Environmental Impact Assessment for Nanotechnologies: Integrating the Ecological and the Chemical Perspective

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Research Focus:
Environmentally Benign Nanomaterials

Nanotechnology

- Exponential growth since ≈ 10 years
- Very recent – hardly any knowledge
- Uncertainty very high
- Very basic principles not understood

➢ Mainly science in this talk – and little policy

Why we Need an Integrated View in Nanoparticle Risk Assessment

Ecotoxicology has been interdisciplinary ever since:

How does the substance behave?

Substance properties, e.g.
- Structure formula
- Solubility
- Lipophilicity
- Vapour pressure

Analytics - Detection

Environmental fate, e.g.
- Sorption
- Transport
- Bioavailability

Effects on organisms, e.g.
- Mortality
- Reproduction
- Behaviour

Why did an effect occur (or not)?

Why we Need an Integrated View in Nanoparticle Risk Assessment

Chemistry and biology mutually support each other:

What substances are in the the environment?

Analytics are difficult and expensive

Organisms are sensitive

Scenario A: The communities look healthy and services are provided ☺ – no action needed!

Scenario B: Something is wrong here ☹ - what could it be?

Nanoparticles in the Environment

Nanoparticles have existed ever since…

However, **engineered** nanoparticles have not

What is so Special about Nano?

Thus far, the aforementioned chemical properties (and some more) have been sufficient – but engineered nanoparticles

- Have new properties,
  e.g. proven potential to cross the blood-brain barrier
- Very high surface → extremely increased reactivity
- Are often very complex (composite particles)
- Are often not alone (coatings, stabilizers)

What is so Special about Nano?

- „So small that the particles are just surface“
- Surface properties differ strongly from bulk properties

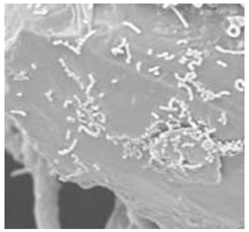
Important questions:

- How small?
- Which shape? (e.g. spherical or nanotubes, asbestos)
- Are they stable? Do they dissolve? ($Ag^0 \leftrightarrow Ag^+ + e^-$)
- What is their behavior in suspension?
- What happens in biological media?

A Short Story about Silver Nanoparticles

- In many consumer products, e.g. clothing, cosmetics, curtains, packings
- Long tradition as „bulk material“
- Human health risk very low
- Excellent antimicrobial action

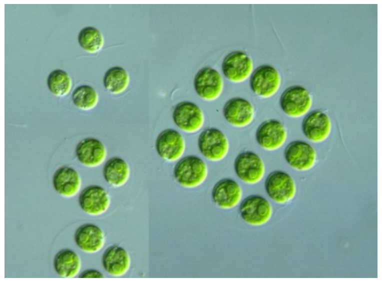
➢ **Up to now no specific risk assessment**



➢ What about the essential services provided by microorganisms?
- e.g. nutrient remineralisation
- breakdown of pollutants

Chemistry is difficult enough...

Calculated speciation (MINEQL+) and cellular uptake of Ag species by *Chlamydomonas reinhardtii* as affected by silver and chloride concentration



Fortin & Campbell 2000, Environ Toxicol Chem

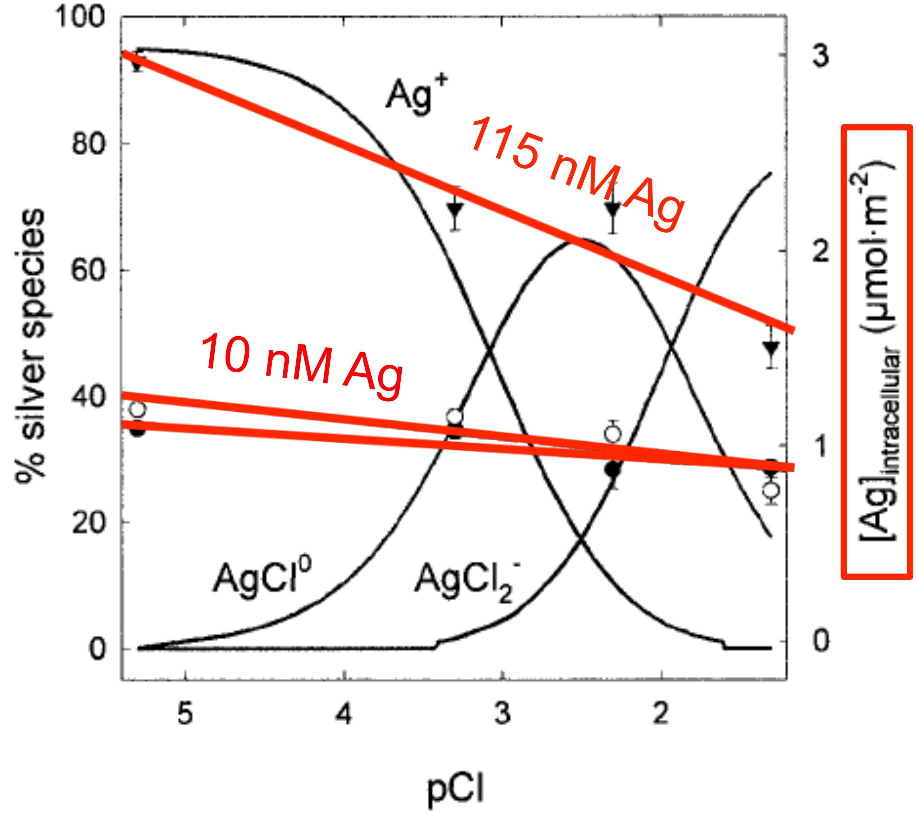


Fig. 6. Calculated distribution of silver species as a function of pCl using MINEQL+ (solid curves referring to the left axis). Short-term (15 min) intracellular silver uptake as a function of pCl for 10 nM (●, ○) and 115 nM (▼) total silver (right axis). Dark symbols represent results obtained with the same cell culture; straight dashed lines were drawn as linear regressions of the data. Error bars represent standard deviations for the average of three measurements.

Composition of Biological Media

Test	Ionic strength [mM]	Main Components
Lemna	9.2	KNO_3, potassium phosphate buffer, Ca $(NO_3)_2$
Algae	26	NaCl, KNO_3, sodium phospate buffer
Arthrobacter	28	NaCl, Glucose, proteins
Acetylcholin Esterase	50	Sodium phospate buffer
RPMI	168	NaCl, sodium phosphate buffer, $NaHCO_3$, Glucose, KCl, amino acids

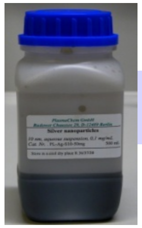
Particle Diameter and Ionic Strength

d (DCS) [nm]	AgNP, 50 mg/L	Ionic strength [mM]
Water	14 ± 4	≈0
Lemna	25 ± 7	4.6
Algae	42 ± 25	13
Arthrobacter	67 ± 27	14
AChE	38 ± 10	25
RPMI	96 ± 26	84

Particles agglomerate at high ionic strength

Growth Inhibition of Algae

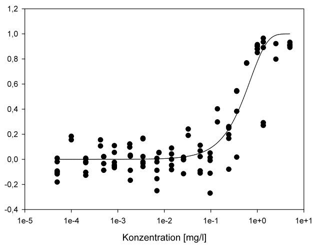
- Silver nanoparticles and silver nitrate in comparison



