

# Ecotoxicity effects of multi walled carbon nanotubes (MWCNTs)

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## EU FP7 project

<http://www.sintef.no/Projectweb/BYEFOULING/>

# **BYEFOULING-Low-toxic cost efficient environment friendly antifouling materials**

### Acknowledgements:

- Interuniversity Institute for Marine Sciences in Eilat
- TAU students and staff members
- Israel Nature and National Parks Protection Authority - permits.



**Biofouling**: undesirable accumulation of marine microorganisms, plants, animals on submerged structures, especially ships' hulls

Ships hulls may gather 150 kg of fouling per square m in < 6 months. On oil tanker with 40,000 square m underwater area, this would add ~6,000 tons of fouling.



**Anti-fouling**: process of removing the accumulation, or preventing it

# Biofouling





# Marine structures are under constant attack from the environment being biofouled

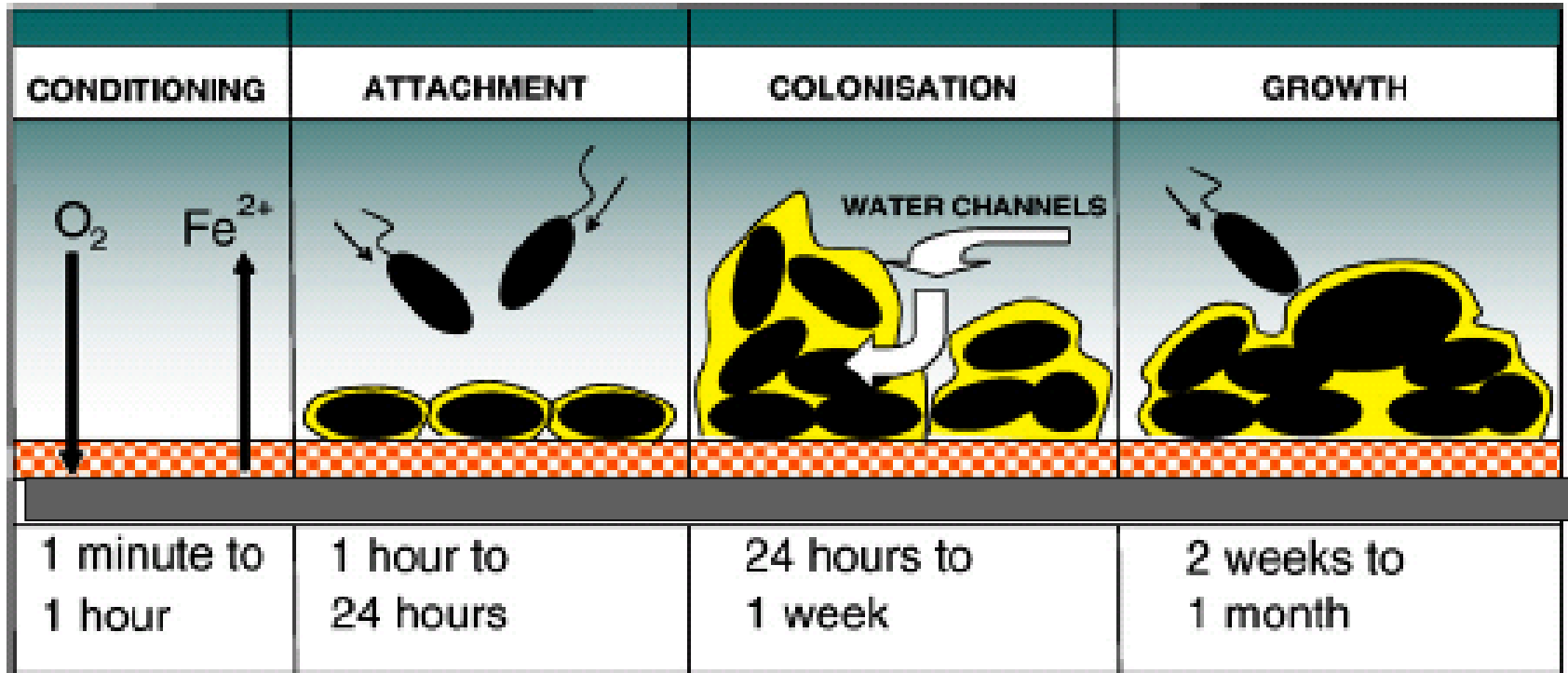
- Ship hulls
- Marine platforms
- Docks
- Offshore rigs
- Fish cage nets
- Floating buoys



