NANOPARTICLES IN ENVIRONMENT – FRIEND OR FOE?

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Toxic metals
Radionuclides
Organic xenobiotics
Air
Water
Soil

PHYTOREMEDIATION

Toxic metals

Organic xenobiotics

Explosives, pharmaceuticals

Radionuclides

Caesium, uranium

“NEW” CONTAMINANTS

- **Four groups of micropollutants were included in this list:**
  the Linear Alkylbenzene Sulfonates, (LAS), Polycyclic Aromatic Hydrocarbons (PAH), Nonylphenols, Ethoxylates (NPE), and Diethylhexyl-Phthalates (DEHP) (Knudsen et al. 2000).

- **The fate of pharmaceuticals or personal care products in sludge is rarely addressed.**

- **Today, up to 50% of the sludge used for agricultural purposes does not comply with the given standards.**

- **Nevertheless, this sludge is used as an amendment to soils and, hence, delivers organic pollutants to soil, surface water and crops**

NANOPARTICLES

- **Nanoparticles are now being used in the manufacture of scratchproof eyeglasses, crack- resistant paints, anti-graffiti coatings for walls, transparent sunscreens, stain-repellent fabrics, self-cleaning windows and ceramic coatings for solar cells**.

- **At the nanoscale, the properties of particles may change in unpredictable ways**.

- **Nanoparticles of titanium oxide used in sunscreens, for example, have the same chemical composition as the larger white titanium oxide particles used in conventional products for decades, but nanoscale titanium oxide is transparent.**

- **Antimony - tin oxide provides another example since nanoparticles of this oxide are incorporated into a coating to provide scratch- resistance and offer transparent protection from ultra-violet radiation, not seen with larger size particles.**

- The appropriateness of existing methodologies to assess the potential risks associated withengineered and adventitious products of nanotechnologies (2006).

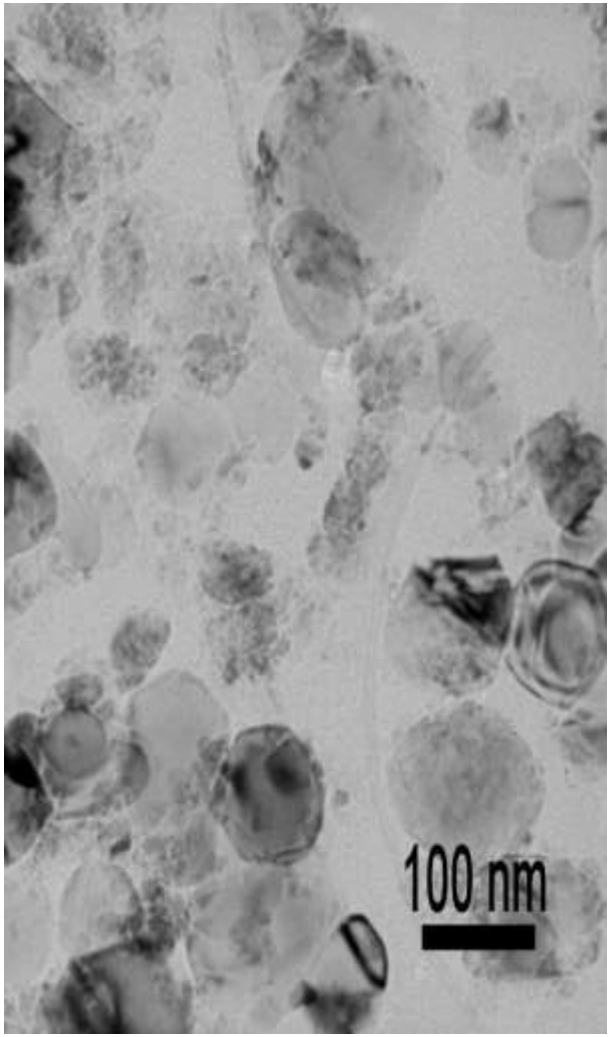
NANOPARTICLES

- **Already more than 800 consumer products containing NPs**

- **Woodrow Wilson database**

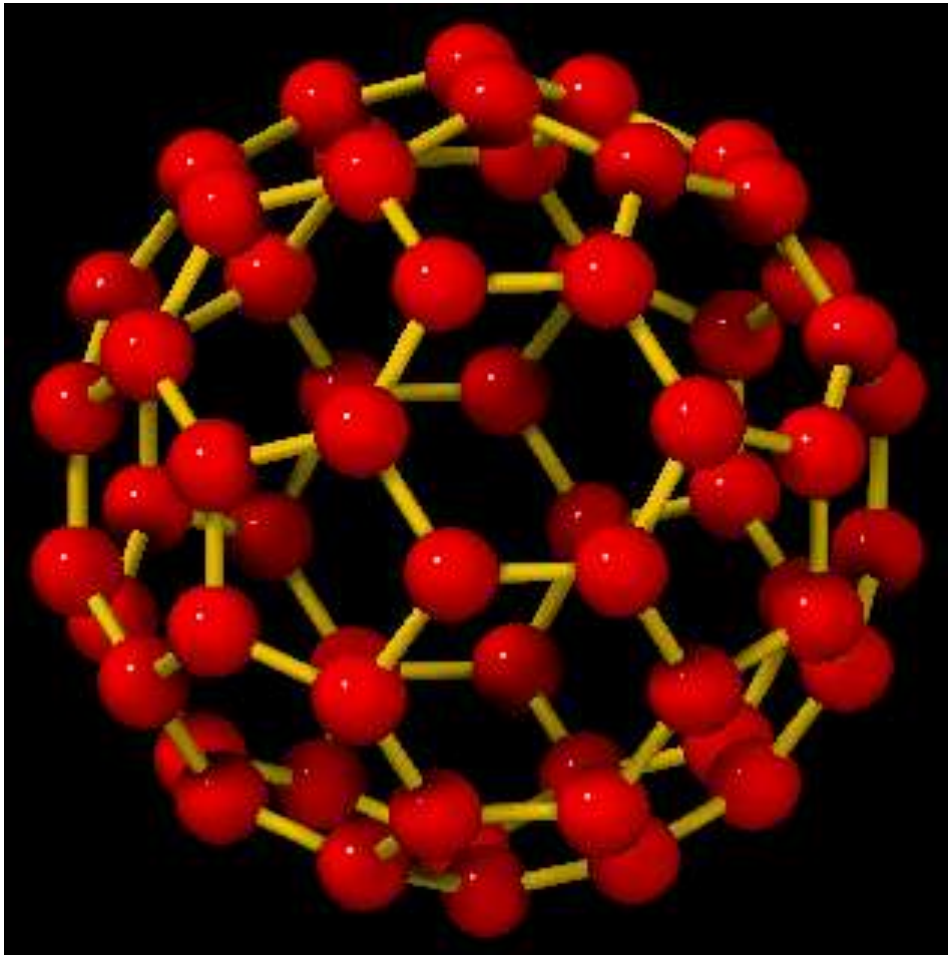
  http//www.nanotechproject.org/inventories/consumer/