Ag-NP 5-0,00005 mg/l

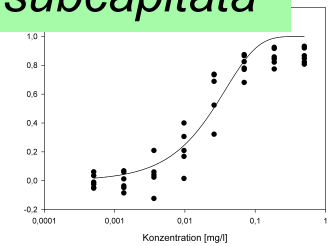
$AgNO_3$ 0,5-0,0005 mg/l

Pseudokirchneriella subcapitata



0,1 mg Ag / L

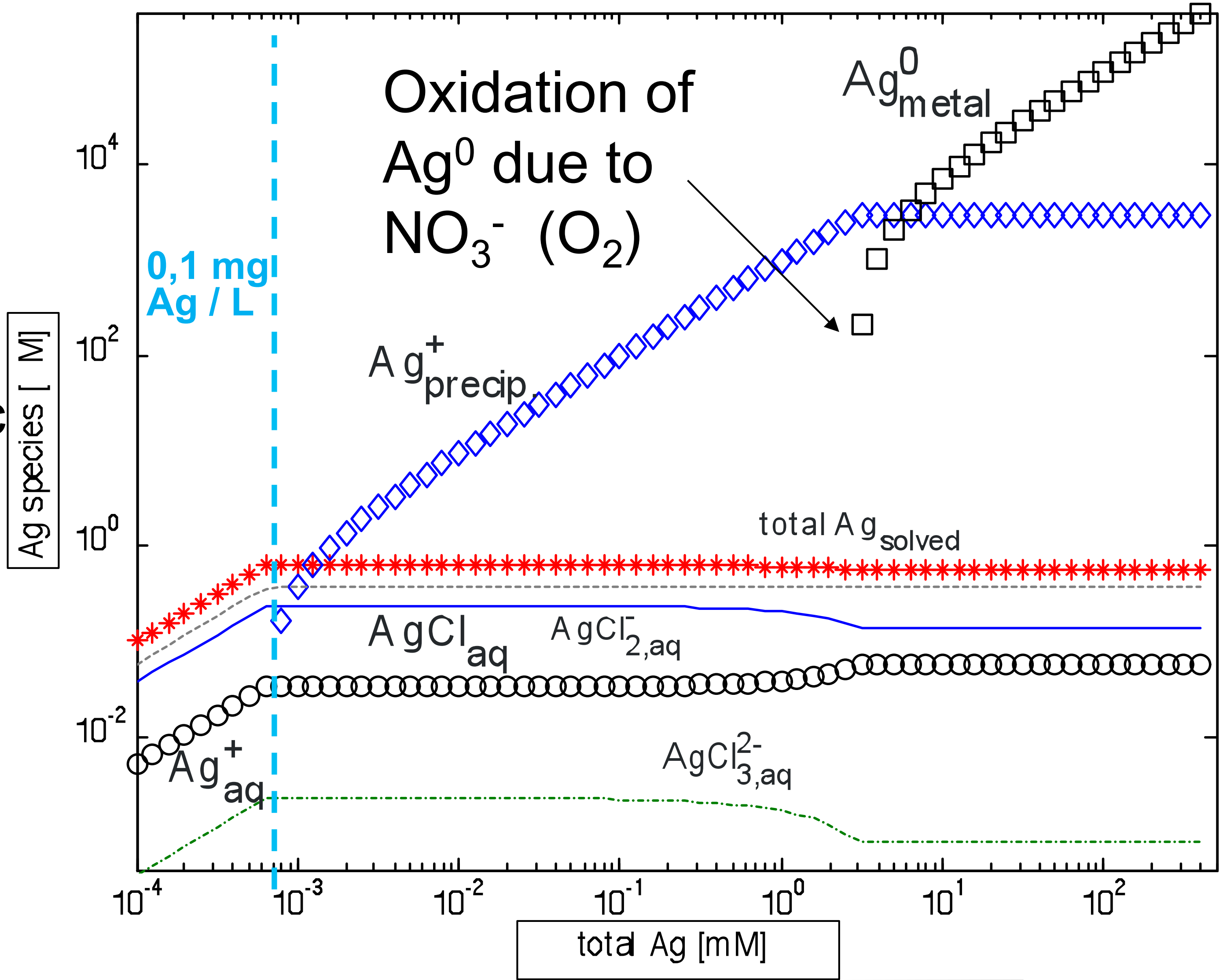
1 mg Ag / L



0,1 mg Ag / L

Algae Medium: Equilibrium Ag Species

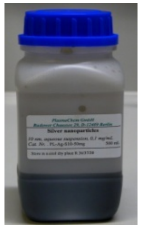
Speciation of metallic silver calculated from thermodynamic data with PHREEQC



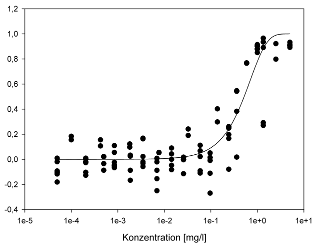
[Liu 2010, ACSNano 4]

Where does the Nanoparticle Effect Come from?

- **There are no equilibria in biological systems!**
- Organisms are part of the reaction *kinetics*



Ag-NP 5 - 0,00005 mg/l



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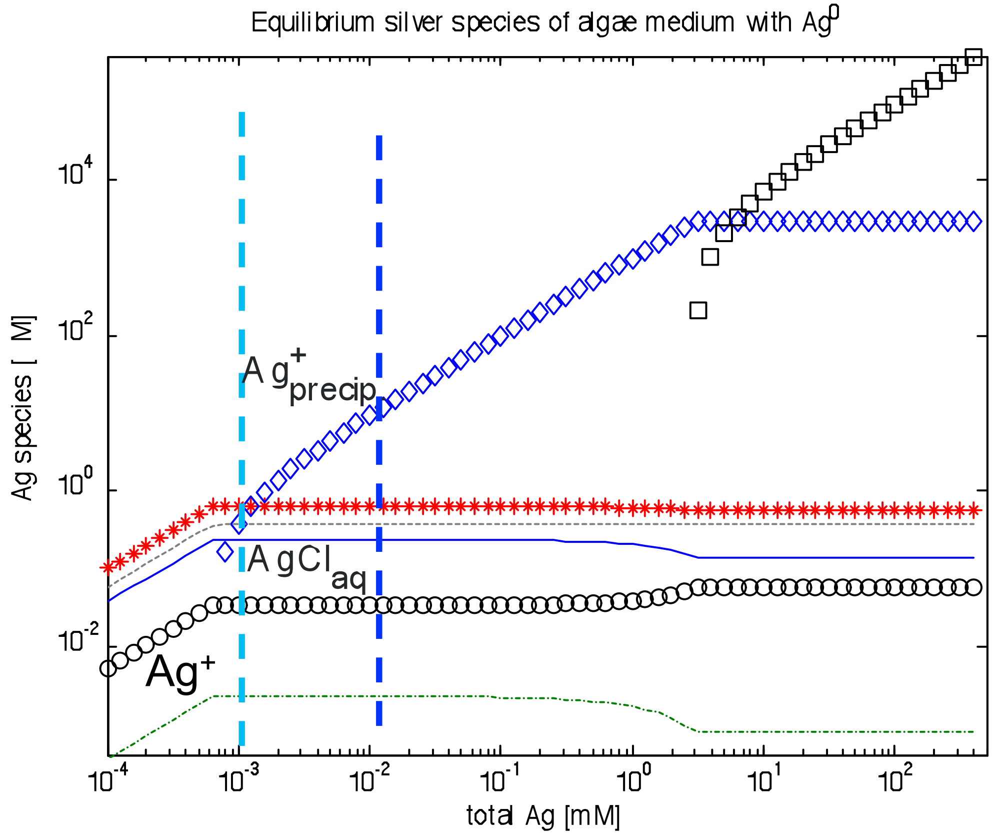
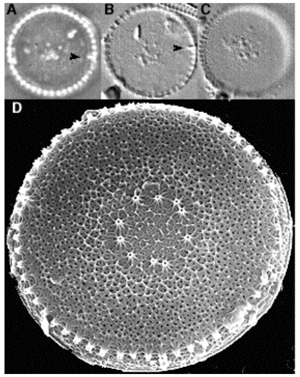




Thalassiosira weissflogii
(marine diatom)