## Need to be protected from biological attack

# Process of biofouling: succession and growth stages

*L.D. Chambers et al. / Surface & Coatings Technology 201 (2006) 3642–3652*

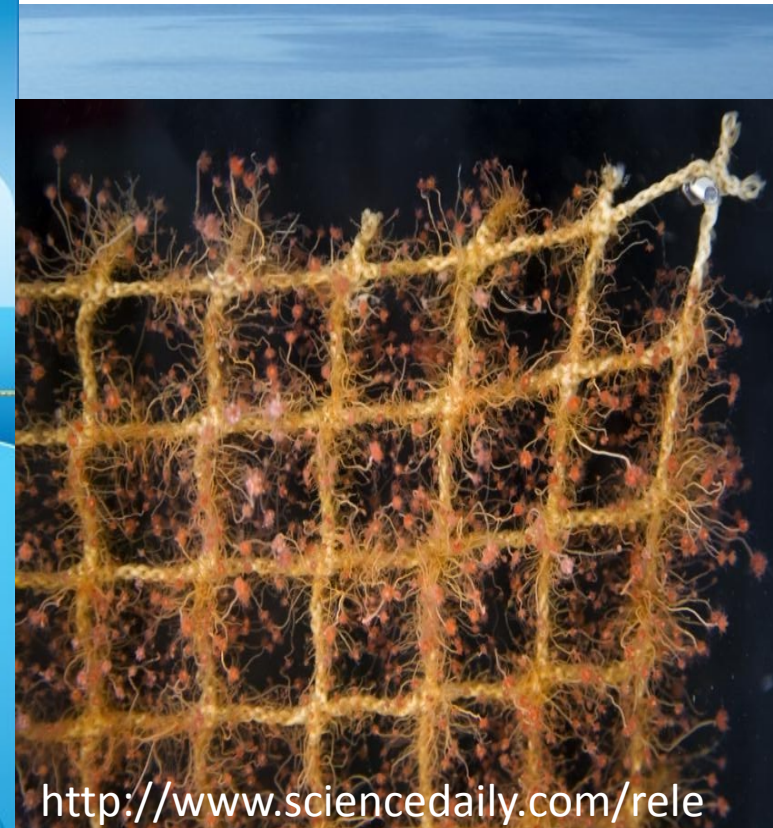
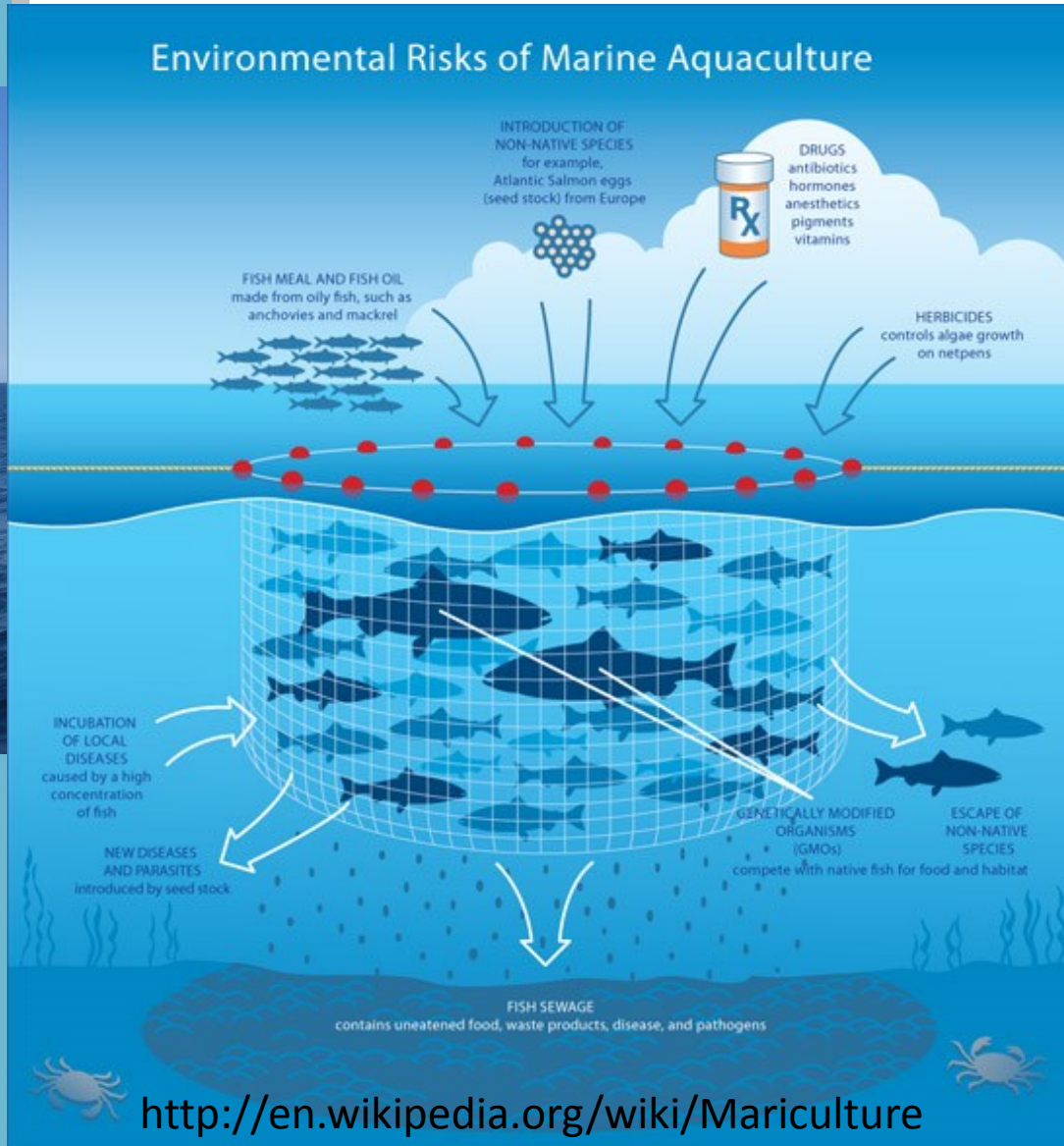


## Sequence of events – the first event: biofilm formation

EU project, FP7-KBBE: Byefouling - Low-toxic cost-efficient environment-friendly antifouling materials

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# Mariculture/ aquaculture – fish net cages





# Oceanographic measuring and navigation buoys



Accumulation of both indigenous and invasive fouling organisms: barnacles, mussels, sponges, algae, etc.

# Anti-fouling systems

Barnacles are quite unlike other crustacean, because they permanently attach to the substrate by means of an adhesive cement, and secrete calcareous shell

Anti-fouling paints using metallic compounds slowly "**leach**" into the sea water, killing marine life that have attached to substrate

Planktonic larvae



Settled barnacles



# Fouling effects

- Hydrodynamics - negatively affected
- Increase hull roughness
- Increased fuel consumption
- Degradation of ship performance
- Frequent docking
- Spread of invasive species





Revolutionary self polishing  
copolymer technique  
employing TBT (tributyltin)  
to deter fouling

Considered as the most toxic  
substance ever deliberately  
introduced into aquatic  
environment



**Imposex** – endocrine disruption  
causing development of male  
organs in female snails –  
bio-indicator of TBT

- 2003: ban on TBT based paints  
application
- 2008: ban on TBT based paints

# Modern antifouling alternatives

- Ban on TBT created a gap in the market.
- Research began into environmentally acceptable replacements