Titanium oxide

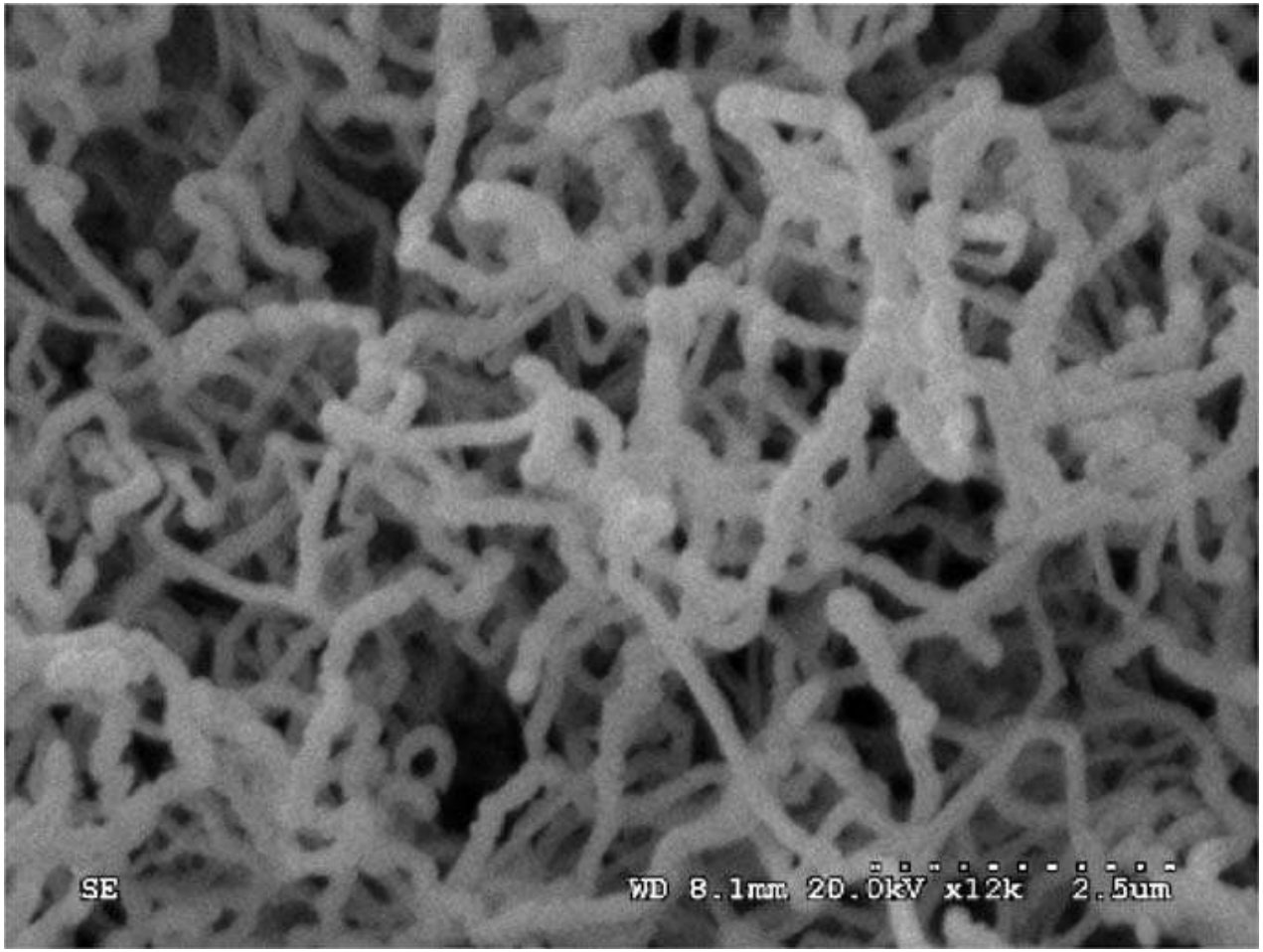


Fullerenes

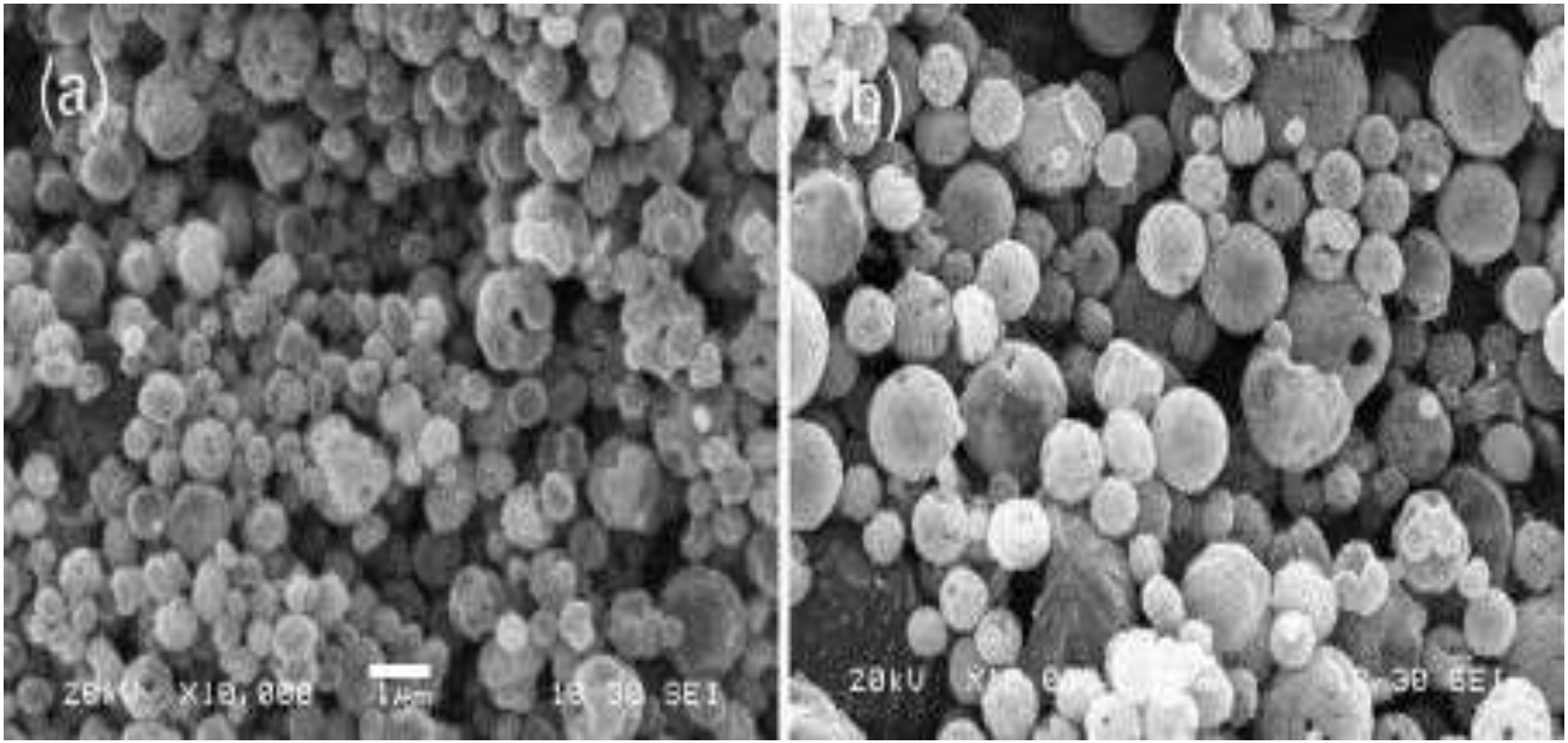
C60 mean ball diameter 6.83 Å



Carbon nanofibers



ZnO



- **Are Nanoparticles Safe? What Are The Risks?**

Theresa Phillips, About.com, Jul 30 2009

- **Nanoparticles, human health hazard and regulation**

Anthony Seaton, Lang Tran, Robert Aitken, Kenneth Donaldson, Journal of the Royal Society, print September 2, 2009, doi: 10.1098/rsif.2009.0252.focus

PROBLEMS??

Nanoparticles In Sewage Could Escape Into Bodies Of Water

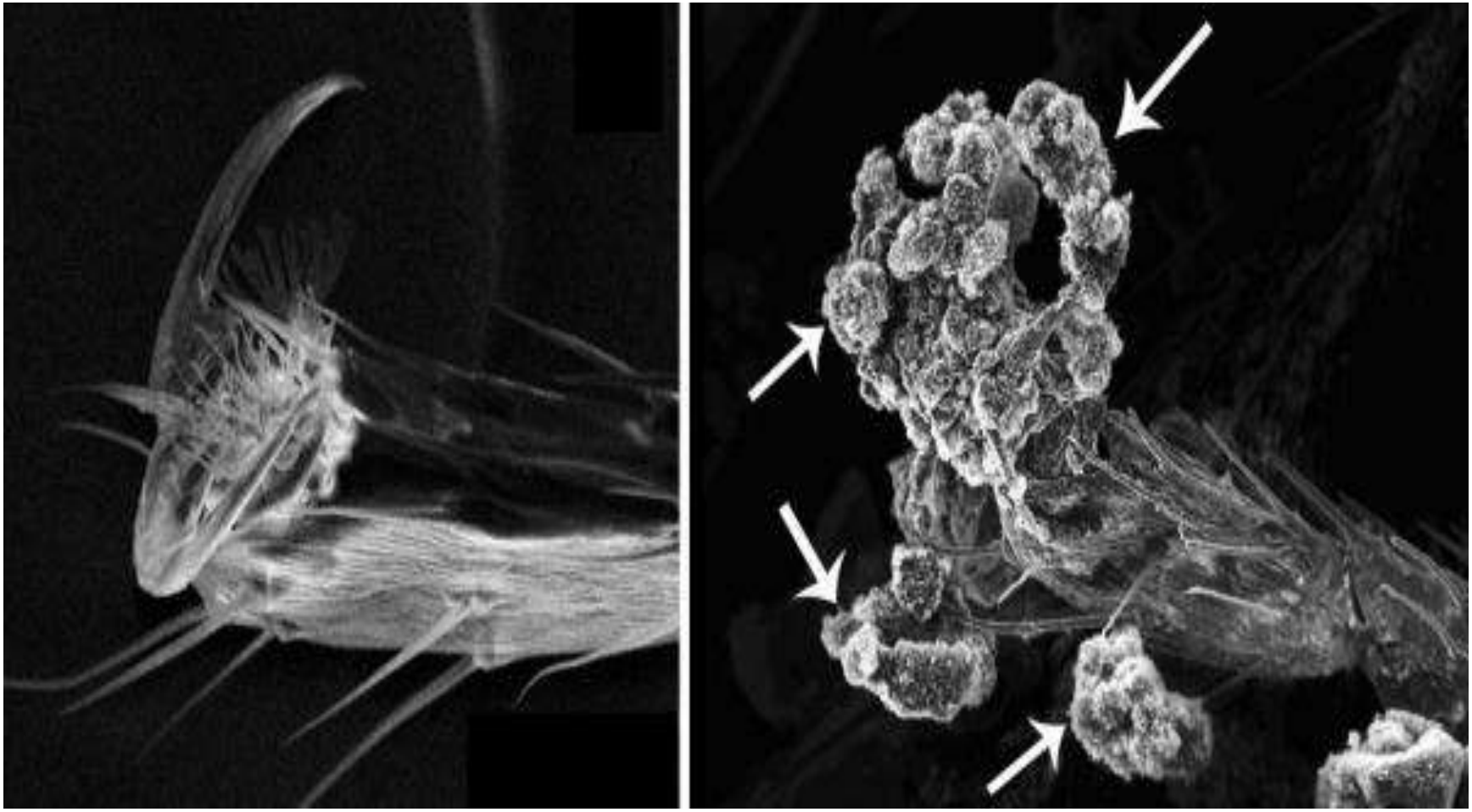
ScienceDaily (July 28, 2008)

Limbach et al. Removal of Oxide Nanoparticles in a Model Wastewater Treatment Plant: Influence of Agglomeration and Surfactants on Clearing Efficiency. *Environmental Science & Technology*, 2008;

DOI: 10.1021/es800091f

Carbon Nanoparticles Toxic To Adult Fruit Flies But Benign To Young

ScienceDaily (Aug. 17, 2009)



- Tests showed adults immersed in tiny pits containing two varieties of carbon nanoparticles died within hours.
- Analyses of the dead flies revealed the carbon nanoparticles stuck to their bodies, covered their breathing holes, and coated their compound eyes.
- Scientists are unsure whether any of these afflictions led directly to the flies' death.

Plants?

- **Insignificant acute toxicity of TiO_2 nanoparticles to willow trees**

Seeger, Eva · Baun, Anders · Kästner, Matthias · Trapp, Stefan, Journal of Soils and Sediments, 2009

YES or NO?

