanosilver
    - **dissolution rate** in the specific test media
    - information on **uses** of nanoAg
  - **TIER2** (*only if in TIER1: toxicity nanoAg > ionic Ag!)*
    - quantitative info on **fate** of nanoAg in soil

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Poster **TH081** '*REACH Substance Evaluation of silver – justification of read-across from ionic silver to nanosilver*'

  - toxicity to soil microorganisms (OECD 216) in 3 different soils
  - proper **characterisation** nanosilver
  - **dissolution rate** in the specific test media
  - information on **uses** of each registered Ag nanoform
- **TIER2** (*only if in TIER1: toxicity nanoAg > ionic Ag!)*
  - quantitative info on **fate** of nanoAg in soil porewater & solid fraction

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Poster **MO410** '*The aquatic ecotoxicity of a marketed nanosilver product – a direct comparison with ionic silver*'

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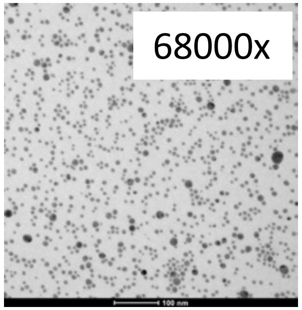
Focus of this presentation

cfr. '*Transformation-dissolution reactions partially explain adverse effects of metallic silver nanoparticles to soil nitrification in different soils*'

by Bollyn et al

(*accepted for publication in Environmental Toxicology and Chemistry*)

Test setup (1/3)



TEM image

- **Test compounds**:
  - nanosilver ('***nanoAg***'):
    - aqueous suspension (37% Ag), spherical particles
    - mean prim. particle size 8.4 nm, volume SSA: 714 m^2 cm^{-3}
  - **$AgNO_3$** as silver salt:
    - 63.5% Ag (purity >99.9%)
- **Soils**:
  - European arable soils (0-20 cm)

	pH	OC *[%]*	Sand/silt/clay *[%]*	CEC *[$cmol_c$ kg^{-1}]*	Ag_{bg} *[mg kg^{-1}]*
Rots (FR)	7.3	1.3	20/50/10	14.3	0.4
Lufa 2.2 (DE)	5.4	1.6	76/17/8	9.7	0.4
Poelkapelle (BE)	6.0	3.8	17/66/16	19.7	0.1

! properties: P10-P90 for European soils (Reimann et al 2014)

! pH/OC correlated with Ag tox in soil (Langdon et al 2014)

Test setup (2/3)

- **Ag sampling**:
  - **total** Ag in soil (*hot HNO_3 digestion*)
  - Ag in **porewater** (*centrifugation, double chamber system*)
    - '***total*** dissolved Ag' (filtration, <0.45 µm)
    - '***truly*** dissolved Ag' (ultrafiltration, <1 kDa)
- **Ag dosing**:
  - 1 wk preincubation, triplicate spiking at 7-8 doses, 1 wk incubation

	Ag doses in *[mg kg^{-1}]*	
	$AgNO_3$	***AgNP***
Rots	0 - 1536	0 - 2474
Lufa 2.2	0 - 492	0 - 669
Poelkapelle	0 - 3355	0 - 4563

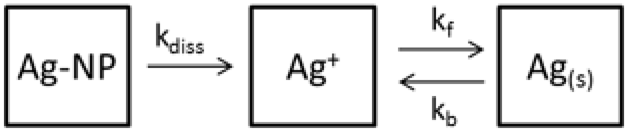
! No leaching cfr Langdon et al 2014

Test setup (3/3)

- **Nitrification assay**:
  - addition 100 mg NH_4-N kg^{-1}
  - measurement NO_3-N concentration at days 0 - 7 - 14 - 28
  - **endpoints**: Potential Nitrification Rate ('***PNR***'; 0-14 d) and Substance Induced Nitrification ('***SIN***'; 0-28 d) [mg NO_3-N kg^{-1} d^{-1}]

    ! NO_3 added via $AgNO_3$ – exclusion data >1000 mg Ag kg^{-1}

- **Transformation-dissolution test**:
  - spiking with 50 mg Ag kg^{-1} (as $AgNO_3$ or nanoAg)
  - pore water sampling at days 1 - 4 - 7 - 14 - 35 - 97 after spiking
  - total dissolved Ag (<0.45 µm) and truly dissolved Ag (<1 kDa)
  - concentration dynamics fitted with a **three-compartment model**