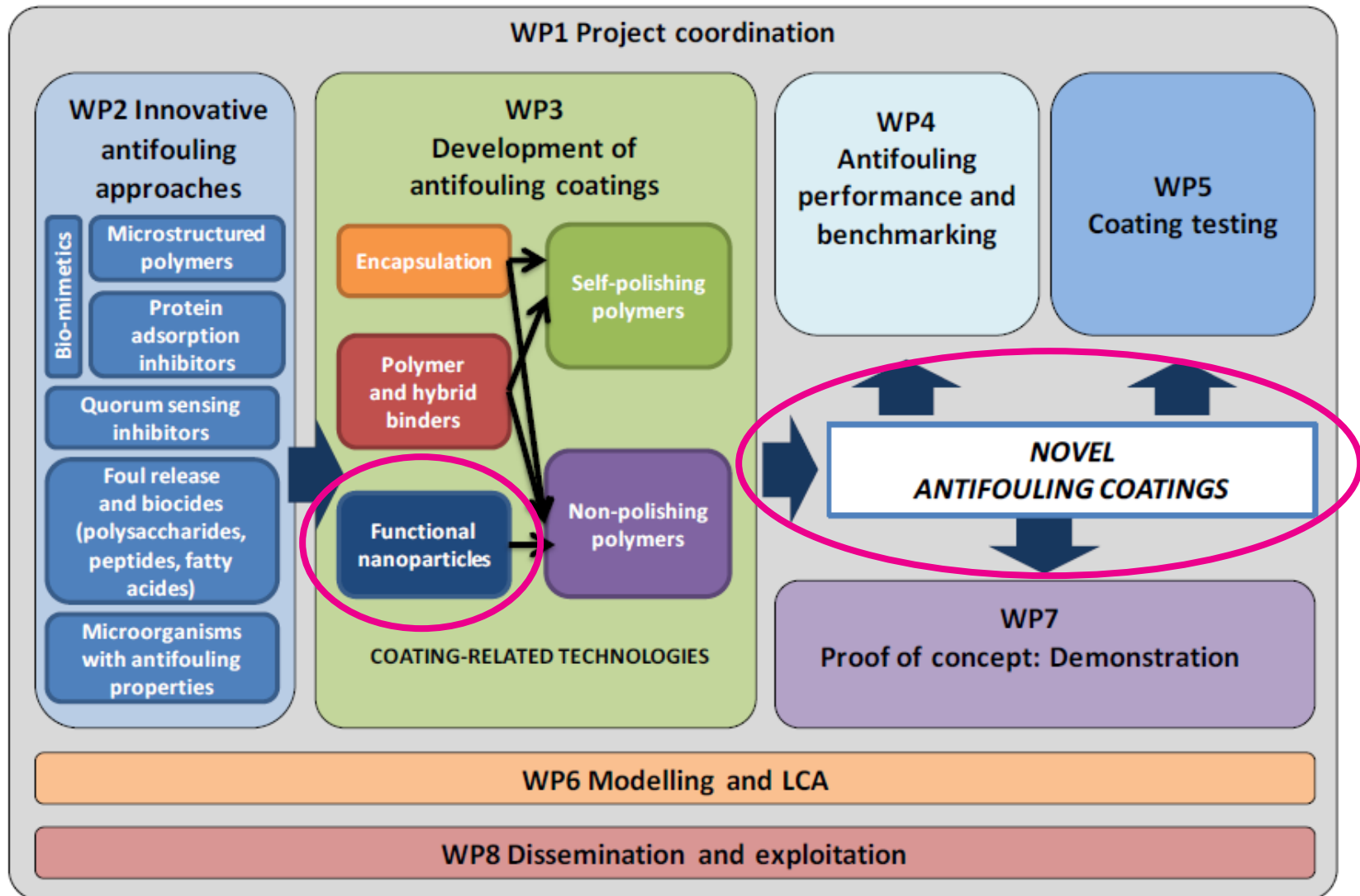
## Interim solutions:

- Copper & zinc self-polishing copolymers
- Booster biocide approach (phenols, phosphorous, sulfurous, aldehydes etc.)

Species specific, influencing non-target organism,  
short life time, slow drying time, expensive, leaching  
rate not constant

# BYEFOULING

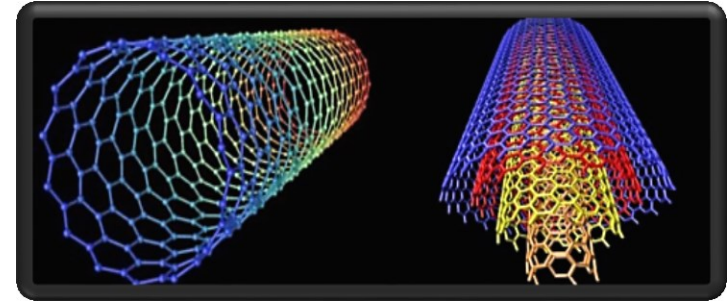
## Low-toxic cost-efficient environment-friendly antifouling materials



# Carbon Nanotubes (CNTs)

Due to their properties are leading materials in technology :

- composites as reinforcements
- coatings
- energy storage
- electronics and sensors.



## Structure

- **Single Walled Carbon Nanotubes (SWCNTs):** A rolled-up, single layer graphene sheet, diameter  $\sim 1$  nm, length several  $\mu\text{m}$ .
- **Multiwalled Carbon Nanotubes (MWCNTs):** 2 or more layers

SWCNTs

MWCNTs

Antifouling activity of CNTs – fouling resistance in shape of nanocomposites with metal, gaseous compounds, metal oxide biomolecules, polymers etc.

## Surface functionalization for lower toxicity

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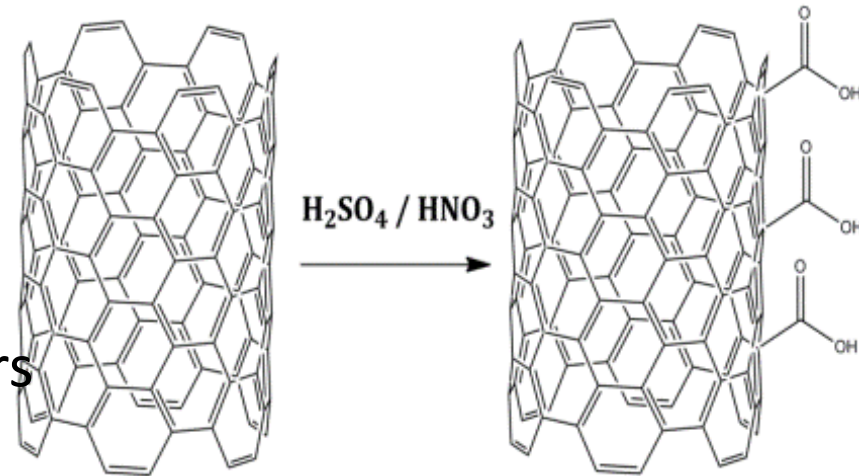
# Production of CNTs