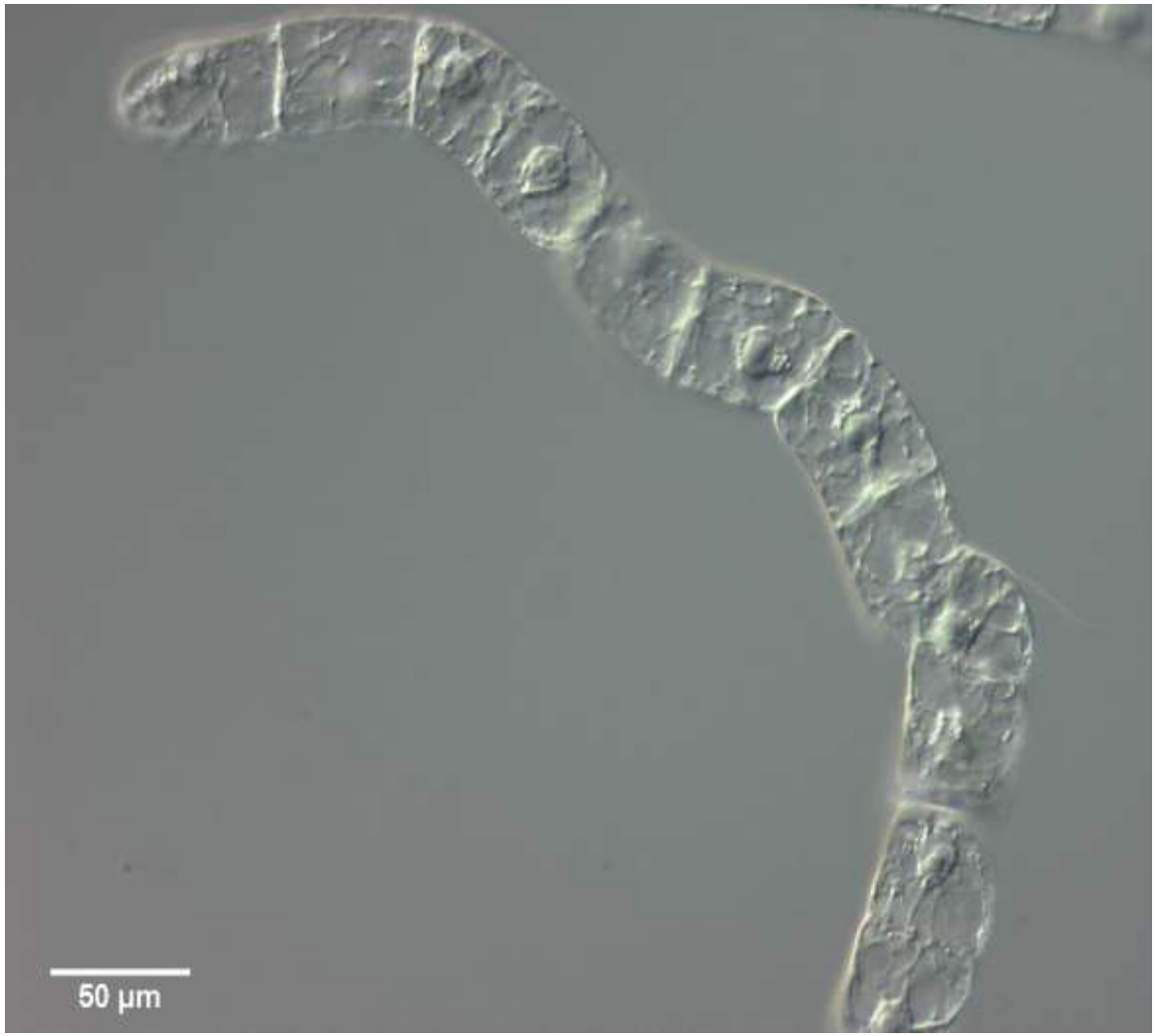
NANOPARTICES SELECTED

- Au (reserved for Lee)
- Ag (reserved for Lee)
- TiO_2
- ZnO
- Al_2O_3
- Graphite fibers
- Fullerenes

EXPERIMENTAL DESIGN

- **Microscopic observation**
- **Viability test**
- **Ethylene formation (ACC)**
- **Peroxidases**
- **Cytokinins**
- **Metabolomic study**
- **DNA Arrays**
- **Hydroponic** experiments
- DIGE proteomics study
- Real-scale experiments

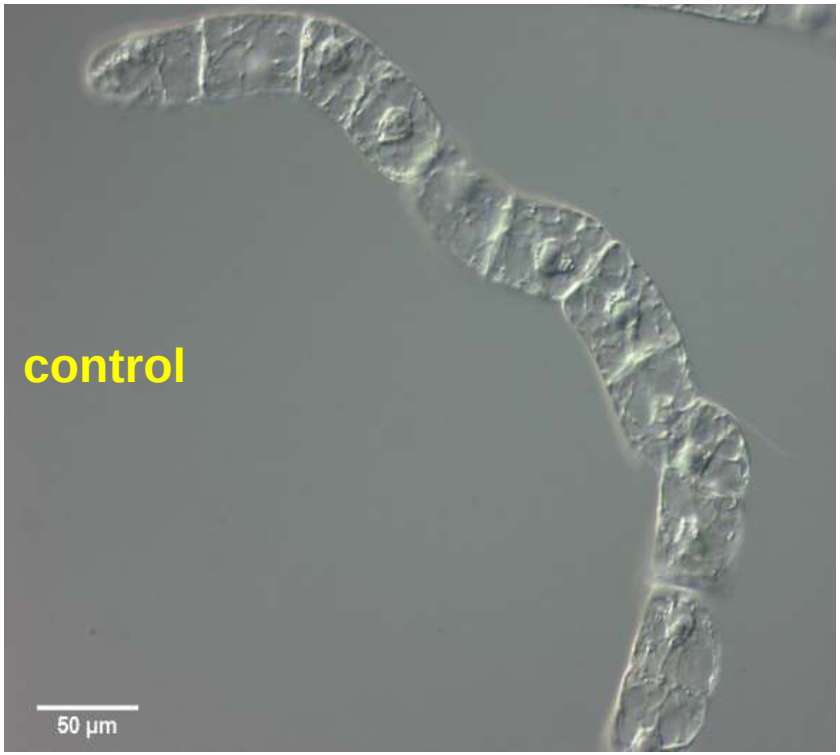
Nicotiana tabaccum CELLS BY2

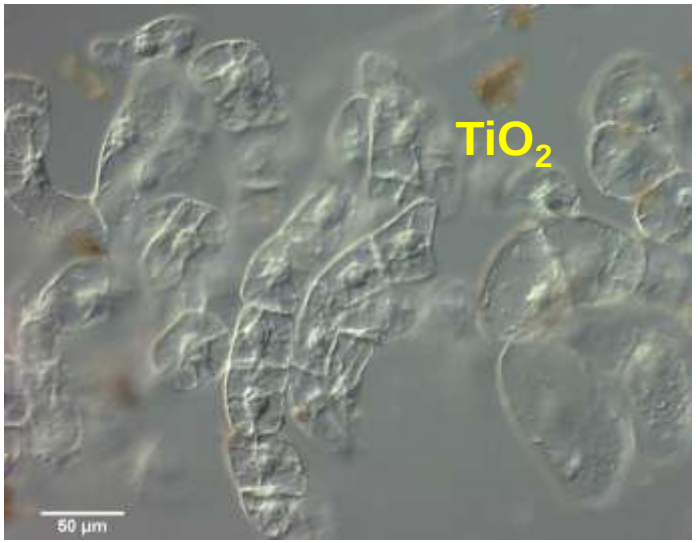


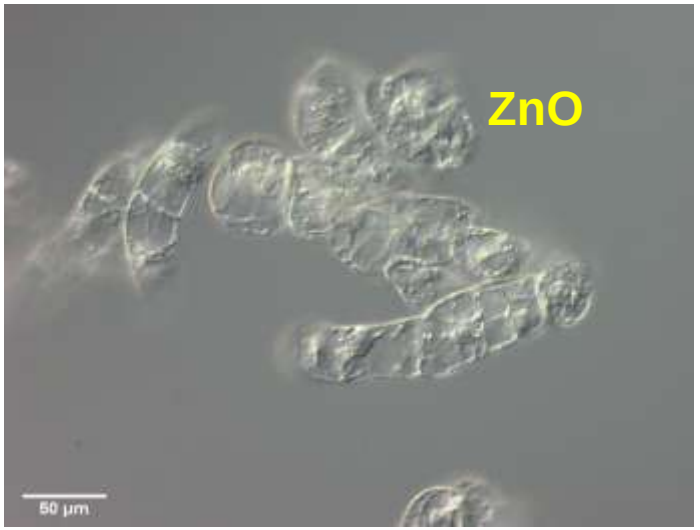
STARTING PARAMETERS

Nanoparticles	Conc	% of dead cells	Viability [% of control]	Perox. Activity [% of control]	pmol/g [% of control]
TiO_2	20ug/ml	4.3	92	57	??
	100ug/ml	8.9	67	108	38
ZnO	20ug/ml	1.2	67	104	60
	100ug/ml	3.3	65	241	42
Al_2O_3	20ug/ml	2.7	87	86	89
	100ug/ml	4.1	85	113	55
Fullerenes	20ug/ml	5.7	85	90	49
	100ug/ml	9	75	82	67
Graphite fibers	20ug/ml	6.5	90	94	120
	100ug/ml	6.6	67	90	67

Microscopic observation





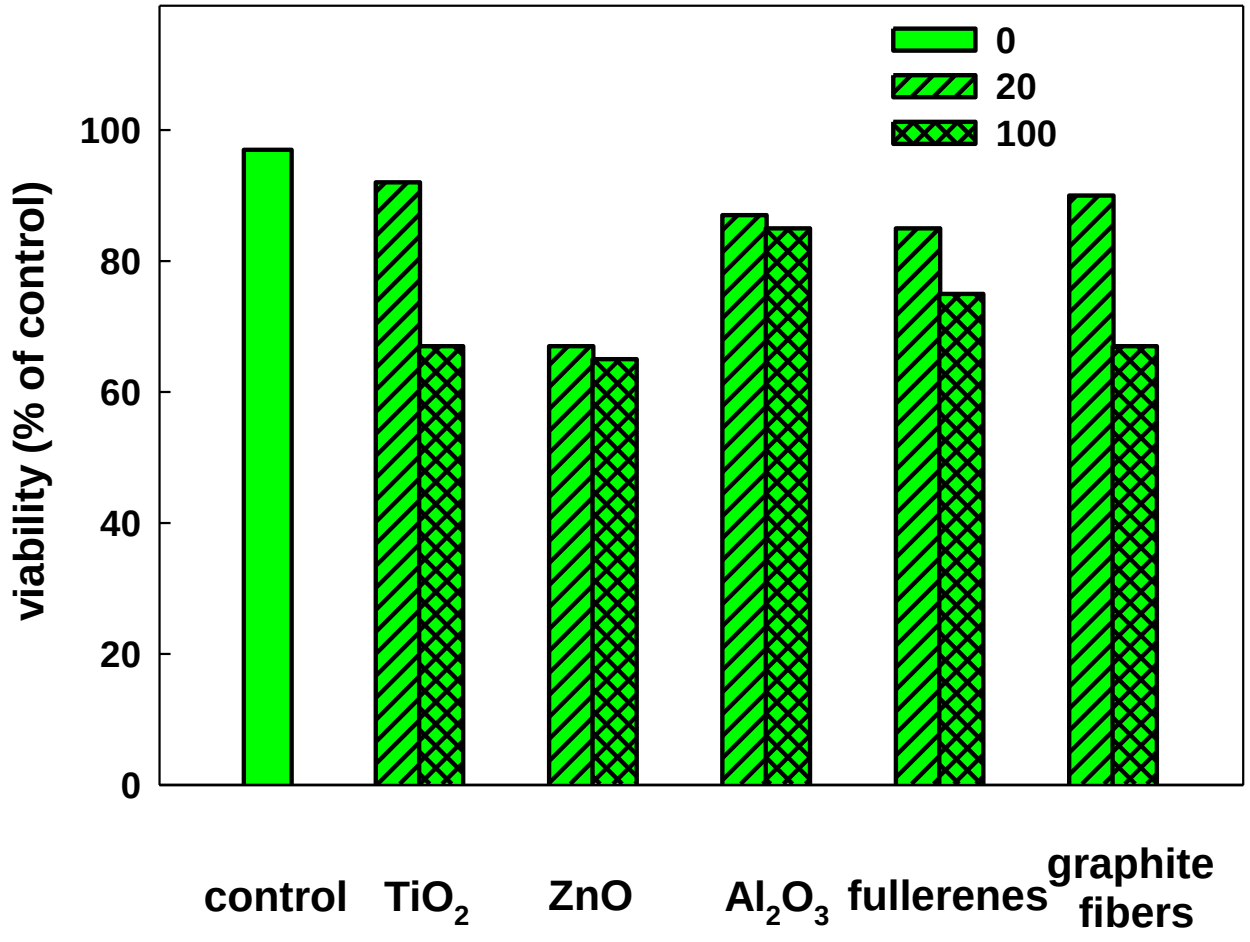


VIABILITY TEST

- The potential toxicity of NPs was assessed following the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to water-insoluble red formazan.