Silver Uptake

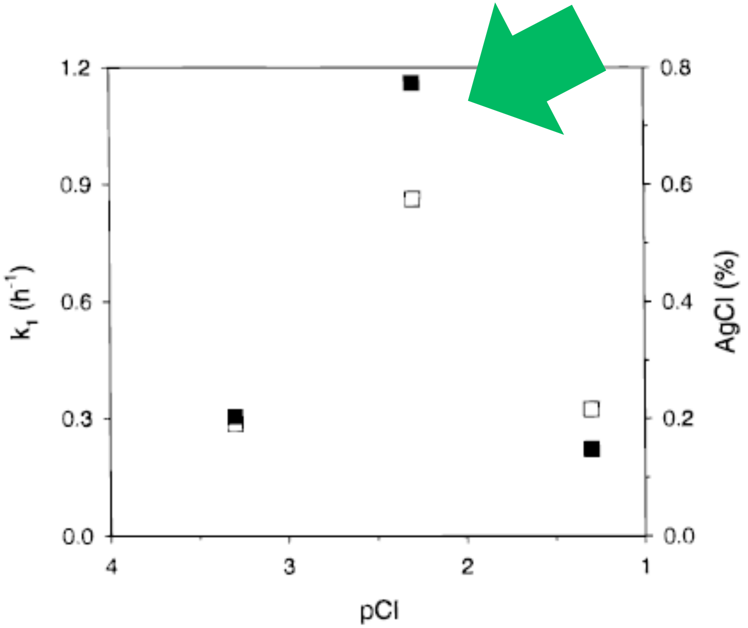


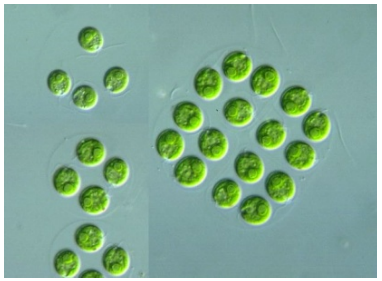
FIGURE 3. Silver uptake rate constants in the diatom *T. weissflogii* (■) and percent Ag present as AgCl(aq) (□) at three chloride concentrations. Percentages of Ag present as AgCl(aq) in the diatom uptake experiments are slightly lower than those in Table 1 because of the formation of $AgSO_4^-$ in the presence of 0.13 M Na_2SO_4.

- Highest Ag uptake at maximum **$AgCl_{aq}$** concentrations
- ➢ $AgCl_{(aq)}$ is the principal bioavailable species of inorganic Ag

Reinfelder & Chang 1999, Env Sci Technol

Chemistry is difficult enough...

Calculated speciation (MINEQL+) and cellular uptake of Ag species by *Chlamydomonas reinhardtii* as affected by silver and chloride concentration



Fortin & Campbell 2000, Environ Toxicol Chem

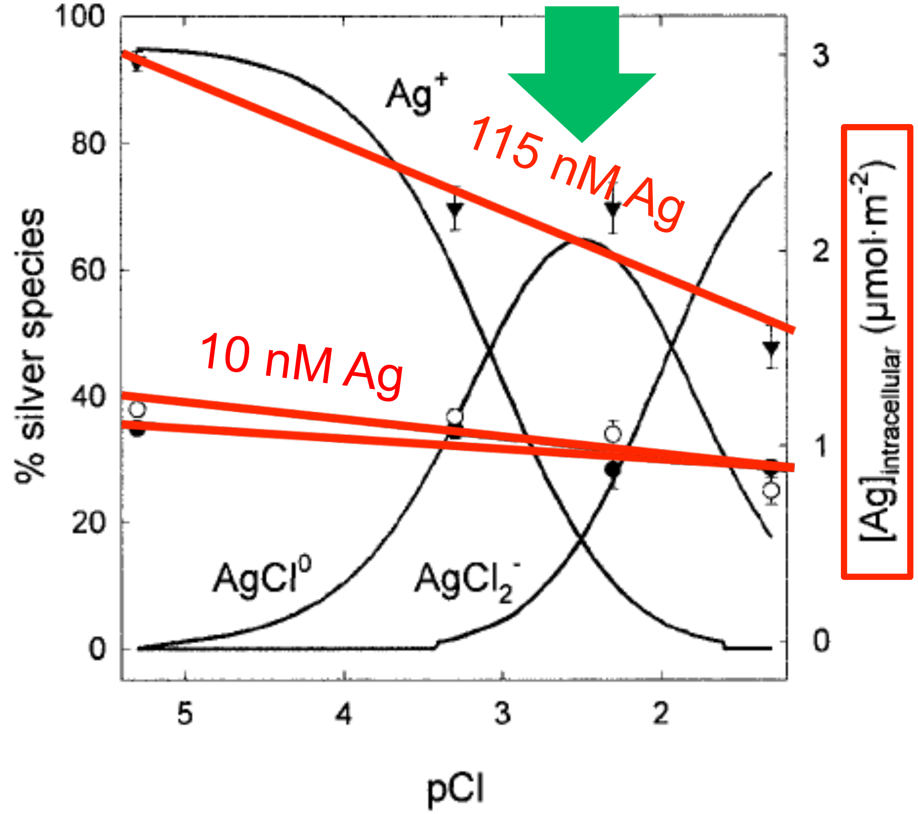
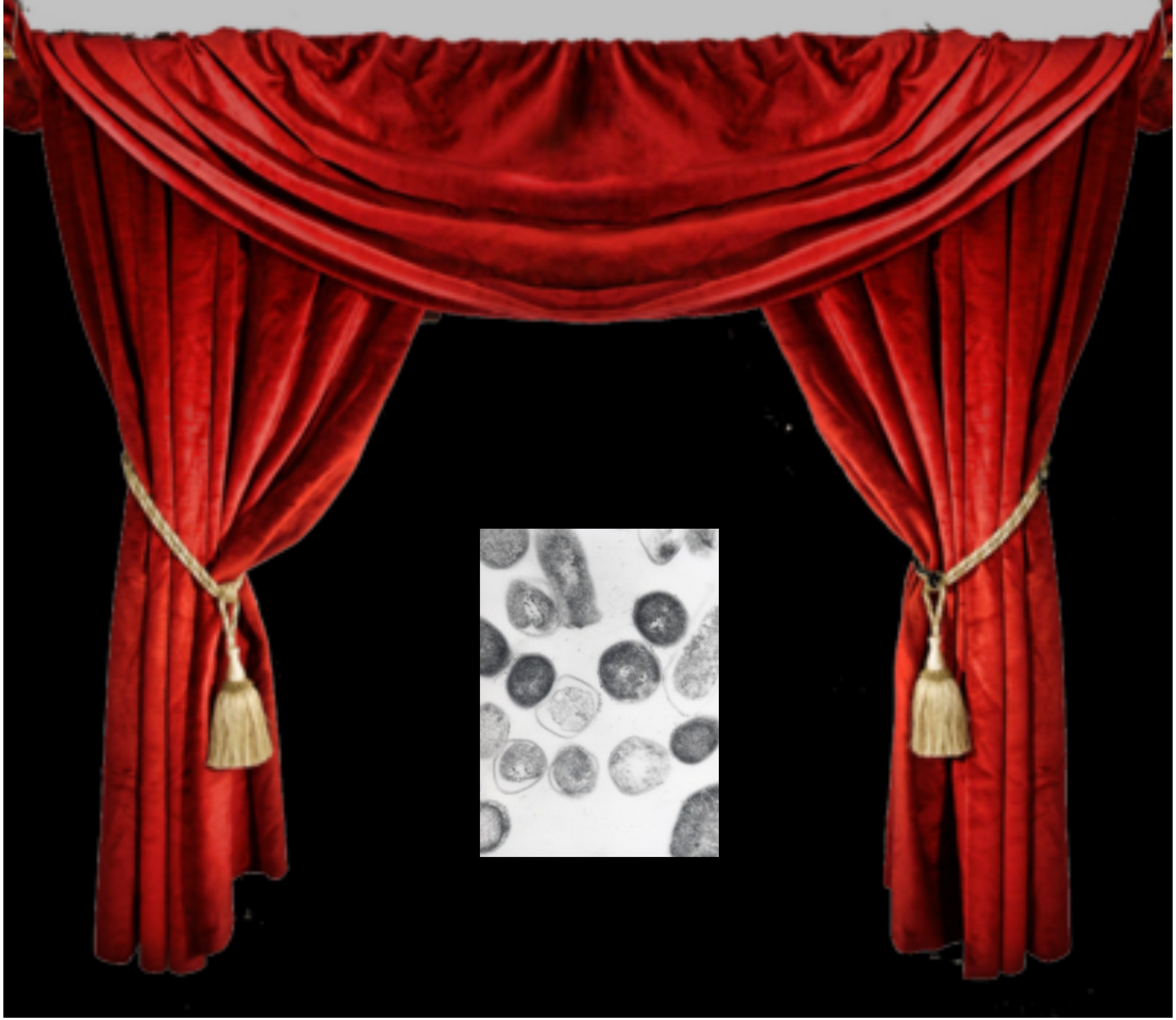
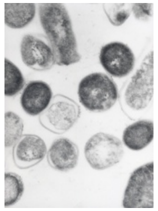


Fig. 6. Calculated distribution of silver species as a function of pCl using MINEQL+ (solid curves referring to the left axis). Short-term (15 min) intracellular silver uptake as a function of pCl for 10 nM (●, ○) and 115 nM (▼) total silver (right axis). Dark symbols represent results obtained with the same cell culture; straight dashed lines were drawn as linear regressions of the data. Error bars represent standard deviations for the average of three measurements.