## ❑ Chemical Vapor Deposition (CVD) method

- Camphor as carbon source & ferrocene as catalyst compound (20:1 ratio)
- Growth temperature: 850°C
- Silicon wafers substrate

## ❑ *Functionalization*

- Better compatibility with polymers matrices
- Better dispersability in polar solvents
- Lower toxicity



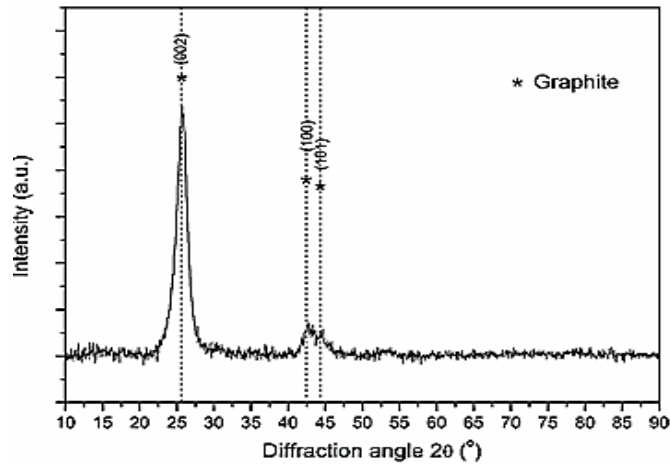
## ❑ Characterization techniques

- SEM, TEM, X-Ray Diffraction XRD, Thermogravimetric Analysis (TGA), etc.