- This viability test is based on determination of reductase activity and is frequently used in plant physiology

PLANT CELLS VIABILITY

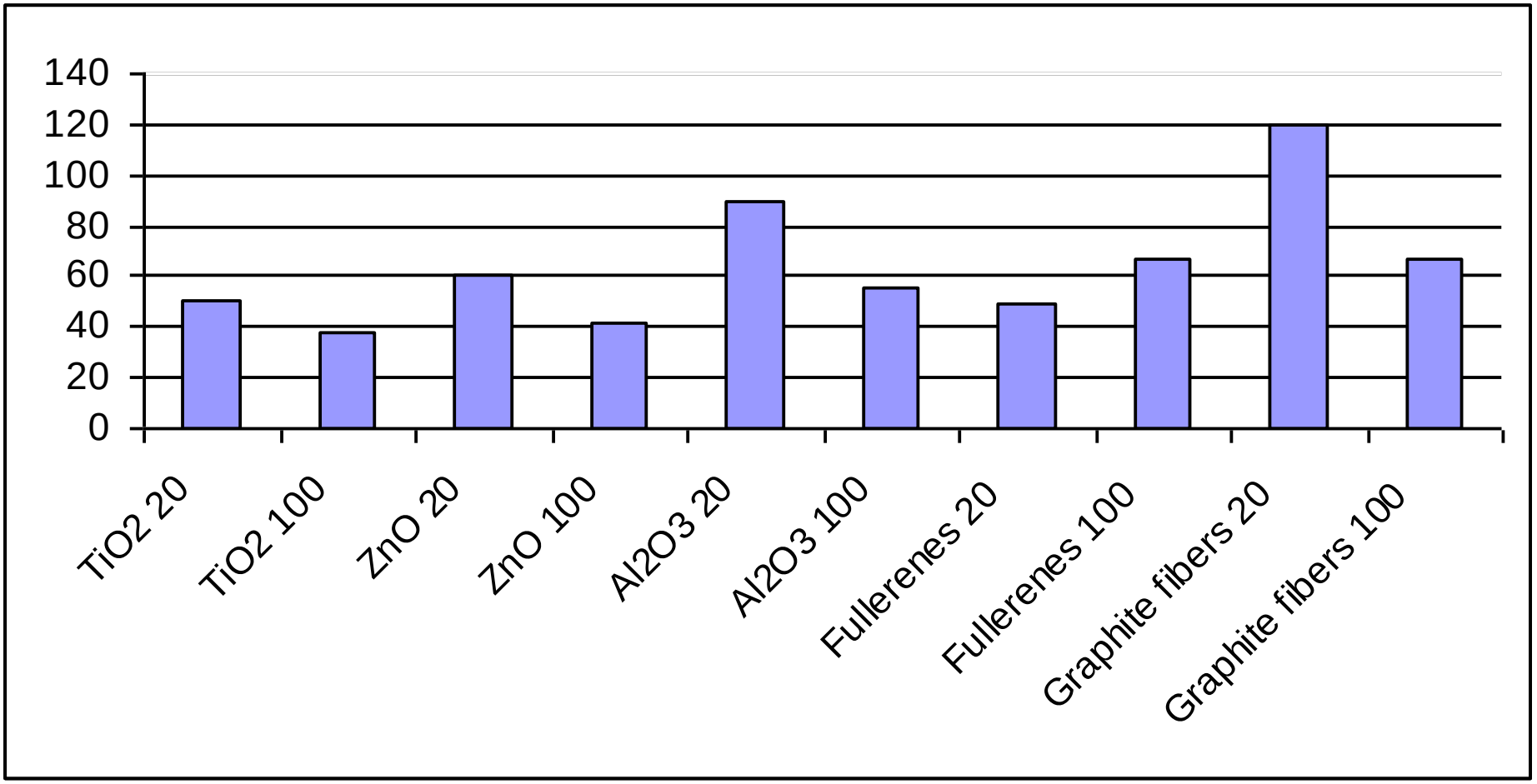


ETHYLENE

- general stress hormone induced in abiotic as well as biotic stresses, can induce program cell death

ETYLENE FORMATION

control = 100%



ETHYLENE (ACC)

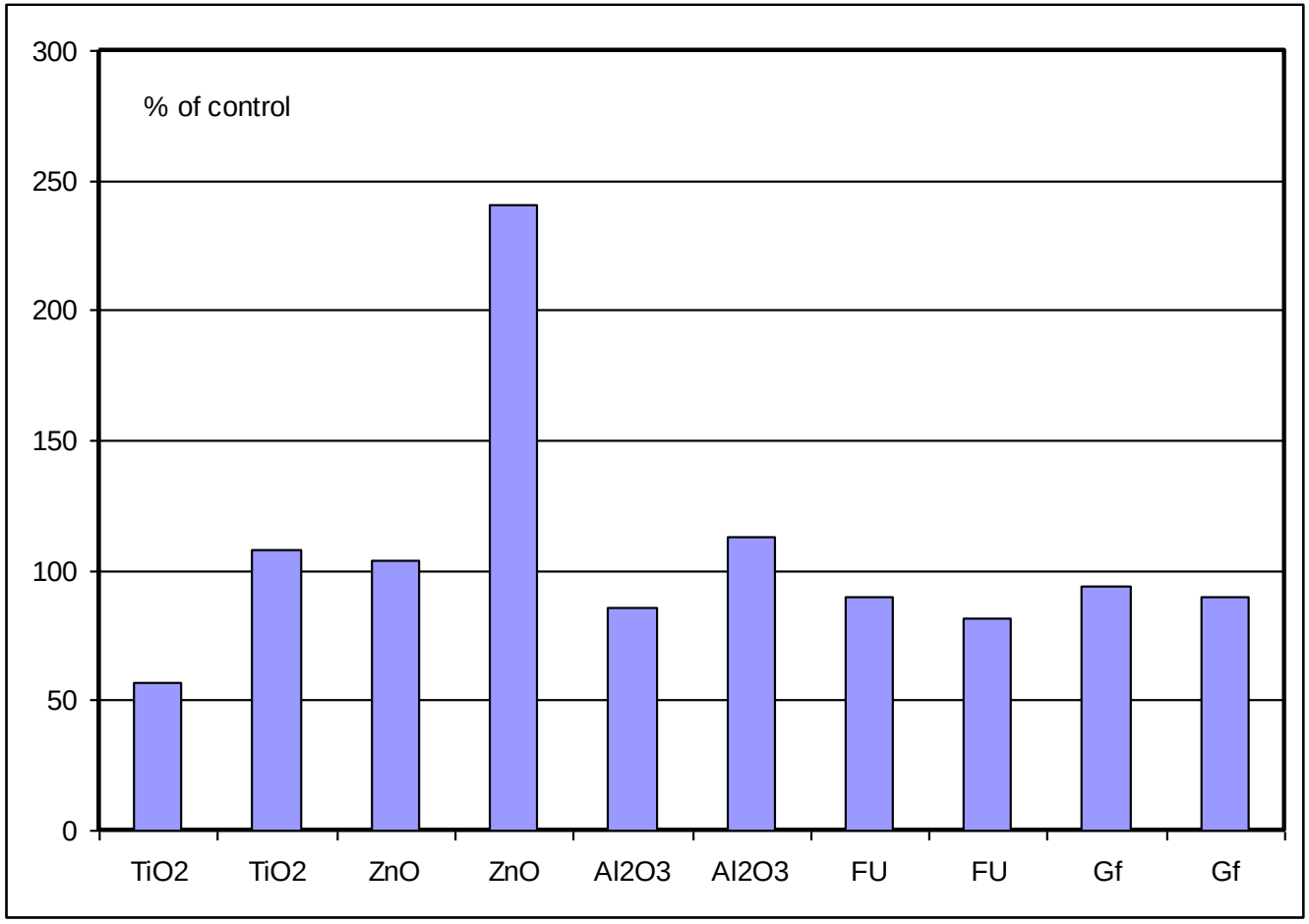
- **ACC aminocyclopropan carboxylic acid (ACC) the level of this precursor may be used as indicator of ethylene formation.**
- **Stimulation of ACC was found only in case of graphite fibers (at lower conc.). All other NPs decreased the production of ethylene.**
- **Part of their unfavourable effects might be disturbance of defense pathways in tobacco cells, probably via the unbalance of ion homeostasis.**

PEROXIDASES

- Belong to antioxidant enzymes involved the regulation of free radicals which in low concentration serve as a stress signals.