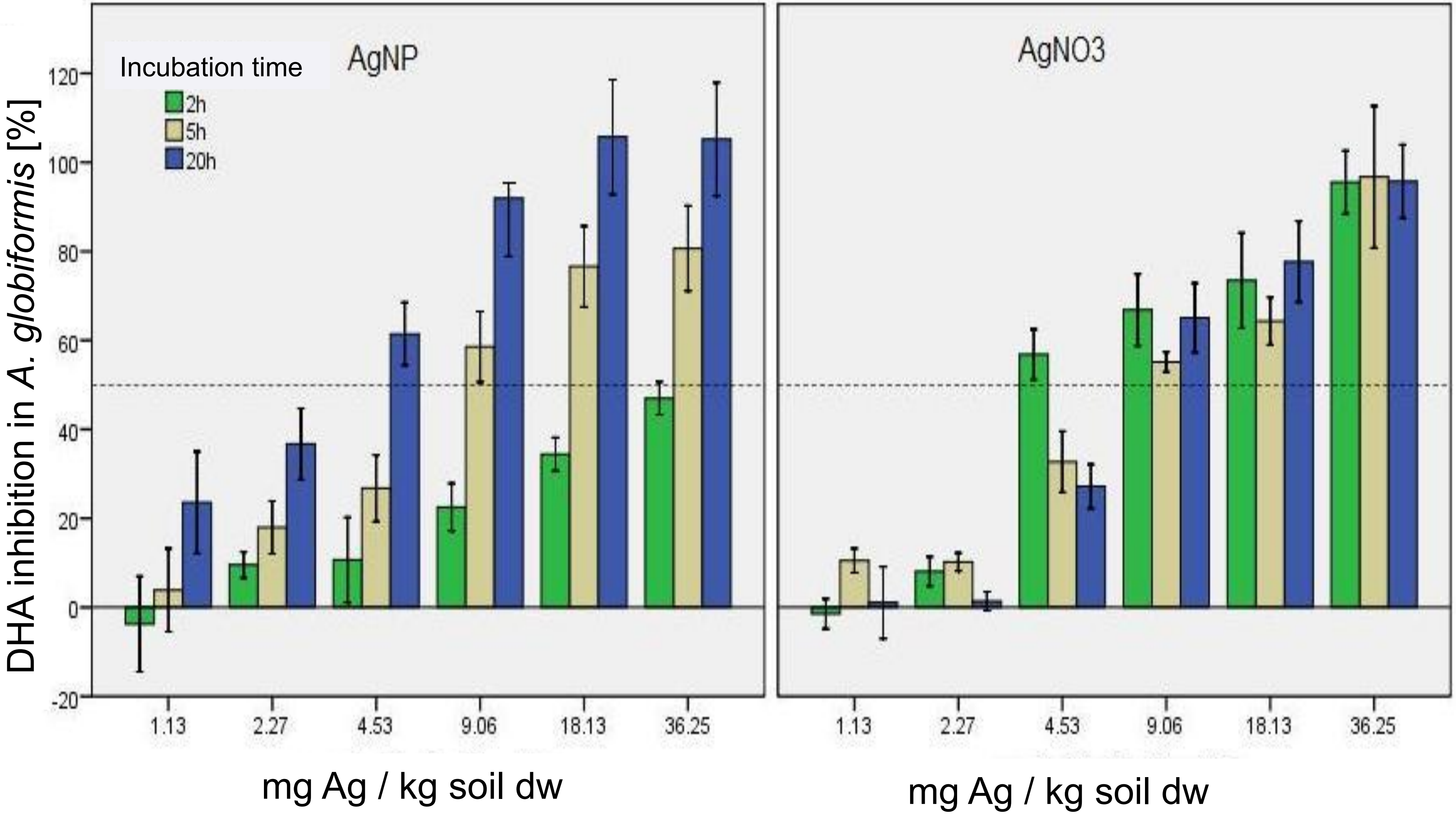

And now for
something
completely
different !





Activity of Bacteria - Test Duration

- Ag-NP become increasingly toxic – even more than $AgNO_3$!



Why? Silver Analytics

Why does the effect increase?

Assumptions and hypotheses

- Only Ag^+ has a toxic action
- In the case of $AgNO_3$, all Ag^+ is present immediately
- Part of it reacts with Cl^- → AgCl - no effect (?), but possibly improved uptake
- Reservoir effect: steady, continuous release of Ag^+ from AgNP – perhaps inside the cell?
- Catalytic effect upon contact with cell?

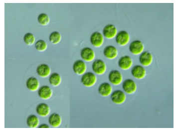
EC_{50} Values in Comparison

mg Ag/L or mg / kg dry soil	**$AgNO_3$**	**AgNP**
Algae / 24 h	0.04	0.08
Bacteria / 2 h	4.2	41.6
Bacteria / 5 h	9.6	7.6
Bacteria / 20 h	6.9	3.6

- Dissolution and uptake kinetics are important
- However,
  the duration of the bacteria standard assay is 2h!

Chemistry is difficult enough...

... But it tells us only half the truth!



Uptake and toxicity in organisms are affected by, e.g.

- Occurence and behaviour
- Nutrition and life cycle
- Membrane properties
- Transport proteins
- Detoxification proteins
- Excretion
- Biotic interactions

Conclusions

Many engineered nanoparticles may be harmless and even have a great potential for beneficial effects on the environment - but:

- Without taking chemistry into account we have no idea what nanoparticles actually do in biological media
- We need to understand basic principles of toxic action of ENPs, especially with respect to surface properties
- We badly need long-term studies!

➢ **Present regulation schemes are not satisfactory**

For the time being let‘s include economy in our interdisciplinary view…

Conclusions and Research Needs

...we should better listen to assurances:

According to the principle „better safe than sorry“ **necessary measures to protect men and the environment should be introduced at an early stage**, although the scientific uncertainties with respect to risks are not yet ultimately clear.

Nanotechnologie – kleine Teile, große Zukunft? Swiss Reinsurance 2004 (translated)

Swiss Re

Allianz

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

OECD

Allianz calls for **a precautionary approach based on risk research and good risk management** to minimize the likelihood of nanoparticles bringing a new dimension to personal injury and property damage losses or posing third party liability and product-recall risks.

Small Sizes That Matter: Opportunities and risks of Nanotechnologies, Allianz & OECD 2005

Thanks…

… for your attention!!

GEFÖRDERT VOM
Bundesministerium für Bildung und Forschung

Hans Böckler Stiftung

BIG Bremen
(now WFB)

Stephan Hackmann
Andrea Knauer
Elena Lesnikov
Marianne Matzke
Lena Röhder

Zentrum für Umweltforschung und nachhaltige Technologien

Two Large Interdisciplinary Projects

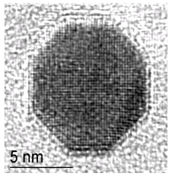
Toxic Combination Effects of Nanoparticles – Graduate School

nanoToxCom

2009 - 2012

8 Partners

Ag, Fe NP



UMSICHT

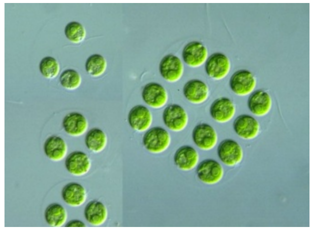
2010 - 2013

16 Partners

UM	weltgefährdung durch
SI	lber-Nanomaterialien: vom
CH	emischen Partikel bis zum
T	echnischen Produkt

Assessing Environmental Hazards of Silver Nanomaterials: from Chemical Particles to Technical Products

Chemistry is difficult enough...