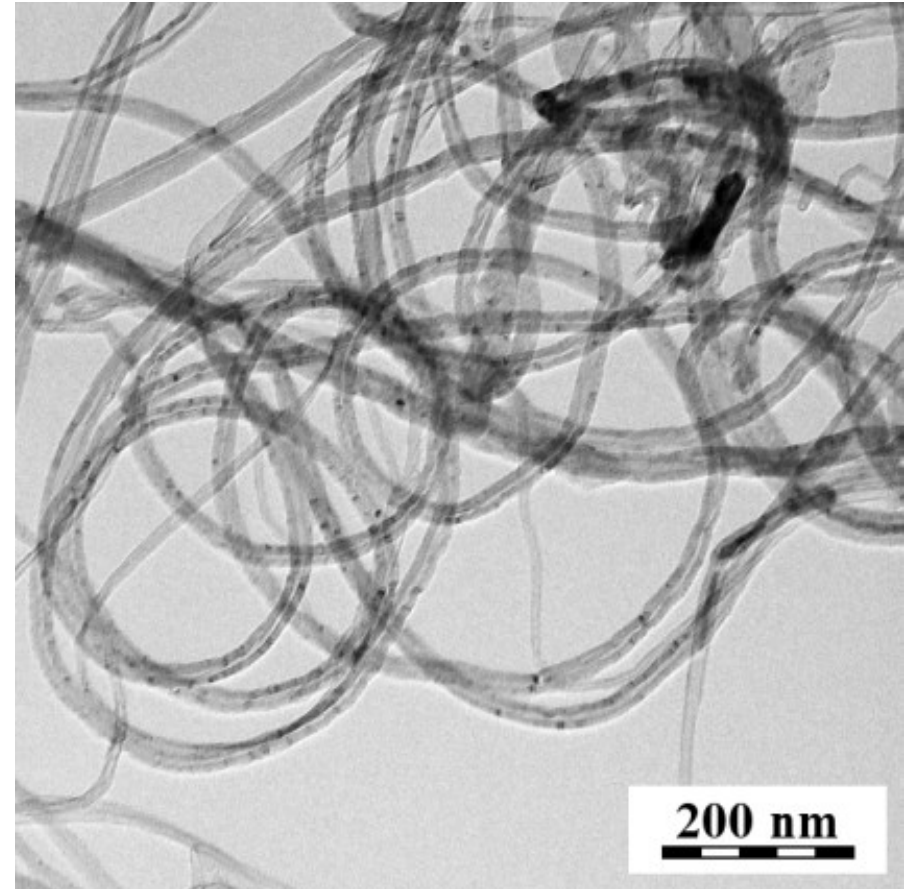


# Characterization of MWCNTs

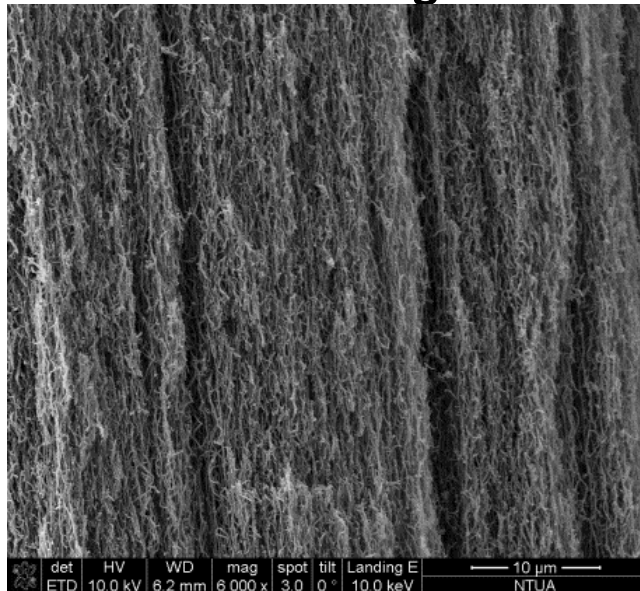
## XRD pattern



## TEM image



## SEM image



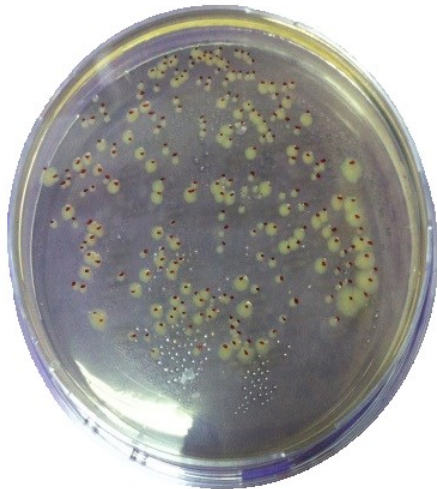
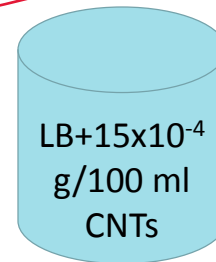
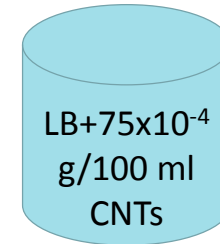
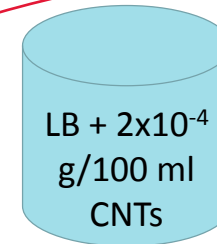
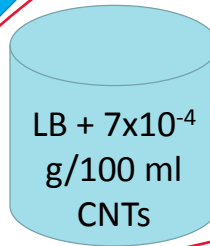
# Antibacterial – *E. coli* - testing

*E. coli*



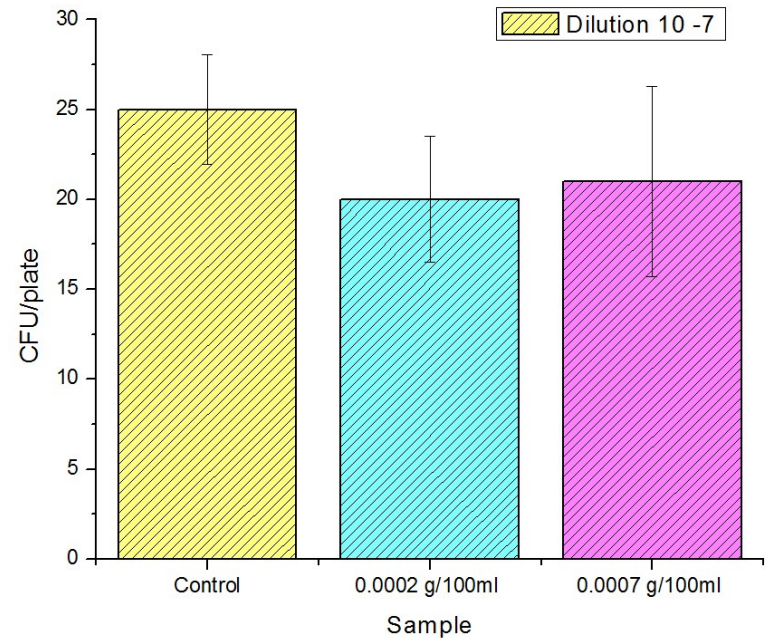
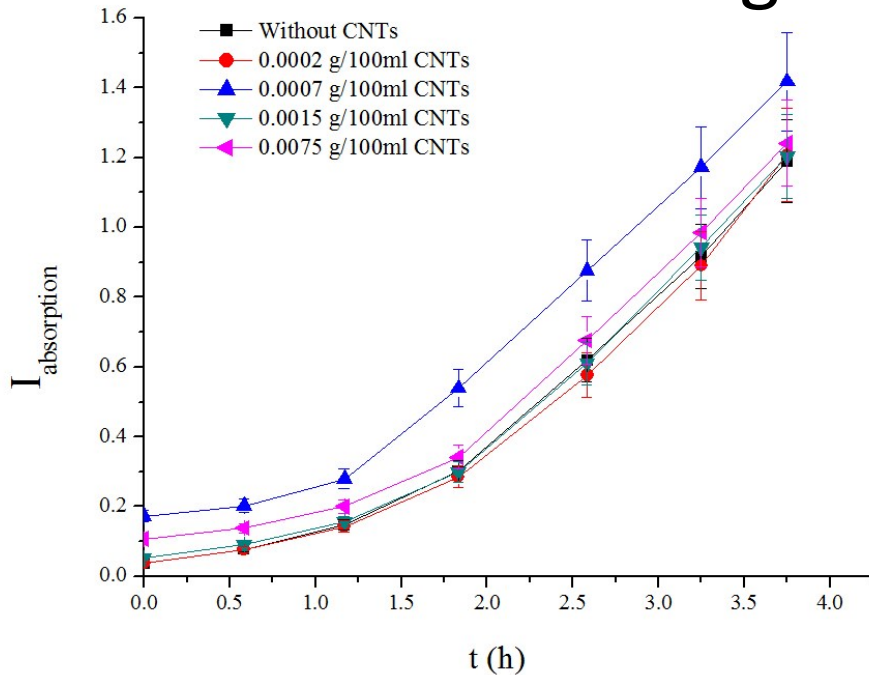
When  $OD_{595} = 0.5$

2.5% v/v *E. coli*



$OD_{595}$  measurement every 30 min

# Antibacterial Testing



**Increase of CNTs concentration - lowered growth rate ( $\mu_{max}$ ) & cell growth ( $x_{max}$ )**

Without CNTs :  $\mu_{max} = 1.0361 \text{ h}^{-1}$

0.0002g/100ml MWCNTs:  $\mu_{max} = 1.0075 \text{ h}^{-1}$

0.0007g/100ml MWCNTs:  $\mu_{max} = 0.9797 \text{ h}^{-1}$

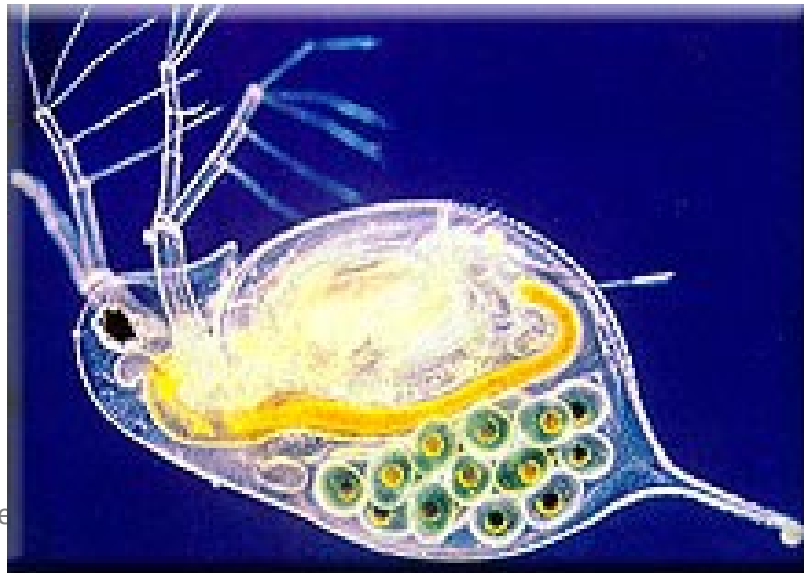
0.0015g/100ml :  $\mu_{max} = 0.9566 \text{ h}^{-1}$