- Harmful in higher amounts

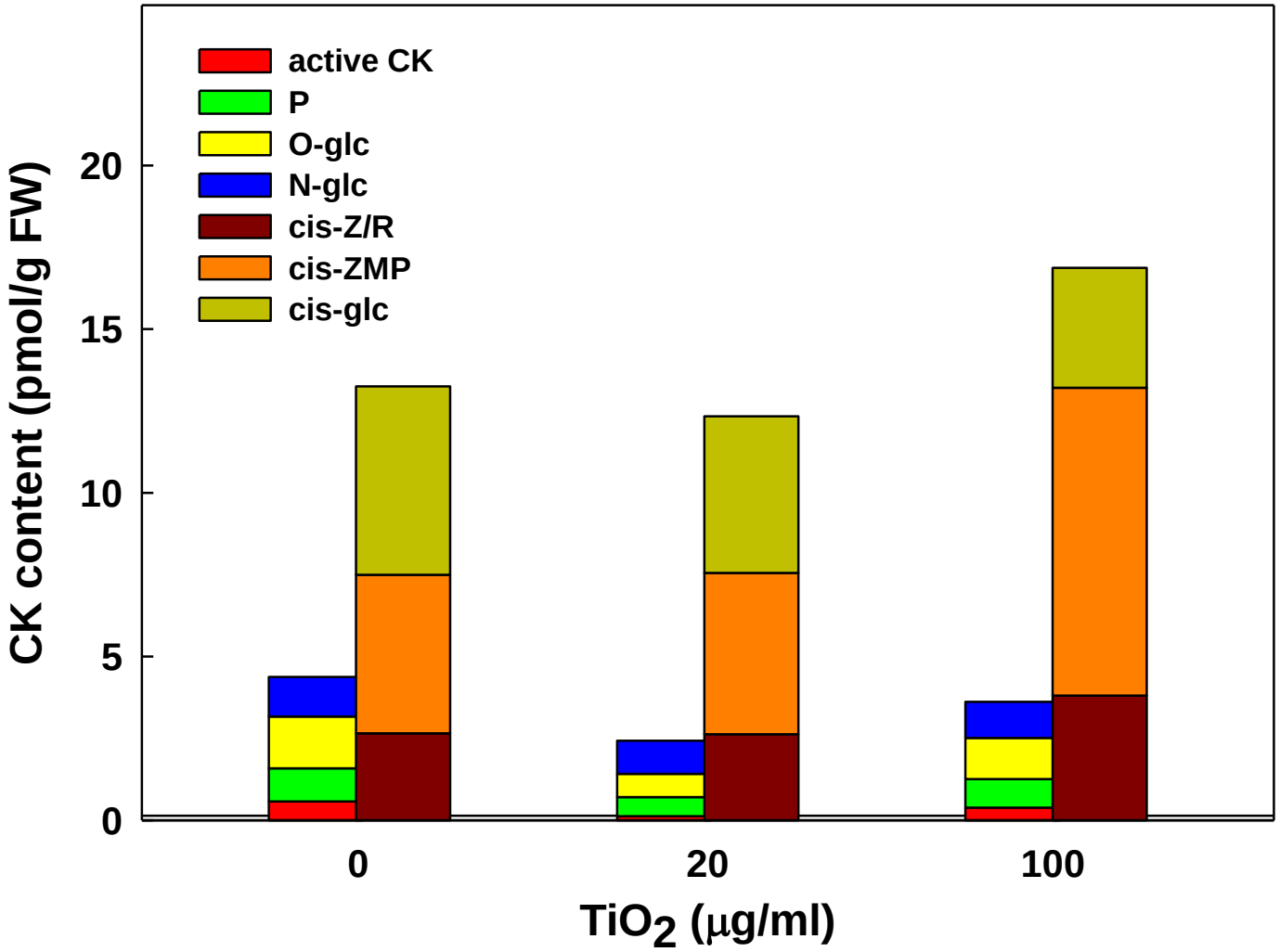
PEROXIDASE TEST



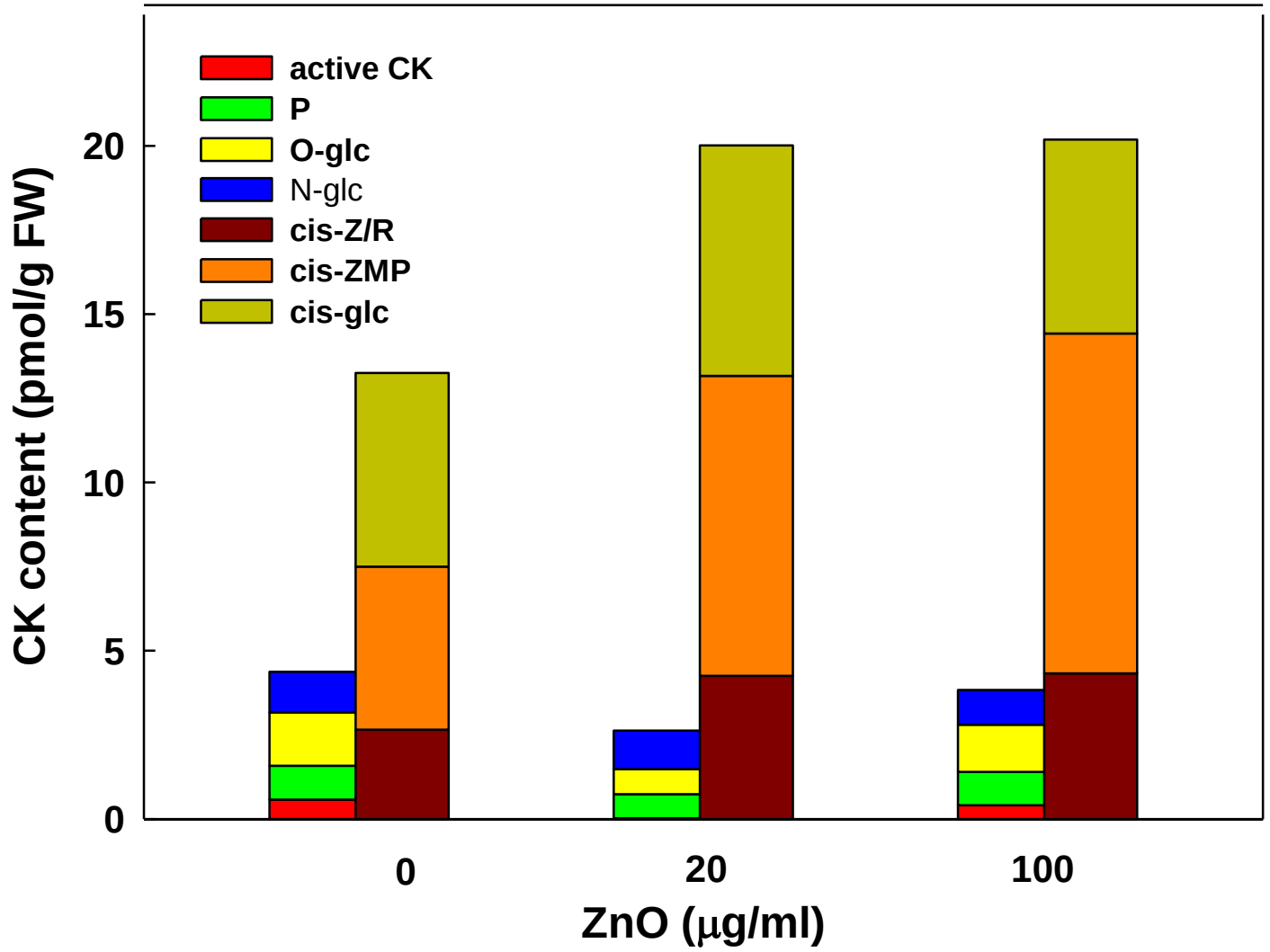
CYTOKININS

- Positive regulators of cell division
- Positive effect on photosynthesis – can preserve energy formation
- Mild stress – elevation cytokinines (energy formation).
- Strong or prolong stress – cytokinines decrease (growth supression).

TiO_2


active CK
P
O-glc
N-glc
cis-Z/R
cis-ZMP
cis-glc
CK content (pmol/g FW)
0
5
10
15
20
0
20
100
TiO2 (μg/ml)

ZnO


active CK
P
O-glc
N-glc
cis-Z/R
cis-ZMP
cis-glc
CK content (pmol/g FW)
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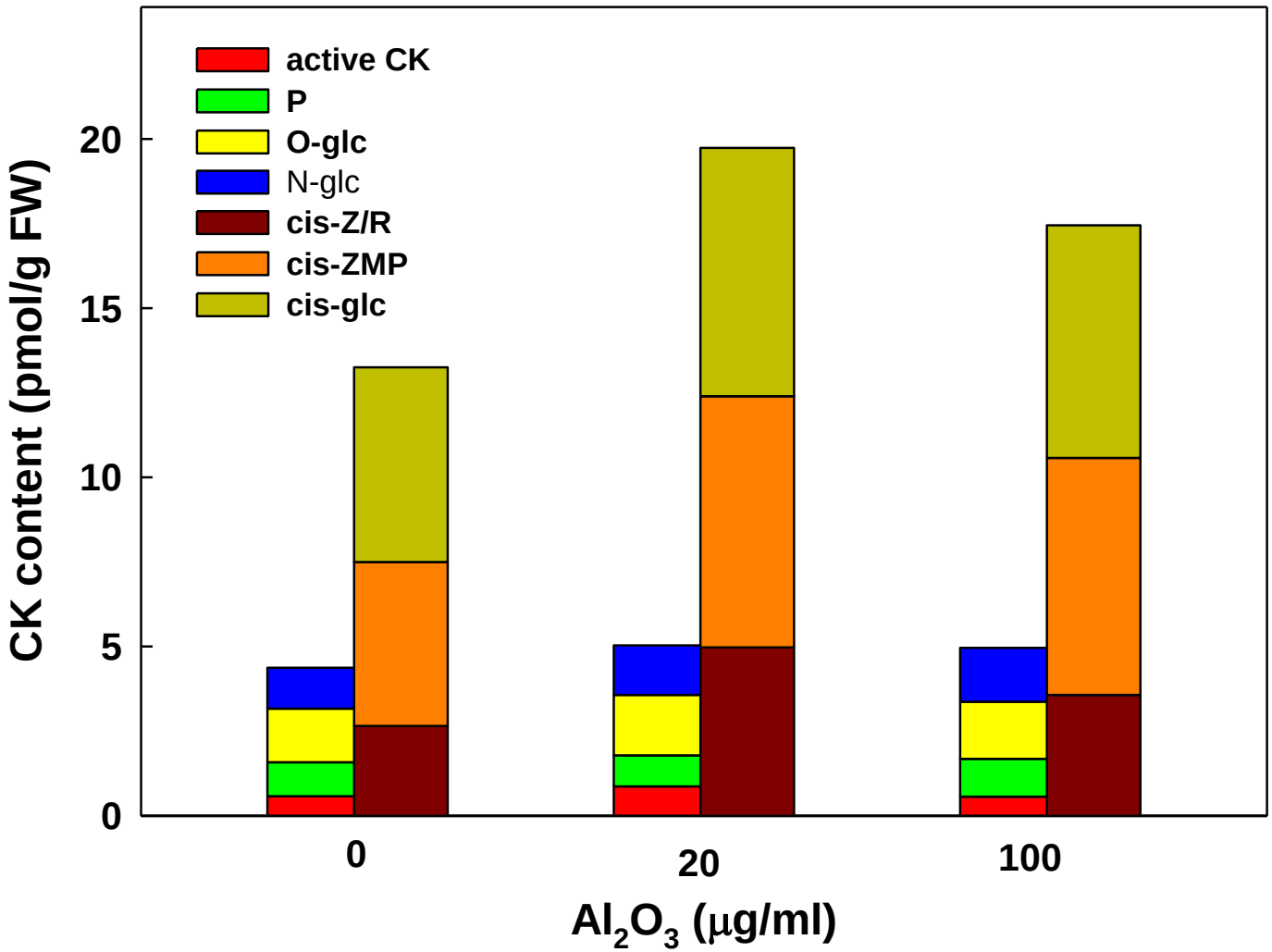
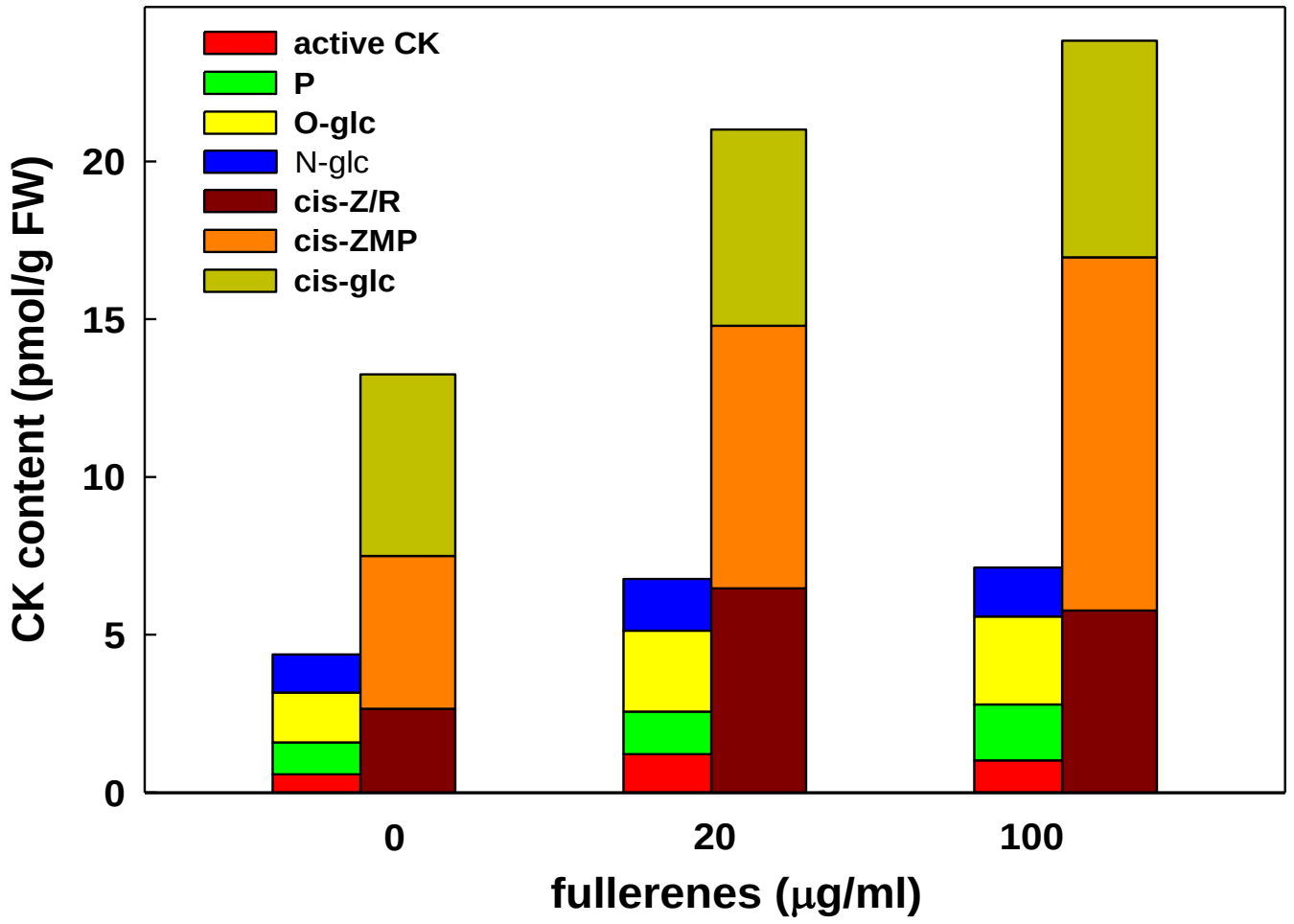
5
10
15
20
0
20
100
ZnO (μg/ml)