Nitrification test

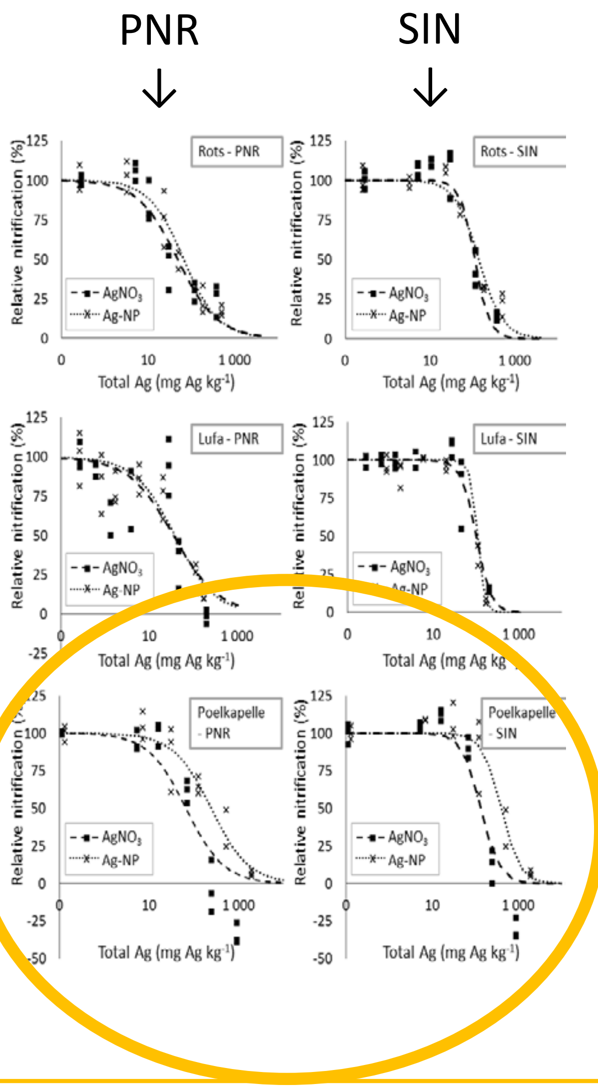
- Nitrification in **control soil** [*mg N kg^{-1} d^{-1}*]:

	PNR	SIN
Rots	6.0	3.1
Lufa 2.2	2.9	2.5
Poelkapelle	5.7	3.0

- Nitrification **decreases with increasing Ag** doses:

PNR	**EC10** *(in mg kg^{-1} d^{-1})*		**EC50** *(in mg kg^{-1} d^{-1})*	
	AgNO$_3$	***nanoAg***	***AgNO$_3$***	***nanoAg***
Rots	4.8	9.0	49	68
Lufa 2.2	3.8	3.8	36	38
Poelkapelle	**8.1***	29	**66***	242