**0.0075g/100ml :  $\mu_{max} = 0.7624 \text{ h}^{-1}$**

**Lower CNTs concentration reduced colony growth  
Fewer & smaller colonies**

# *Daphnia similis* assays

- Planktonic freshwater crustaceans
- “Water fleas”
- Filter feeders
- Short life cycle
- Model organism for ecotoxicity assays



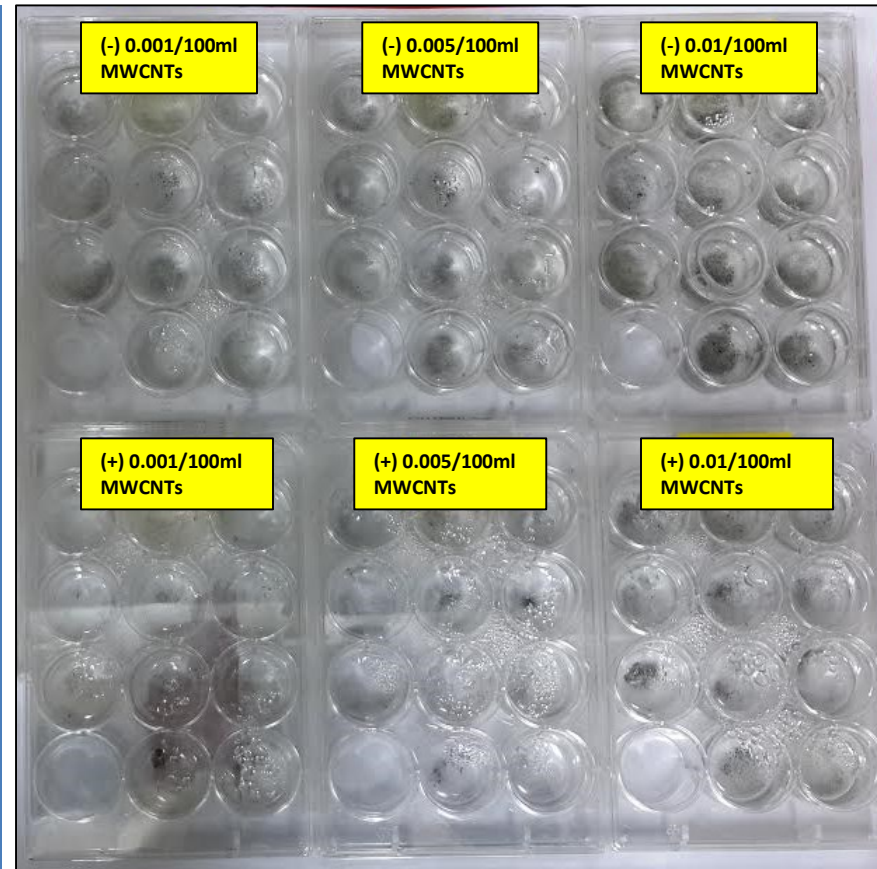
EU proje



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# MWCNTS ASSAY: LAYOUT AND CONDITIONS

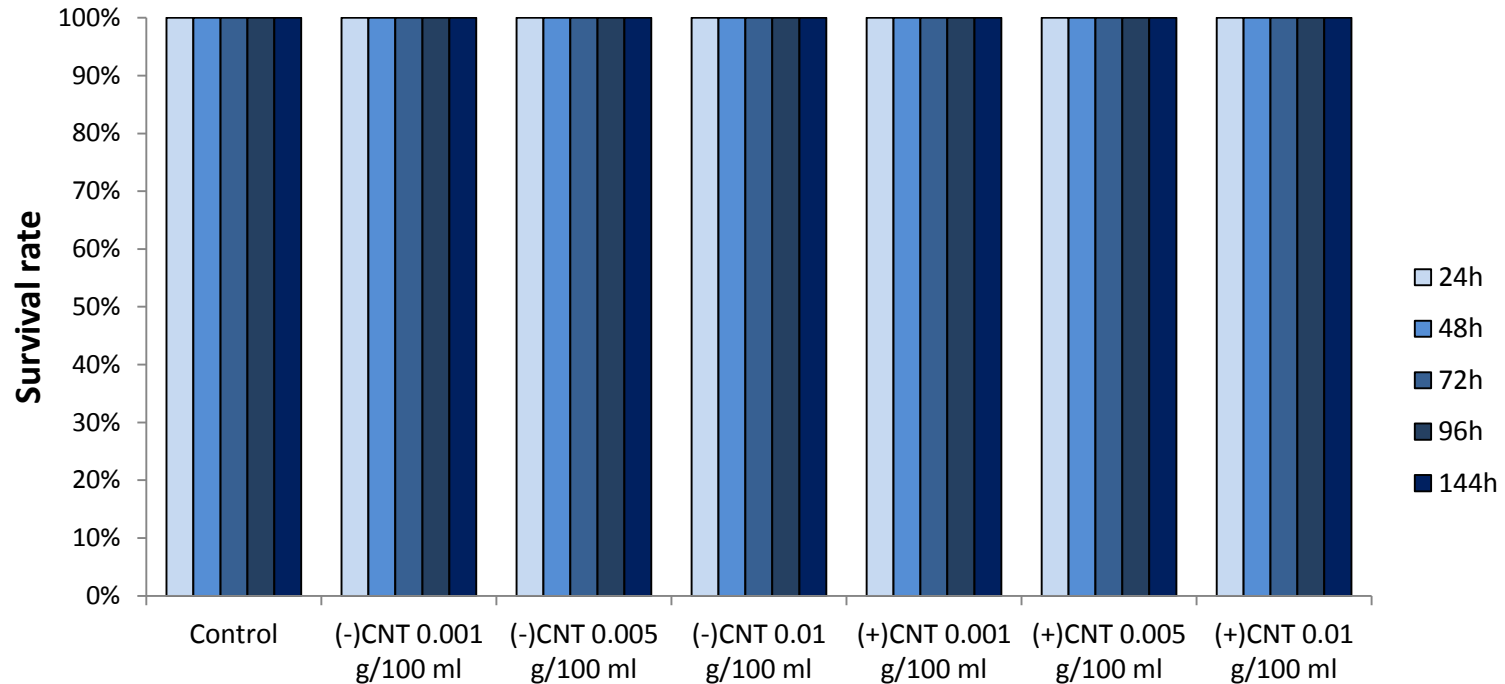
Criteria	Conditions
Organisms	<i>Daphnia similis</i> 7 days post-hatch Same clade
Acclimation period	No acclimation
Temperature / Photoperiod (L:D)	Ambient / 12:12 (L:D)
Test chambers and support equipment	12-multiwell test plate
Medium	ADaM medium
Dilutions	3 concentrations x 2 different types of MWCNTs + 1 control (ADaM medium)
Replicates	12 for each concentration tested
Observation points	24, 48, 72, 96 and 144 h
Endpoints	% mortality
Feed	Yes- <i>Scenedesmus gracilis</i>
Duration of the experiment	144 h