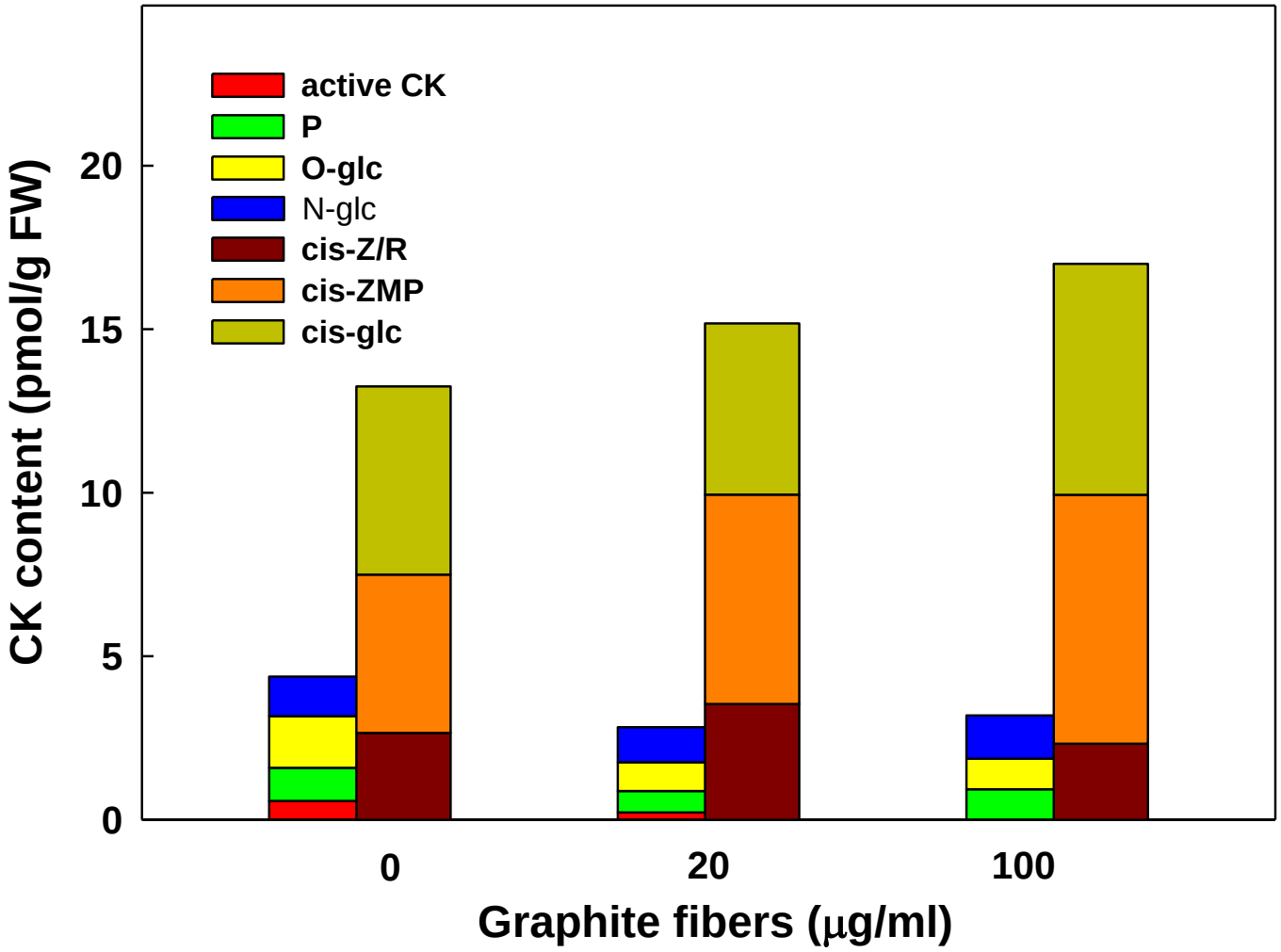
Al_2O_3


active CK
P
O-glc
N-glc
cis-Z/R
cis-ZMP
cis-glc
CK content (pmol/g FW)
0
5
10
15
20
0
20
100
Al2O3 (μg/ml)

Fullerenes


active CK
P
O-glc
N-glc
cis-Z/R
cis-ZMP
cis-glc
CK content (pmol/g FW)
0
5
10
15
20
0
20
100
fullerenes (μg/ml)

Graphite fibers

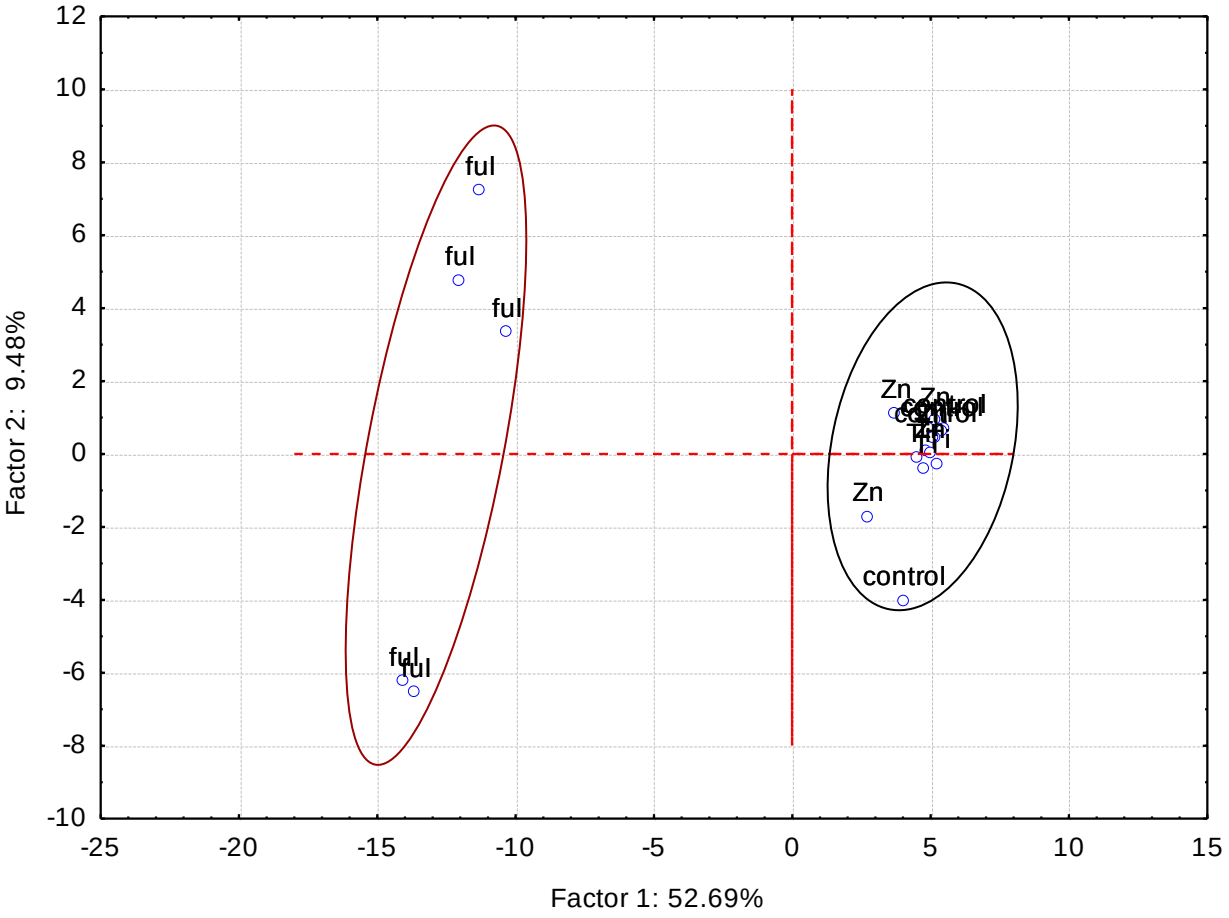


METABOLOMICS EXPERIMENTS

Analyzed samples:	non-polar fraction (CH_2Cl_2) derivatized by transmethylation and silylation
Instrumentation:	Thermo Scientific ITQ 1100™ GC/MS^n ion trap
Conditions:	temperature gradient: 50°C for 5', increased at 5°C/min to 310°C
	Helium flow: 1 mL/min
	injection temperature: 230°C
	interface: 250°C
	ion source: 200°C
	electron impact ionisation at 70 eV