**significantly different value (p<0.05)*



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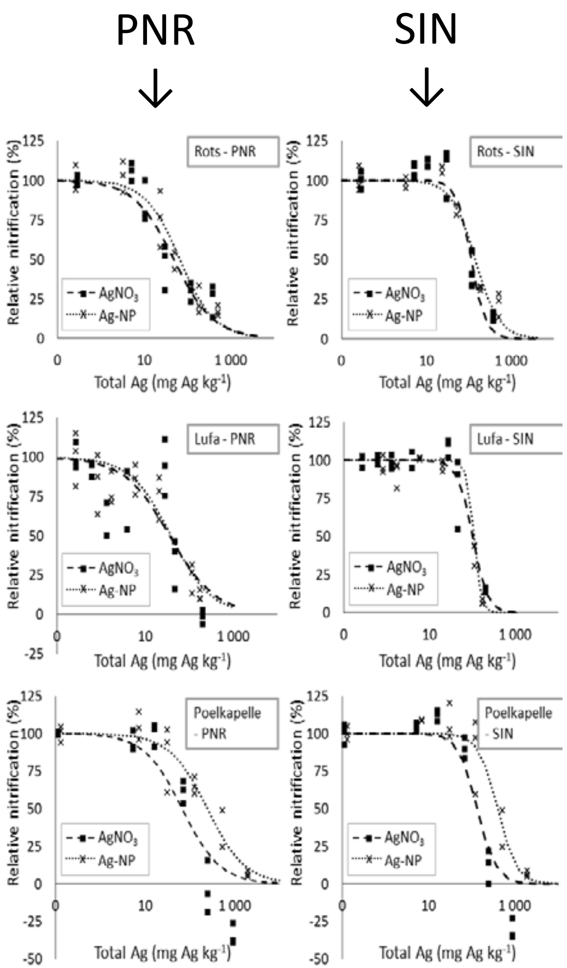
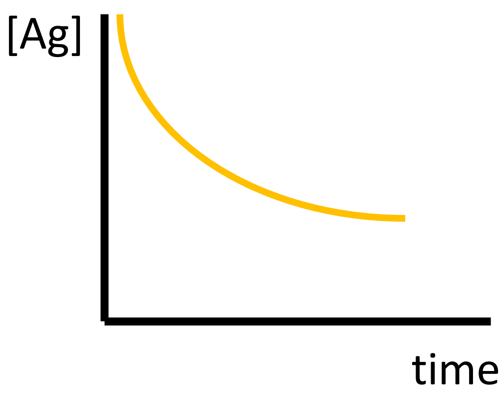
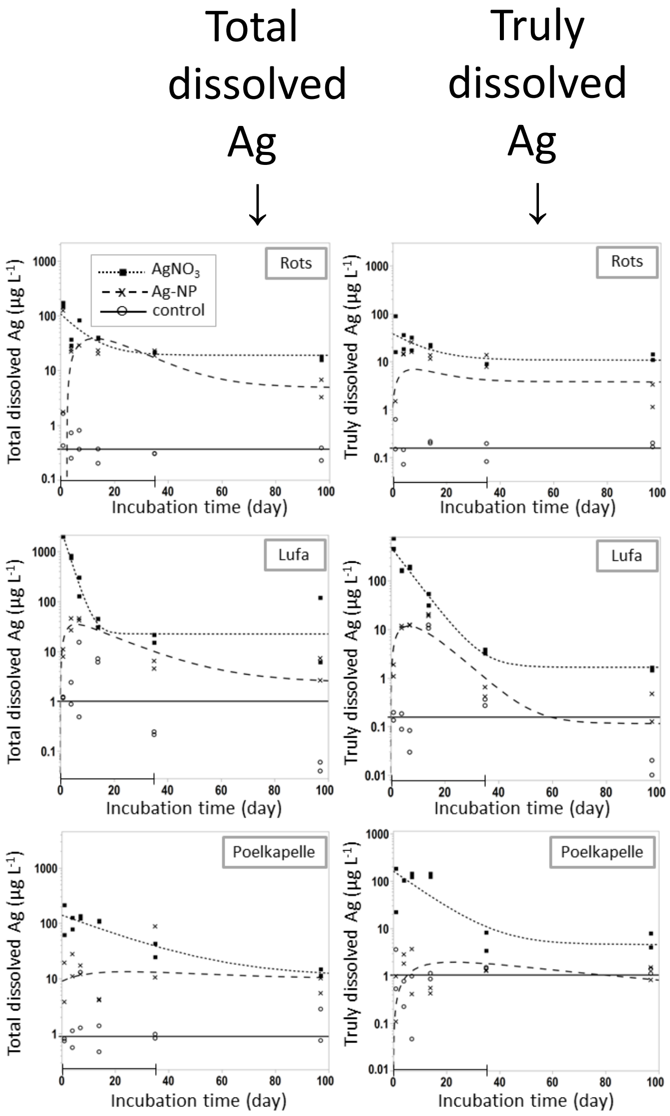


ECx nanoAg <u>similar or higher</u> than $AgNO_3$

Transformation/dissolution

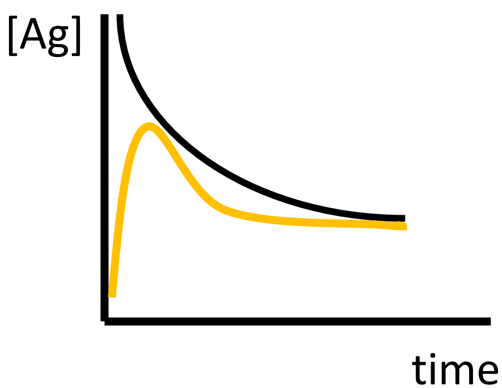
- $AgNO_3$ spiked soils: **decrease** [Ag] over time