(+ ) – With active component  
( - ) – Without active component

\* ADaM = Aachener Daphnien Medium

# Survival rate of *Daphnia* in different concentrations of MWCNTs

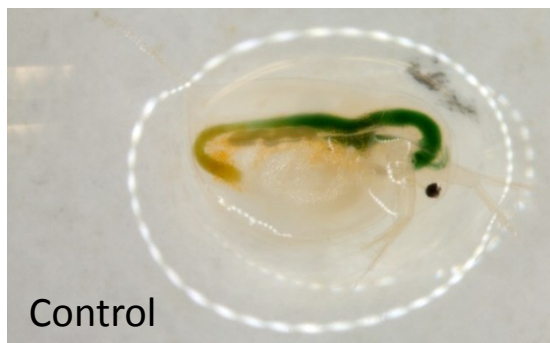
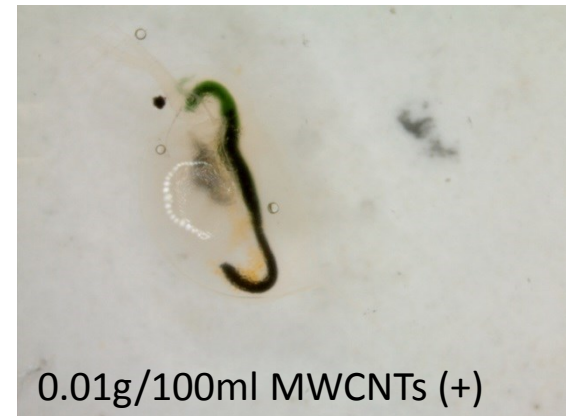
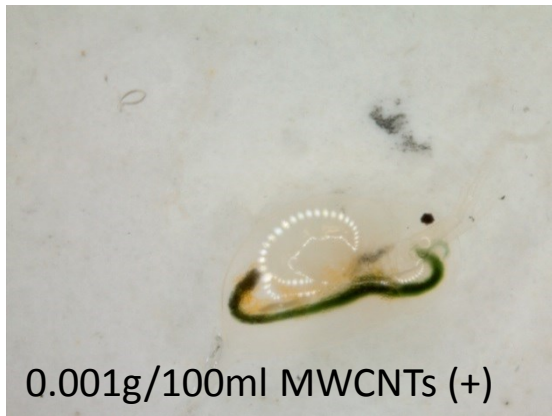
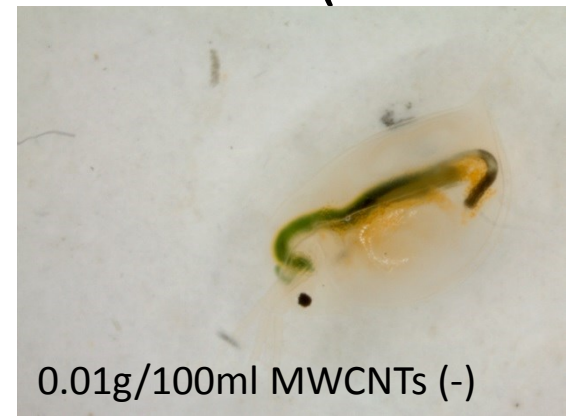
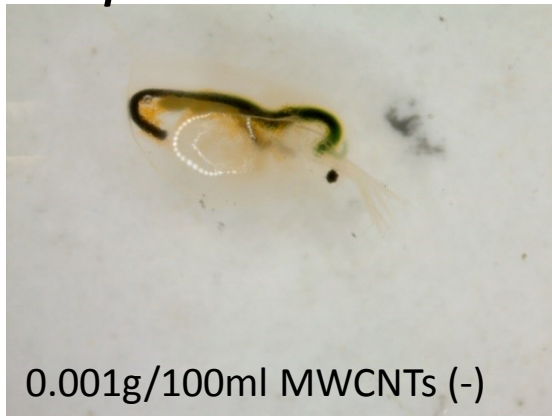


(+ ) – With active component  
( - ) – Without active component

Concentrations of MWCNTs

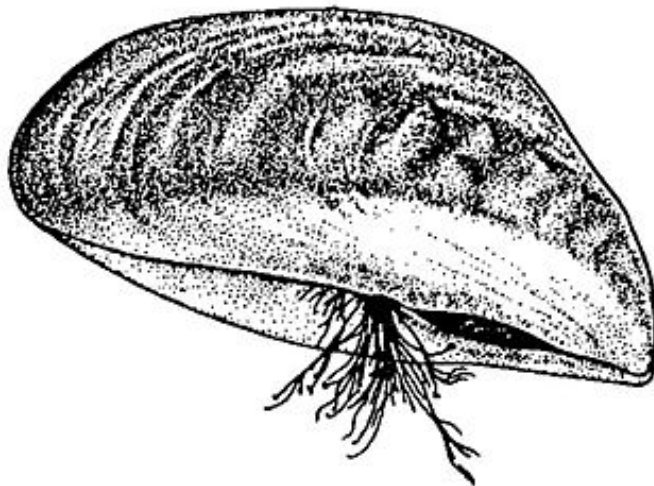
**Conclusion: MWCNTs do not cause mortality of *Daphnia***