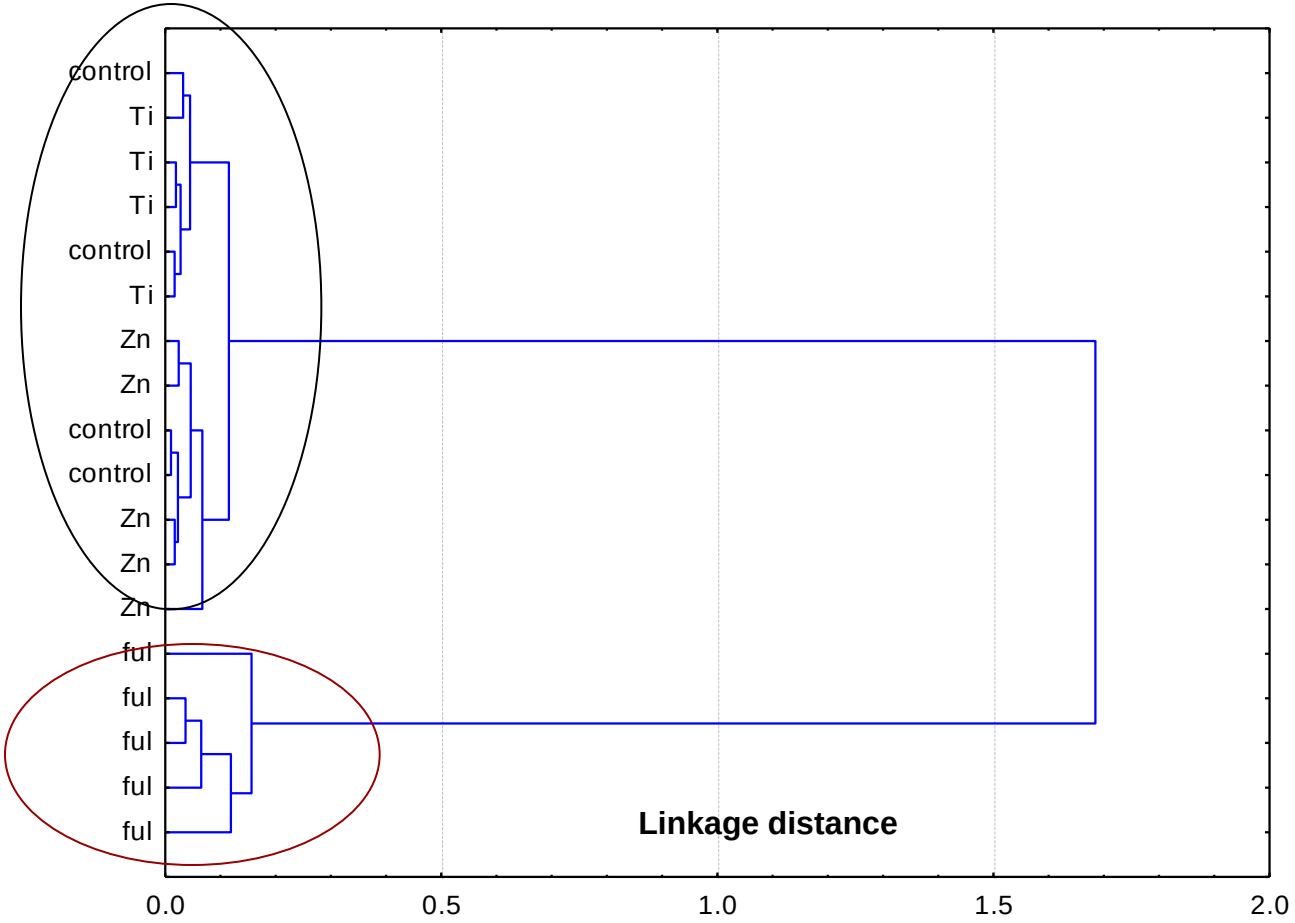
Principal component analysis (score plot)

of selected peaks without identification



Dendrogram of hierarchical cluster analysis

(Ward's method, Euclidean distance) of selected peaks without identification



MICROARRAY EXPERIMENTS

Total RNA labeled by Cy3 and Cy5 using two-color Low RNA Linear Amplification Kit PLUS (Agilent). Hybridized at 65°C for 17 hours.

Acquisition and processing of the microarray data was achieved by TM4 software (Saeed et al. 2003).

Agilent Tobacco Gene Expression Microarray

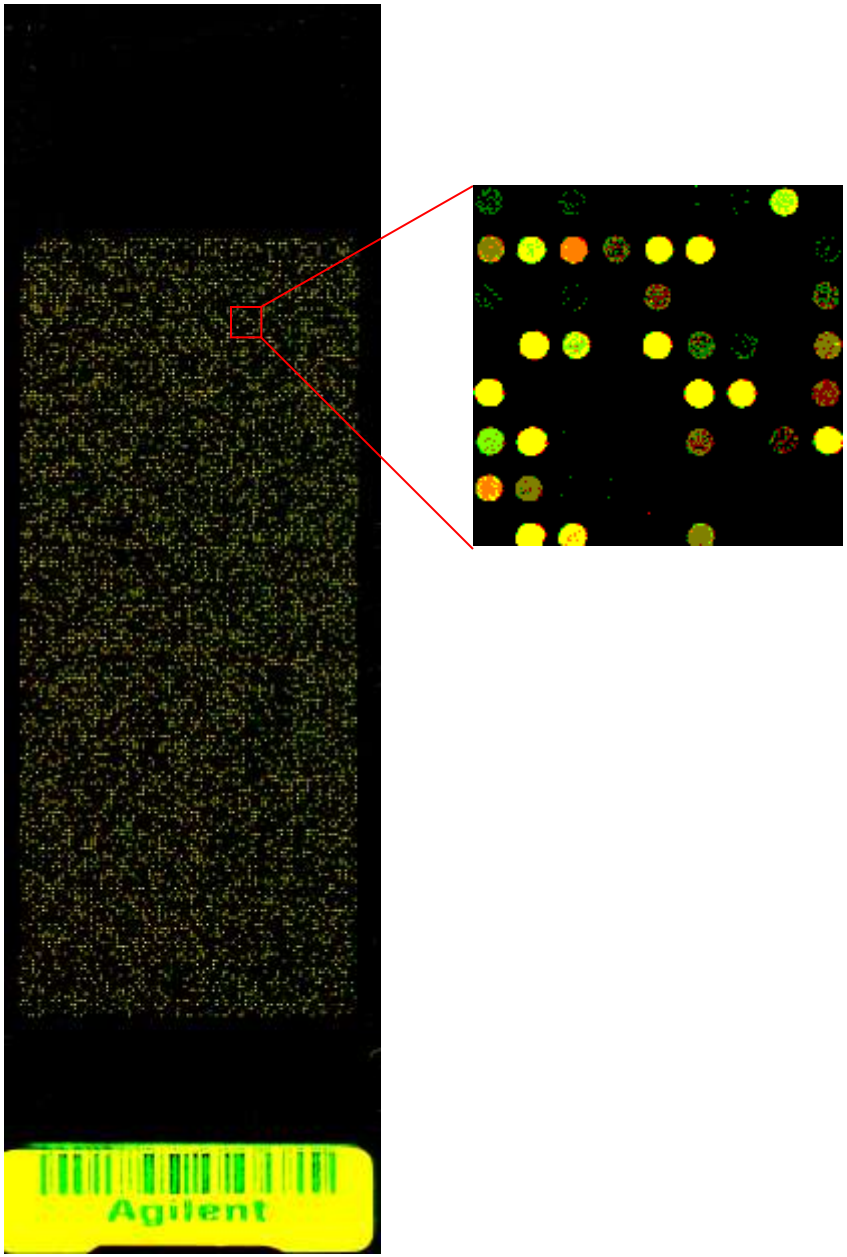
- **using 60-mer SurePrint technology**
- **43 803 Tobacco probes represented**
- **Content sourced from UniGene, TIGR, IGR Plant Transcript Assemblies**

Gene annotations were downloaded from Agilent, NCBI database, and DFCI Nicotiana tabacum Gene Index

Tobacco arrays

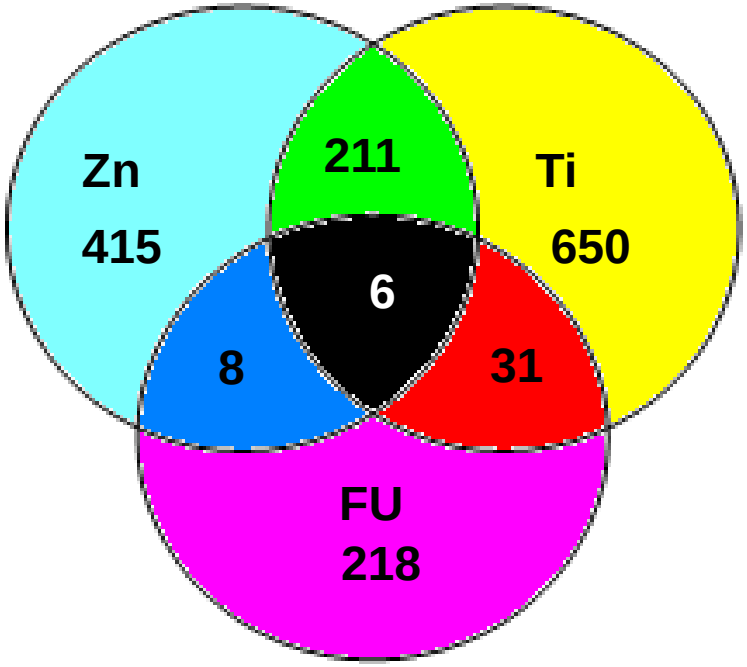
instead of

Arabidopsis…

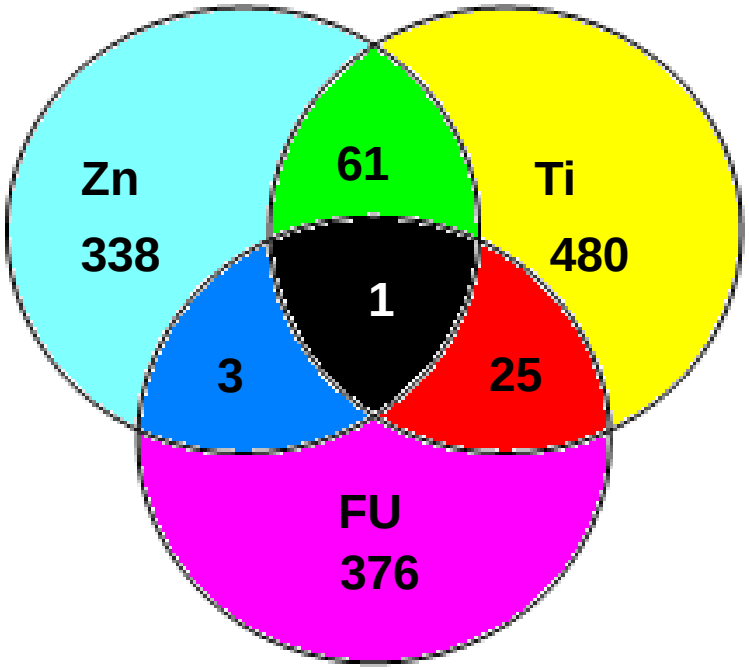


	ZnO	TiO2	Fullerenes
Up-regulated > 2:	415 genes	650 genes	278 genes
Down-regulated > 2:	338 genes	480 genes	376 genes