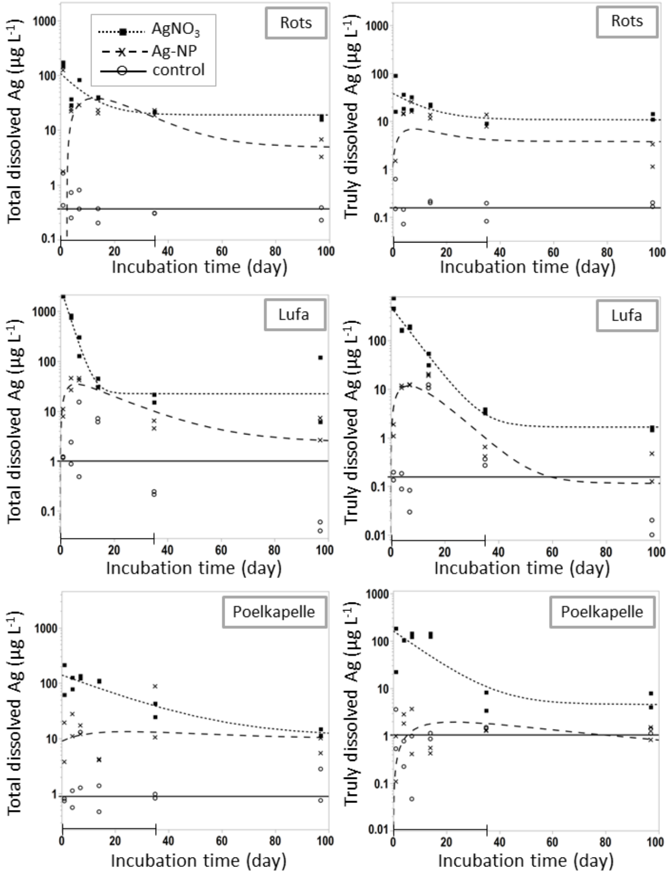
Transformation/dissolution

- $AgNO_3$ spiked soils: **decrease** [Ag] over time
- NanoAg spiked soils: **initial increase, followed by decrease** of [Ag]



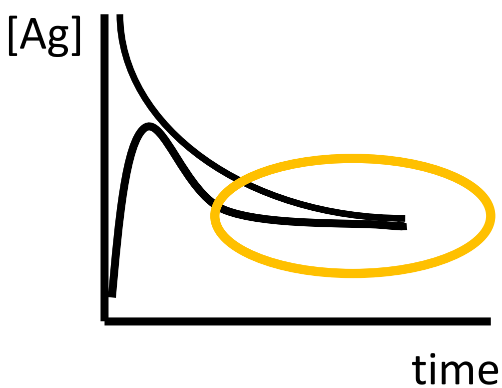
Total dissolved Ag ↓

Truly dissolved Ag ↓



Transformation/dissolution

- $AgNO_3$ spiked soils: **decrease** [Ag] over time
- NanoAg spiked soils: **initial increase, followed by decrease** of [Ag]
- Dissolved Ag initially higher in $AgNO_3$ vs AgNP, but **similar over time**



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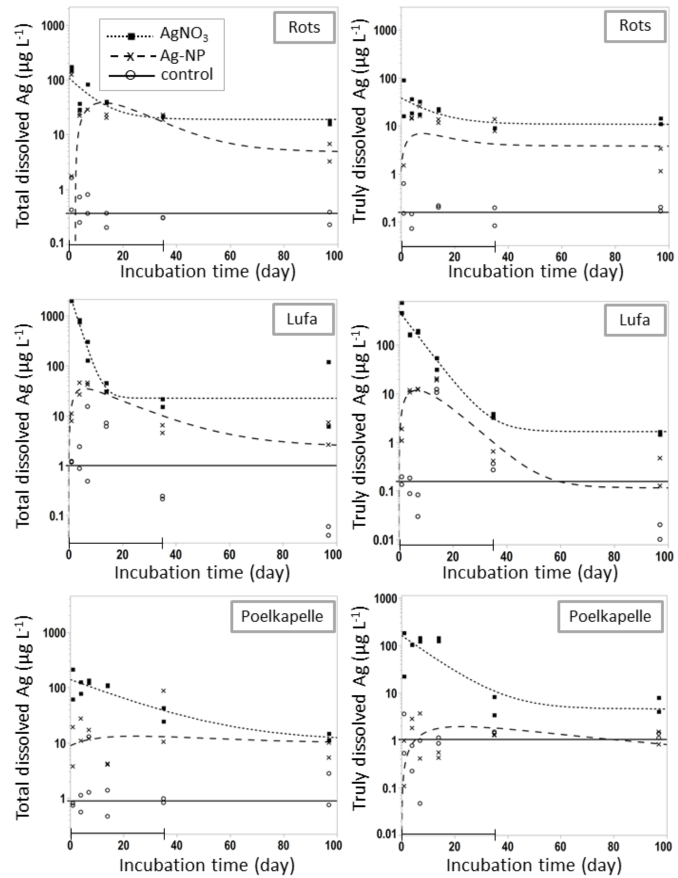