Cellular uptake of Ag by *Chlamydomonas reinhardtii* as affected by of various ligands and their concentration

Fortin & Campbell 2000, Environ Toxicol Chem

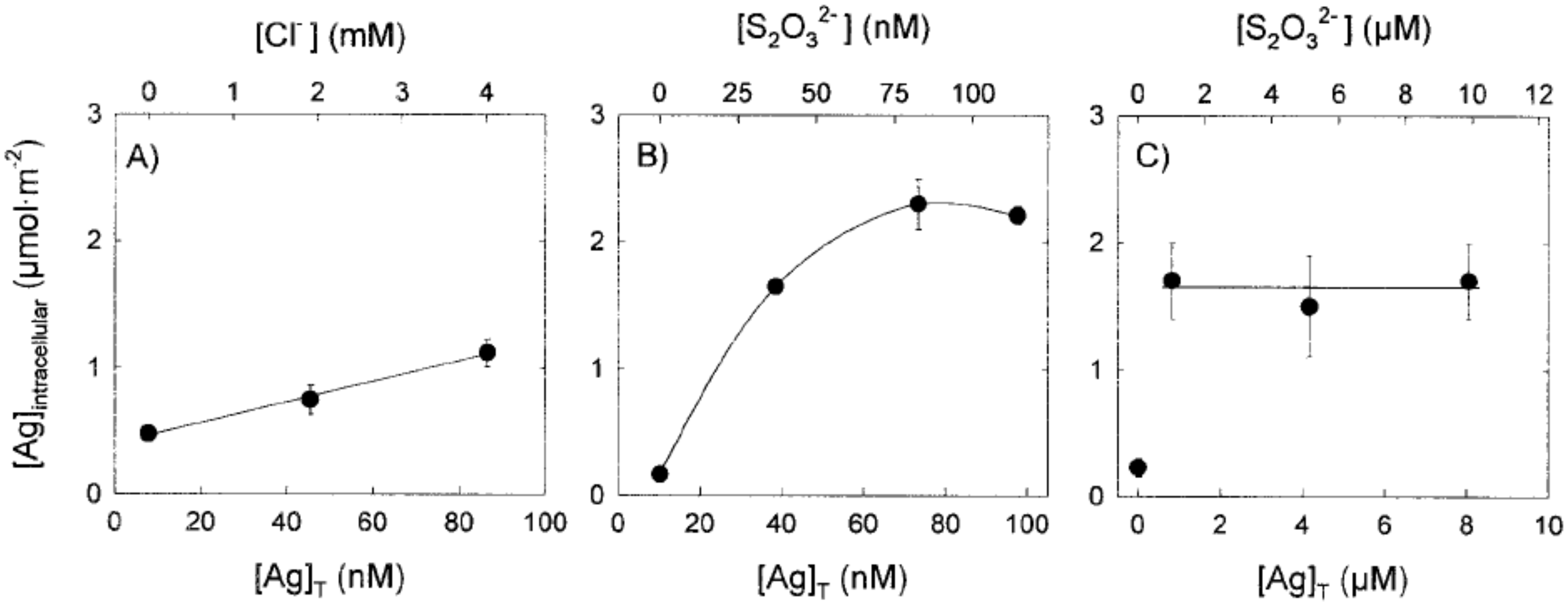
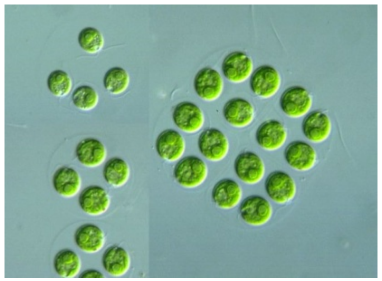


Fig. 8. Intracellular silver uptake as a function of ligand concentration. (**A**) Varying chloride concentrations for an exposure time of 10 min and $[Ag^+]$ = 8 nM; (**B**) varying thiosulfate concentrations (0–114 nM) for an exposure time of 12 min and $[Ag^+]$ = 10 nM; and (C) higher thiosulfate concentrations (0–10 μM) for an exposure time of 12 min and $[Ag^+]$ = 10 nM. Error bars represent standard deviations for the average of three measurements.

Chemistry is difficult enough...

Cellular uptake of Ag by *Chlamydomonas reinhardtii* as affected by other metals



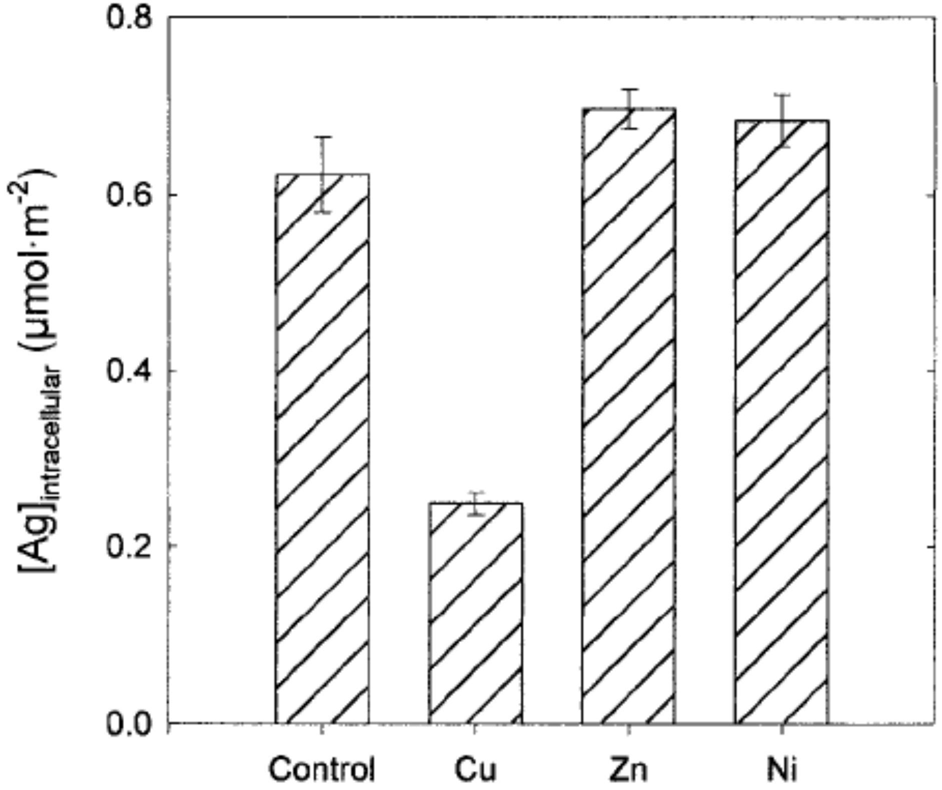
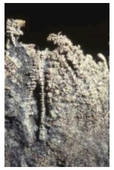


Fig. 3. Effect of copper, nickel, and zinc (500 nM) on short-term (12 min) silver uptake by *Chlamydomonas reinhardtii* at pH = 5 and 10 nM Ag^+ in low-chloride medium. The control experiment was conducted with silver only in the low-chloride medium.

Fortin & Campbell 2000, Environ Toxicol Chem



Ag NP - Community Interactions

Base: Reproduction inhibition test with *F. candida*

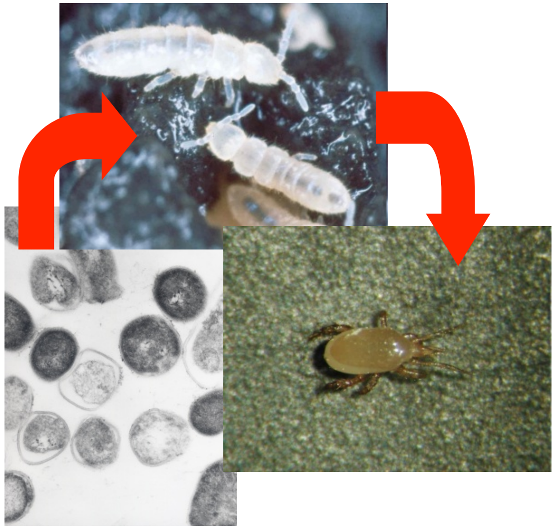
Soil: Lufa 2.2

Organisms:

Arthrobacter globiformis

Folsomia candida

Hypoaspis aculeifer



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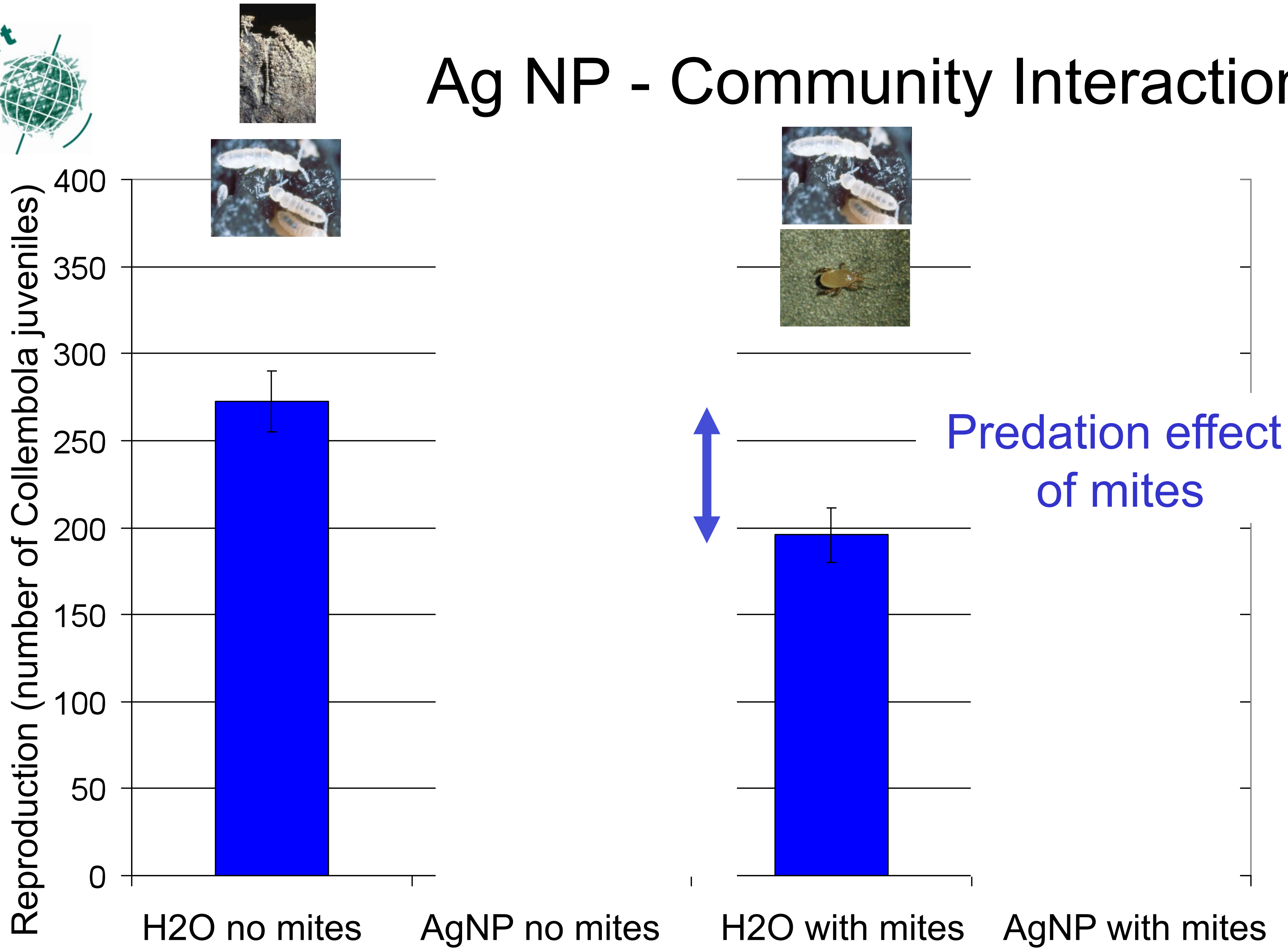

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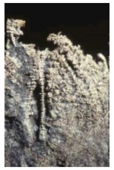
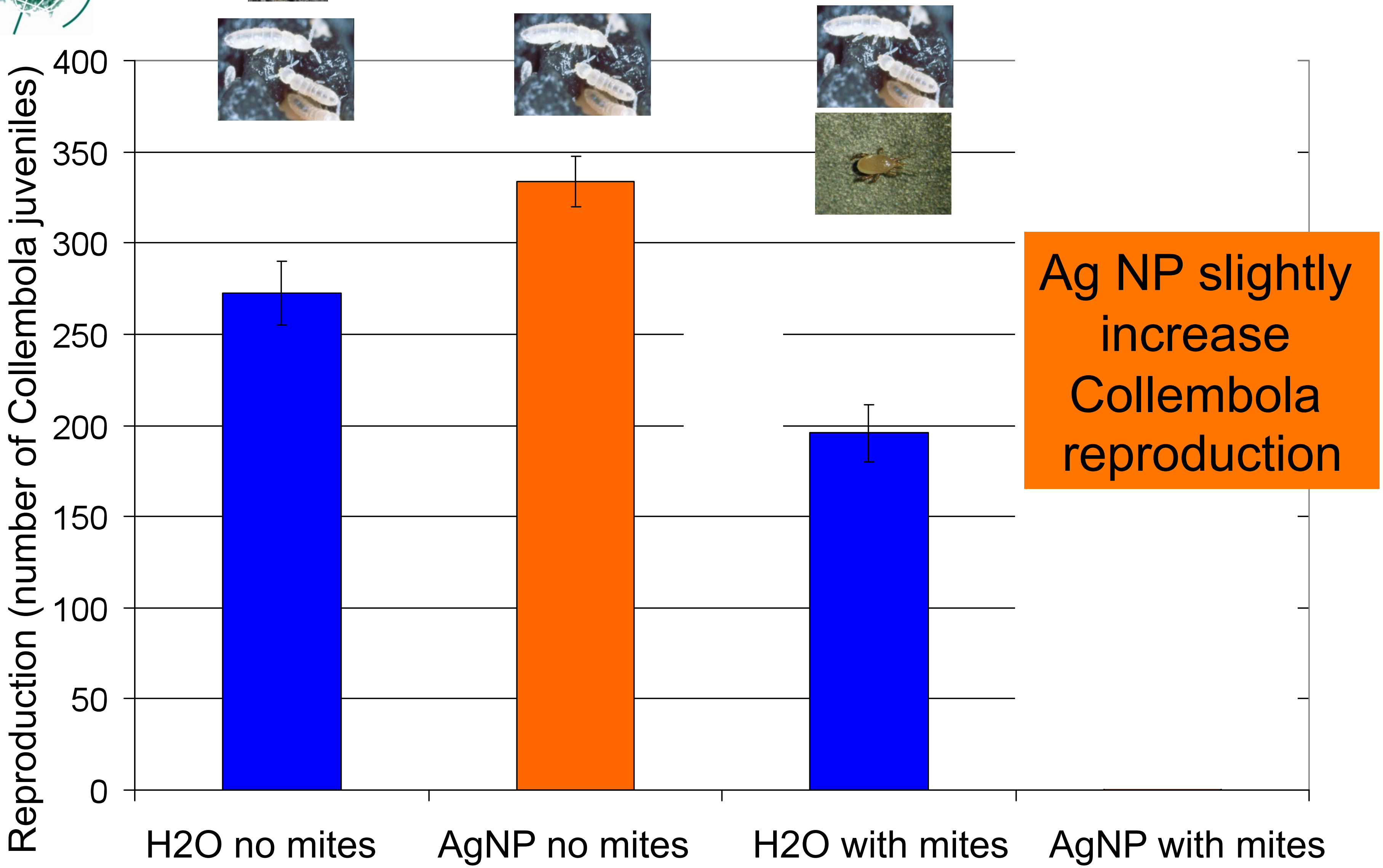
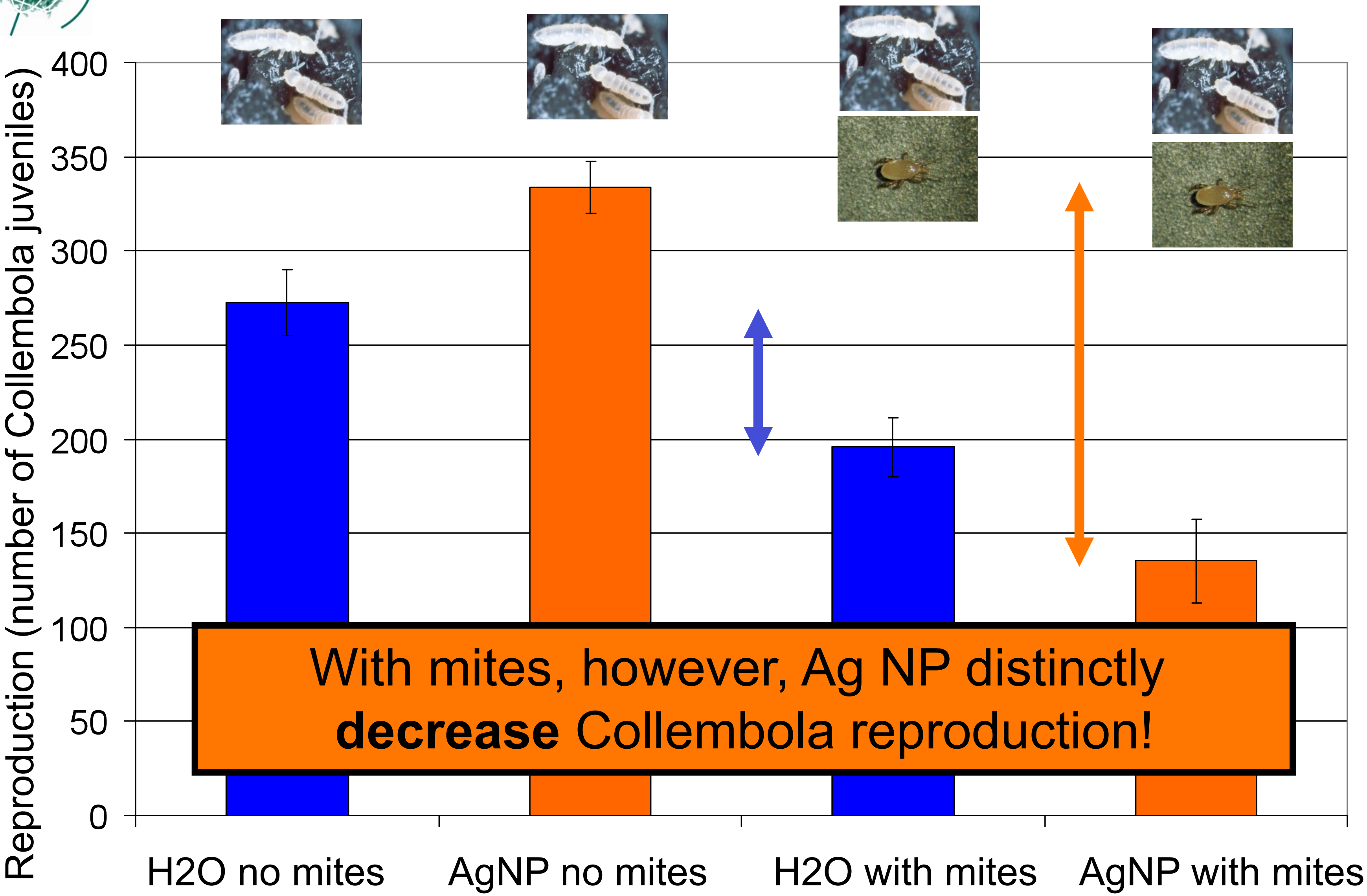