Up-regulated > 2:



Down-regulated > 2:



Up-regulated genes in all three stresses

Name	**Function**
FG640394	**Similar (65%) to Dehydration-responsive element binding protein 2 from *Glycine max***
BP131163	**Unknown**
EH616867	**Homologue (74%) *to* Peroxidase**
EH617918	**Unknown**
X65700	**homolog (68.9%) to *A. thaliana* osmotin=pathogenesis-related protein**
FG164960	**Peroxidase**

Most ten up-regulated genes in Zn and Ti

Name	**Function**
EB451575	**Unknown**
BP134489	**homolog (57.6%) to *A. thaliana* senescence-associated protein-related**
U32644	**immediate-early salicylate-induced glucosyltransferase**
FG640394	**Similar (65%) *to* Dehydration-responsive element binding protein 2 from *Glycine max***
FG638723	**homolog (57.6%) to *A. thaliana* senescence-associated protein-related**
TA17770_4097	**Unknown**
TA20932_4097	**partial similarity (40%) to MTD1(NAD-dependent 5,10-methylenetetrahydrafolate dehydrogenase, plays a catalytic role in oxidation of cytoplasmic one-carbon units)**
DW000416	**homolog (71.8%) to *A. thaliana* STO (SALT TOLERANCE); DNA binding / protein binding / transcription factor/ zinc ion binding**
TA14156_4097	**Unknown**
EH618944	**Unknown**

Eight up-regulated genes in Zn and FU

Name	Function
TA20932_4097	partial similarity (40%) to MTD1(NAD-dependent 5,10-methylenetetrahydrafolate dehydrogenase, plays a catalytic role in oxidation of cytoplasmic one-carbon units)
EH616867	Unknown
X65700	homolog (68.9%) to *A. thaliana* osmotin=pathogenesis-related protein
FG640394	Similar (65%) to Dehydration-responsive element binding protein 2 from *Glycine max*
FG164960	Peroxidase
EH617918	Unknown
EB428467	Unknown
BP131163	Unknown

Most ten up-regulated genes in Ti and FU

Name	Function
BP530375	Unknown
FG640394	Similar (65%) to Dehydration-responsive element binding protein 2 from *Glycine max*
TA20075_4097	Unknown
TA20932_4097	partial similarity (40%) to MTD1 (NAD-dependent 5,10-methylenetetrahydrafolate dehydrogenase, plays a catalytic role in oxidation of cytoplasmic one-carbon units)
DW004480	Unknown
EH618633	Unknown
EB451263	Unknown
CV019614	Similar (59.6%) to *A. thaliana* putative auxin-responsive protein
BP131163	Unknown
EB683621	Unknown

Down-regulated genes in all three stresses

Name	Function
EB443661	Unknown

Most ten down-regulated genes in Zn and Ti

Name	Function
FG155793	Unknown
DW004303	Similar (55.2%) to human v-myb myeloblastosis viral oncogene homolog isoform 3 (Transcription factor required for cell proliferation)
CV020131	Unknown
FG177612	Unknown
AM793637	ribosomal protein L20 from cold overnight cDNA library
BP528116	Unknown
BP532269	Similar (81.5%) *A. thaliana* AT2G27680 gene with aldo-keto reductase and oxidoreductase activity
EB448318	Unknown
BP533121	Unknown
TA13802_4097	Unknown

Three up-regulated genes in Zn and FU

Name	Function
DW004785	Unknown
BP530606	Unknown
EB443661	Unknown

Most ten down-regulated genes in Ti and FU

Name	Function
EB677801	Similar (92.4%) to *A. thaliana AT5G39850* 40S ribosomal protein S9
FG645490	Histone H3 (DNA binding)
FG635626	Histone H4 (DNA binding)
EB677656	Similar (72%) to *A. thaliana* carboxylesterase/ hydrolase, acting on ester bonds / lipase activity
EB444888	Unknown
TA12699_4097	Similar (95%) to histone H2A
EB435434	Similar (77.7%) to *A. thaliana* AT1G78580 alpha,alpha-trehalose-phosphate synthase (UDP-forming) activity/ transferase activity, transferring glycosyl groups
EG650355	Similar (75.1%) to *A. thaliana* AT2G05790 catalytic activity/cation binding/hydrolase activity, hydrolyzing O-glycosyl compounds
AJ717873	Similar (94.9%) to *A. thaliana* putative histone H2B (DNA binding)
EB432418	Unknown

Up-regulated genes

Peroxidases

⬇

oxidative stress

Response to dehydratation

Salt tolerance

Pathogenesis-related

Salicylate-induced glucosyltransferase

Senescence-associated protein

⬇

Various abiotic and biotic stimuli responses

Down-regulated genes

***Histones*- components of chromatin**

- DNA binding

⬇

DNA demage?

Ribosomal proteins

⬇

Decrease protein translation?

Lipase activity

⬇

Impact on lipid metabolism?

HYDROPONIC EXPERIMENTS

SUMMARY

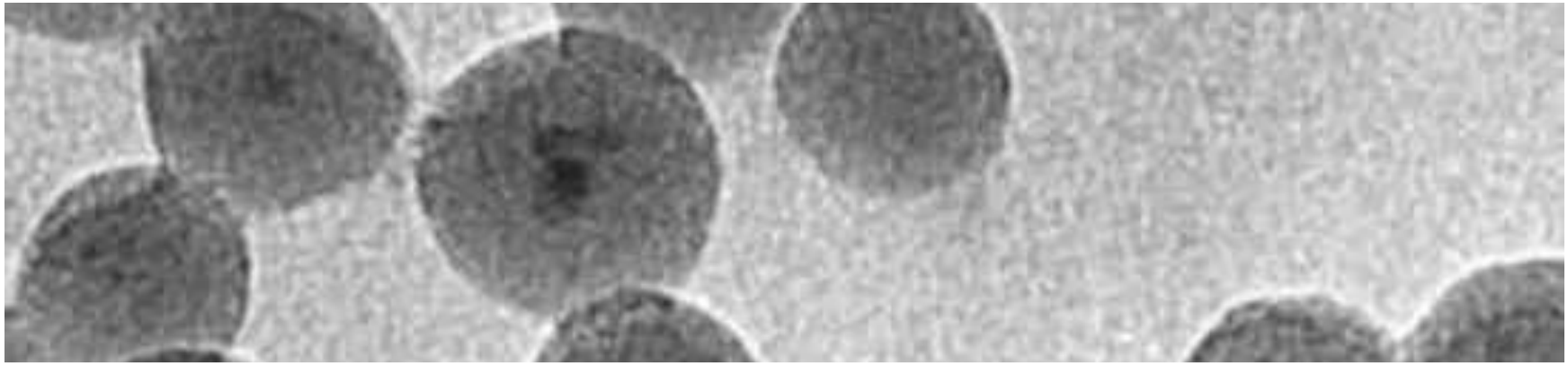
- Mild negative effect on cell division
- Stimulation of stress responses
- Different mechanisms metals x graphite
- Stimulation of antioxidant system (metals)
- Stimulation of ethylene formation (graphite)
- Microaray data confirm stimulation of antioxidant system as well as general stress response and down-regulation of genes related to cell division.

SUMMARY 2

- At this moment preliminary results only
- Complex approach necessary
- Proteomic data missing
- Real experiments missing

SUMMARY 3

- Nanoparticles have high technical potential
- Their impact to the environment is still not fully elucidated
- More research necessary to avoid potential danger



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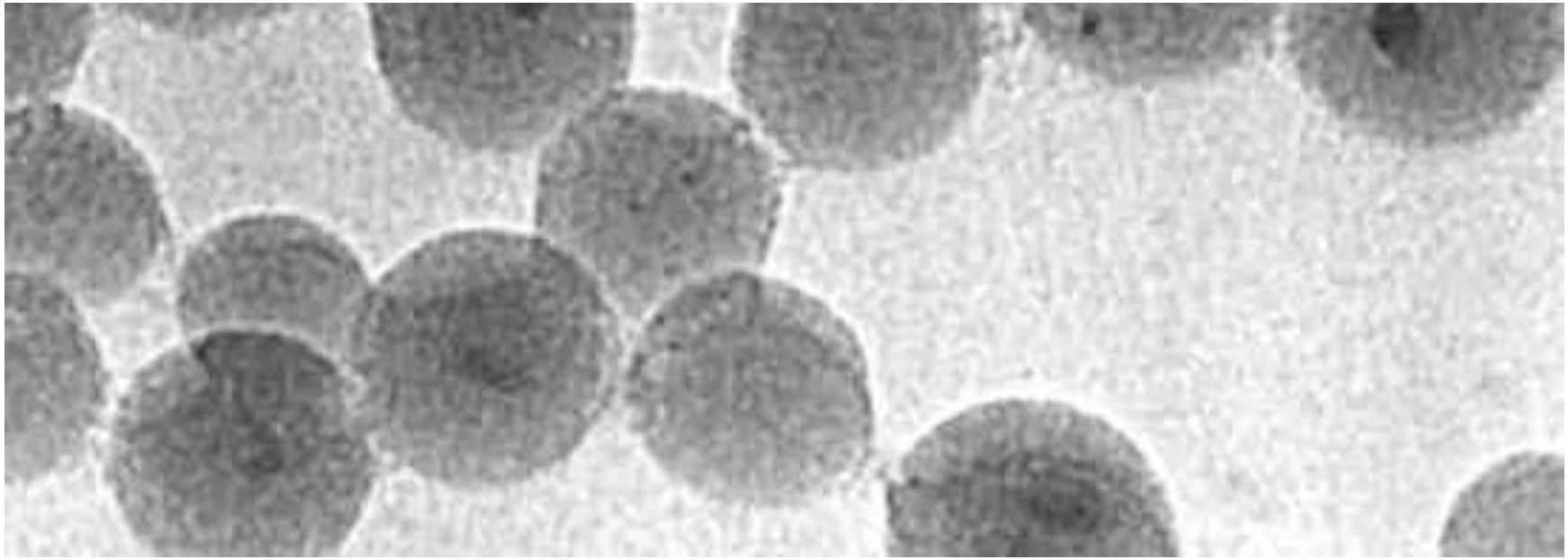

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