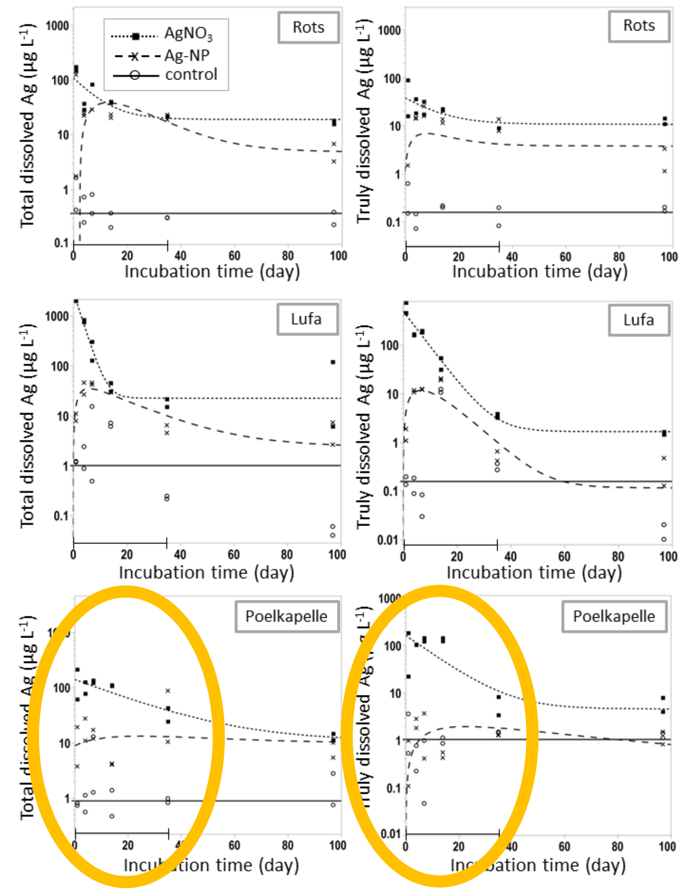
Transformation/dissolution

- $AgNO_3$ spiked soils: **decrease** [Ag] over time
- NanoAg spiked soils: **initial increase, followed by decrease** of [Ag]
- Dissolved Ag initially higher in $AgNO_3$ vs AgNP, but **similar over time**
- **Ratio** $\frac{[Ag]_{AgNO3}}{[Ag]_{nanoAg}}$ day 7-35 (= nitrification test!):

	Total diss Ag	*Truly diss Ag*
Rots	1.7	1.3
Lufa 2.2	3.2	8.4
Poelkapelle	15	140

Total dissolved Ag ↓

Truly dissolved Ag ↓

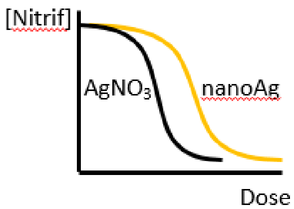


Data interpretation

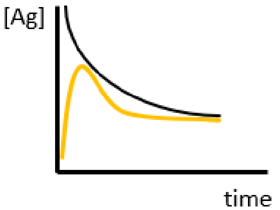
- **ECx nanoAg ≥ $AgNO_3$**

  ⟹ nanoAg **equally or less toxic** than $AgNO_3$

  cfr. conclusions metastudy Notter et al (2014)



- **Transformation/dissolution:**
  - importance of **dissolution + ageing** reactions
  - nanoAg dissolution explaining effects for Poelkapelle and Rots
  - Lufa 2.2: truly dissolved [Ag] **higher** for $AgNO_3$ than nanoAg



Data interpretation

- **Pending questions**:
  - role of local **hotspots** ??

  - further research on **transformation/dissolution** at multiple doses

  - **porewater fractionation:**
    - truly dissolved Ag = ionic Ag & small Ag complexes (org/inorg)
    - re-speciation over time

  - **analytical challenges** associated with Ag determination
    - need for strong dilutions

      → proportional contribution $[Ag]_{bg}$ increases with dilution
    - enhanced dissolution during isolation

Conclusions

- **Experimental & analytical challenges** for Ag detection (~low [Ag] at relevant test conditions)
- Importance of **equilibration / ageing**
- **No indications of nano-effect** when expressed as $[Ag]_{total}$
- **Dissolution** partially explaining observations
- **Read-across ionic Ag to nanoAg conservative!**

Further reading:

'***Transformation-dissolution reactions partially explain adverse effects of metallic silver nanoparticles to soil nitrification in different soils***'

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(*accepted for publication in Environmental Toxicology and Chemistry*)

THANK YOU

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