Universität Bremen

Complex Systems: Microcosms



Why? Silver Analytics

Why is the effect stronger in presence of predators?

Assumtions and hypotheses

- Higher mobility of prey → higher exposure
- Mites try to overcome their own damage through Ag by increased prey consumption

But why do effects vary so much between different test systems?

OK, organisms vary in their properties – but so do the test media!

Zeta Potential

A measure for particle surface ↔ media interaction!

In suspension negatively charged particles have a negative zeta-potential.

Particles in aqueous suspensions react and interact with the media compmonents:

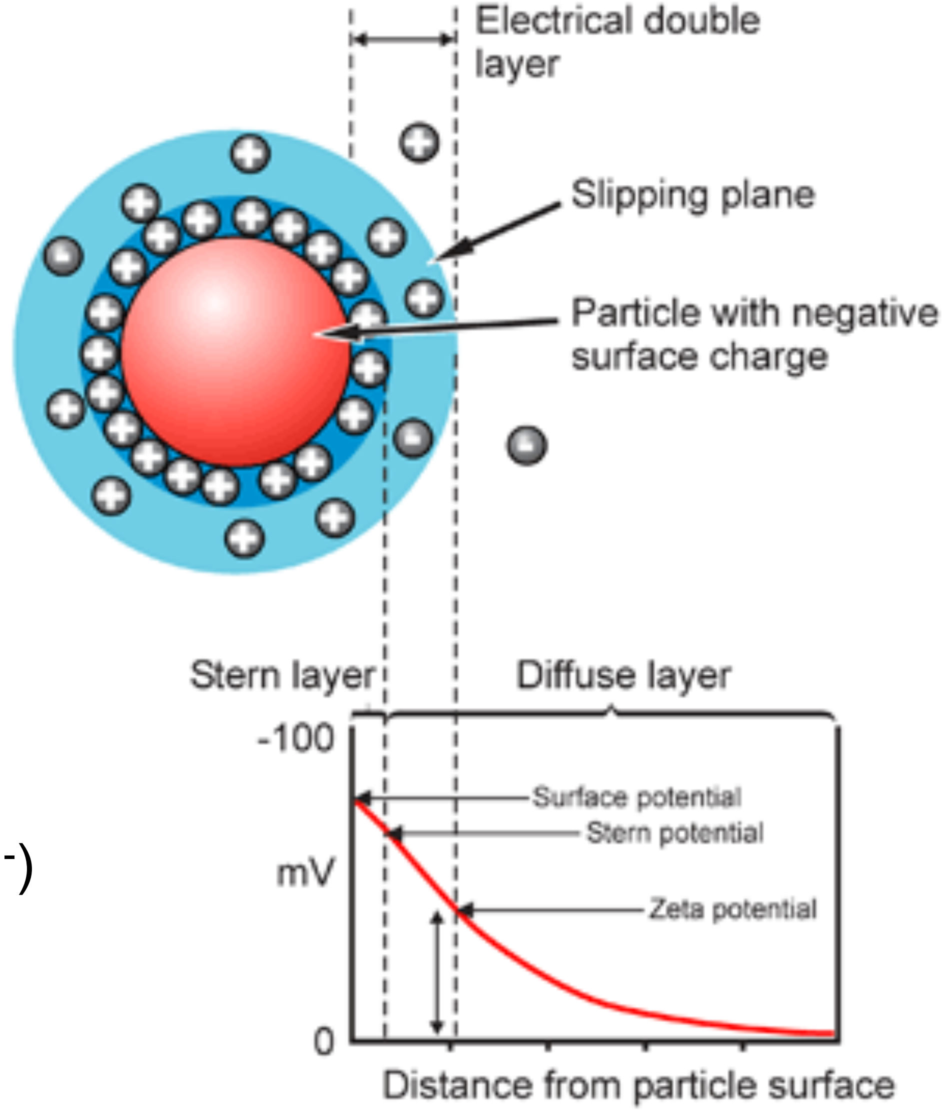
- pH (H^+, OH^-)

- electrolyte

- specific ion interaction (e.g. Ag^+, Cl^-)

- redox eqilibrium (e.g. Ag^0, nitrate)

- adsorption of ligands



[Malvern]