# *Daphnia similis* after exposure to MWCNTs– 96 h (All alive)



(+) – With active component  
(-) – Without active component

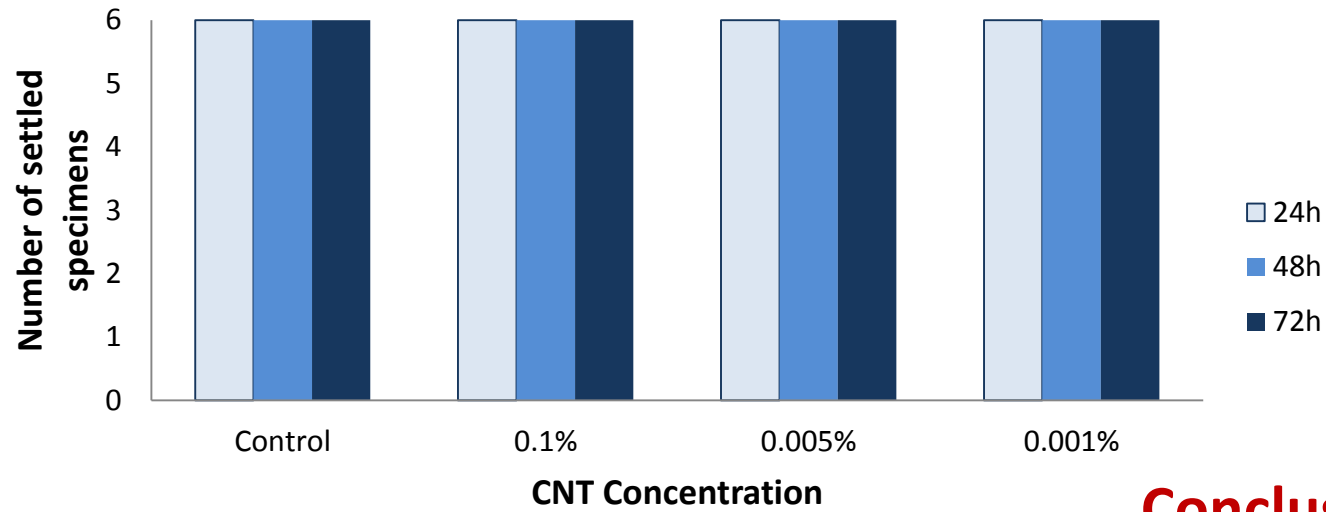
# Mussel assay: *Brachidontes pharonis*



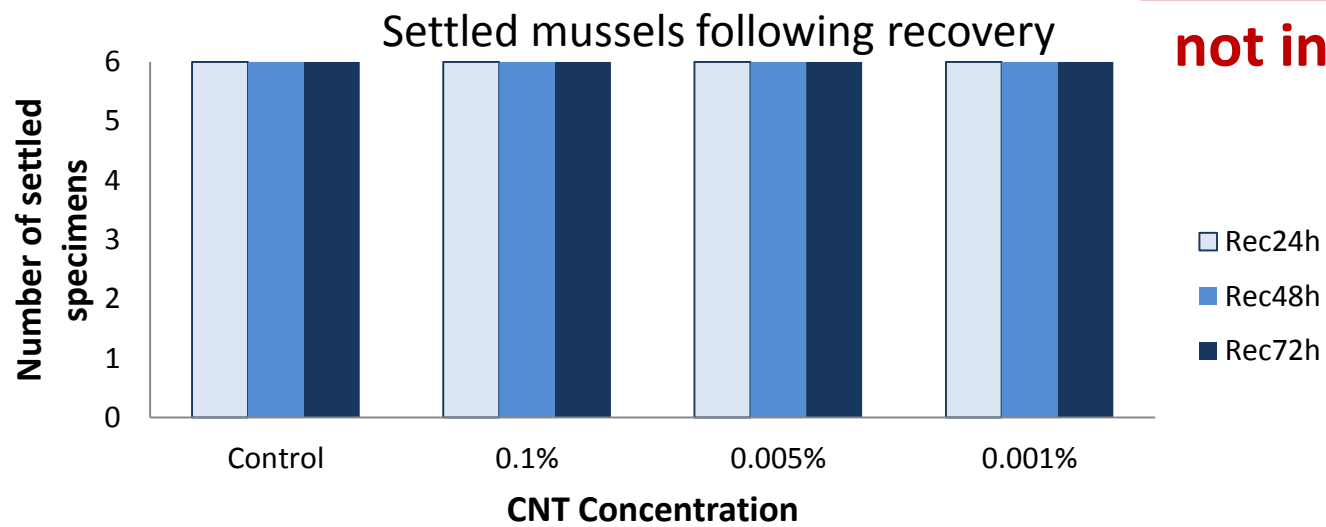


n=6 animals per treatment

# Settled *Brachidontes* mussels during CNTs assay



**Conclusion: MWCNTs do not inhibit settlement**



## Conclusions

- ❑ **Multi Walled Carbon Nanotubes** were synthesized via **Chemical Vapor Deposition Method** and **fully characterized** via SEM, TEM, XRD and Thermogravimetric analysis.
- ❑ MWCNTs with the **following characteristics** were produced:
  - 60 – 100 nm diameter, > 5 $\mu$ m length, 92 – 95 % purity
- ❑ MWCNTs were **further purified** and **functionalized with oxygen containing groups** in order to increase their **compatibility** with different solvents and matrices.
- ❑ **Carboxyl groups have introduced** onto MWCNTs sidewalls.
- ❑ Increased concentration of CNTs led to their **lower dispersion**.
- ❑ CNTs **lowered bacterial growth**.
- ❑ Quantitative plating results indicated that lower CNTs concentration appeared to prevent colonies' development.
- ❑ Higher the concentration of CNTs, the lower interactions with the organisms, probably due to the **creation of agglomerates**.
- ❑ **No effect on *Daphnia* or mussels**.

**Thank you!**



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