Stability of Suspensions: Influence of Electrolyte

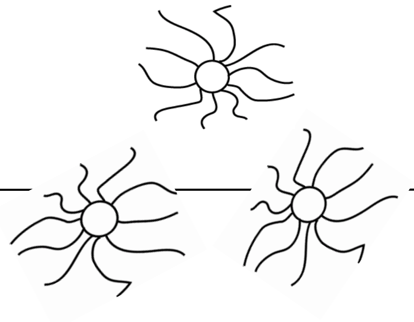
e.g. Polystyrene-Latex

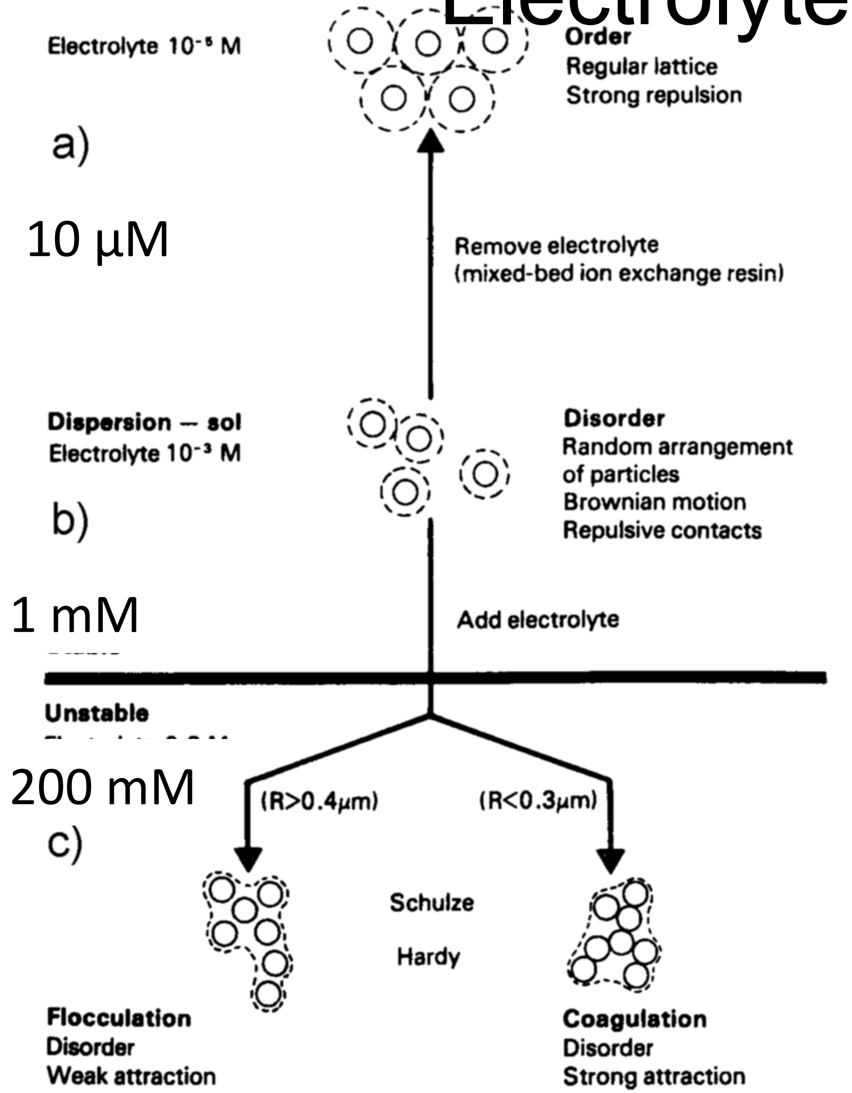
diameter is 83 ± 16 nm,

ζ-potential is -56 mV @ pH7, ionic strength ≈ 0mM

These nanoparticles are electrostaticly stabilised due to their surface charge

Polymeric ligands may have a steric stabilisation effect





[Piirma82]

Major Concerns

Organisms

- Toxicity
- Transfer (cell division, food web)
- Bioaccumulation
- Microbial activity
- Immune reponse
- Long-term effects

Environment

- Mobilisation in soils: groundwater contamination
- Disaggregation
- Catalytic action of NPs
- Degradation?
- No generalisation possible

Major Concerns

"Ordinary" Chemicals	Nanoparticles
Soils adsorb them	Do soils mobilise them?
Microorganisms degrade them	Do NPs degrade microorganisms?
Animals enrich MO's	Do animals enrich NPs?
Many chemicals increase plant production	Do NPs hinder plant growth?
Dose - effect curves	Surface area - effect curves

The UFT Concept

UFT

- Sustainable and biocompatible products and processes
- Green Chemistry
- Focus: Nanomaterials
- Interdisciplinary approaches

Department of Ecology

- Prospective environmental risk assessment
- Focus: novel products and technologies
- Soil ecology

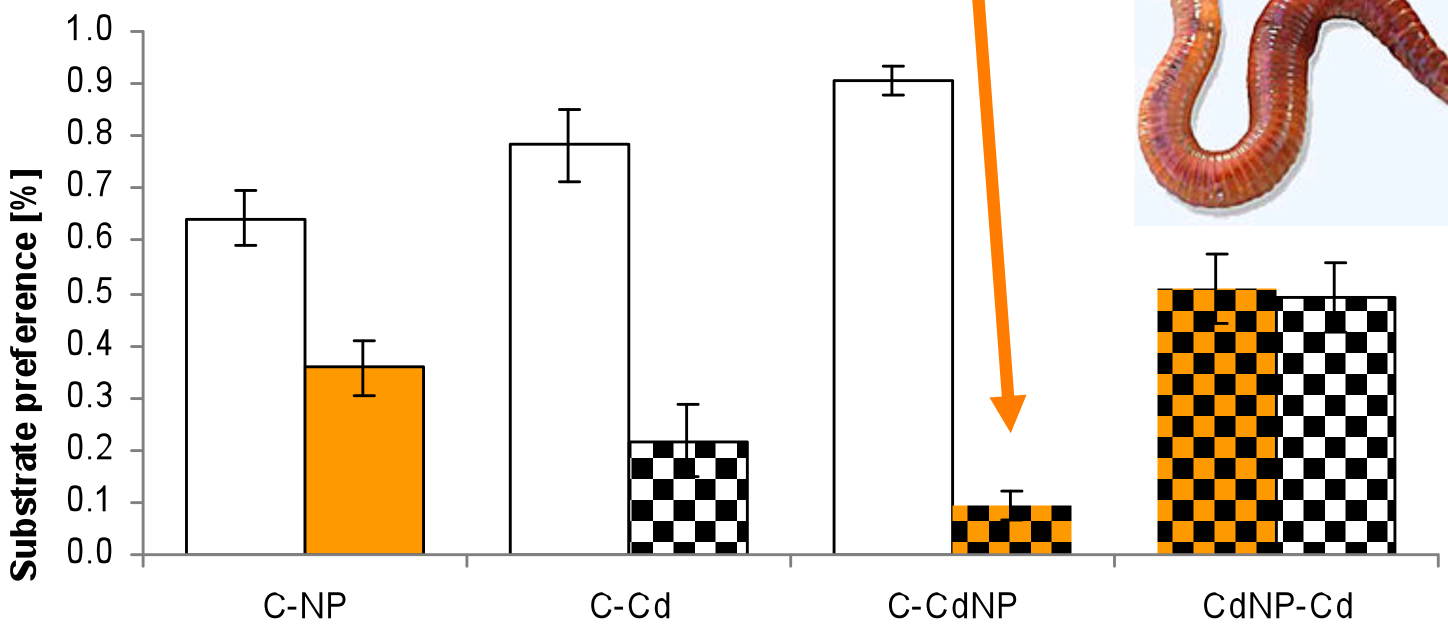
What we have done so far

- Do metal engineered nanoparticles (ENP) affect
  - microorganisms?
  - plants?
  - animals?
- Have metal ENP stronger effects than the metal ions themselves?
- Which mechanisms contribute to the effects?
- Do standardised test protocols reliably protect our ecosystems?
- What do commercial ENP solutions contain?

Organisms as Indicators in Soils

- Earthworms clearly avoided both Cd and RNIP
- Strongest effect with Cd and RNIP

Reactive
Nanoscaled
Iron
Particles,
commercial product
for **decontamination**

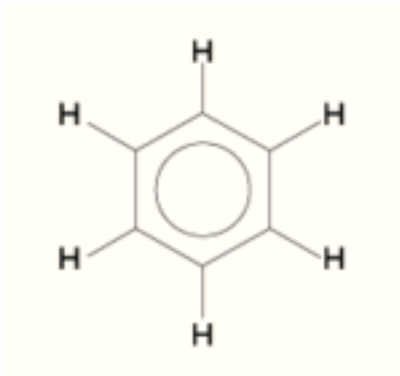


Substrate preference in % after 48h, mean values +/- SE. Cd = 200 mg/kg; NP = NPII of plant tests

Why? GC-MS

Why should iron (oxides) be so toxic?

- RNIP is very smelly...
- Chemical analysis of RNIP revealed 36 different volatile organic compounds (mostly alcohols, alkanes, ketones), among them acetone and **benzene**
- Their composition idiosyncratically changed over time
- Whereas their amount even increased



Conclusions and Research Needs

- We have hardly any idea of ENP exposure
- Nor of their detection under field conditions
- Many ENP have toxic effects
- The metal ENP studied by us do not appear to be more toxic than their ions
- Stabilisators and coatings could be more problematic than the ENP
- Product safety (data sheet) is not sufficient
- **Standard tests are not sufficient**
  - Species
  - Duration
  - Endpoints