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The toxicity effects of Fe_2O_3, TiO_2, ZnO, and Ag engineered nanomaterials (ENMs) on the macrophyte *Spirodela* species

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WHAT IS NANOTECHNOLOGY?

It is the development and application of materials with at least one dimension in 1-100 nano-meter range (10^{-9} m) in any phase of matter. Nanomaterials possess strikingly different properties relative to macroscale materials mainly due to high surface area and increased atom density at the surface. The global nanotechnology industry is growing rapidly.

NANOTECHNOLOGY AND ENVIRONMENTAL CONCERNS

Data indicates increasing nanotechnology use whilst concerns have been raised because ENMs are generally highly reactive and their environmental fate and effects are less understood

BACKGROUND

Nanoecotoxicity is a relatively new toxicology field which started in the mid 2000s. Data so far indicates algae to be relatively sensitive to other groups of aquatic organisms, but there is minimal data on algae and no data exist on macro-algae or macrophytes (Kahru and Dubourguier *et al.* 2010). Nanoecotoxicity data is useful for product safety data sheets, ecological risk assessment and environmental guidelines development. The current uncertainties about ENMs environmental fate and effects limit a wider nanotechnology application. Current literature suggests oxidative stress as a basic form of toxicity induction on biological systems

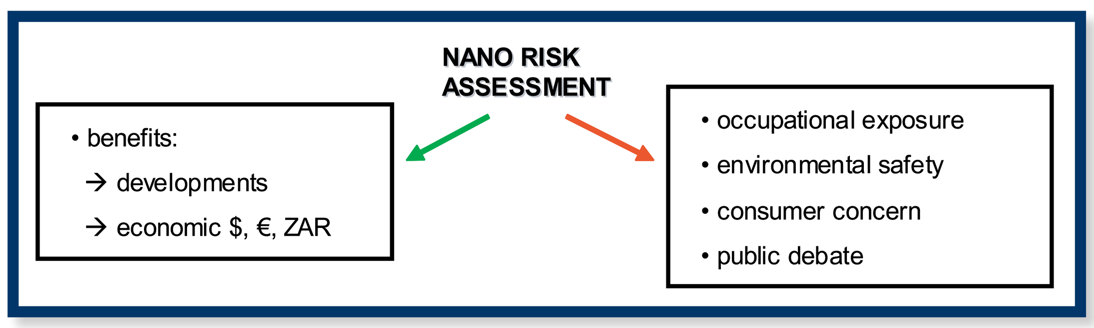

Figure 1: Benefits and current issues to be addressed for nanotechnology to be widely acceptable; commercially and publicly.

STUDY AIM

To investigate the influence Fe_2O_3, TiO_2, ZnO and Ag engineered nanomaterials on oxidative stress, structural cellular integrity and photosynthesis on the algal *Spirodela* species.

Selected Engineered NanoMaterials

Hansen et al. (2008) indicated that silver (Ag), carbon nanotubes, Zinc (Zn), Silica (Si) and Titanium dioxide (TiO_2) respectively, were five of the most used ENMs in consumer nanoproducts. ENMs used in high volumes are likely to end up in the environment during application and also waste disposal where they will pose some level of risk. Four of these highly-used ENMs are selected for the current study.

Fe_2O_3 (iron oxide)

Nanoscale iron is extensively used in bioremediation (Zhang 2003) and its use in environmental clean-up operations is increasing. Therefore a significant portion of nanoscale iron entering the environment will be introduced intentionally during environmental bioremediation. Considering the potential benefits and increasing usage of nanoscale iron and also that it is one of the highly-used ENMs, raises concerns about the potential toxicity of nanoscale iron.

TiO_2 and ZnO (titanium dioxide and zinc oxide)

Nanoscale TiO_2 and ZnO are currently few of the ENMs that are already widely used in market products, such as beauty care products. Some known toxic effects of TiO_2 and ZnO include oxidative stress induction (Hartmann *et al.* 2009). A high proportion of TiO_2 and ZnO introduced into the environment will be unintentional as waste from domestic (mainly cosmetic) and also industrial applications.

AgNP (silver nanoparticles)

Silver has long been used for its microbiocidal properties to treat microbial infections and burn wounds. Engineered nano-silver is used to sterilise fabrics, medical equipment and facilities (Amro et al., 2009). Nano-silver is known to possess other toxicity tendencies including the generation of reactive oxygen species. Nano-silver will mostly enter natural ecosystems unintentionally as waste; literature has already reported nano-silver release from washing fabrics.

STUDY ENDPOINTS

Oxidative Stress (OS)

Oxidative stress is a condition during which the generation of reactive oxygen species (ROS) within a cell exceeds metabolic detoxification mechanisms. These mechanisms include the activation of antioxidant enzymes such as superoxide dismutase, catalase, ascorbate peroxidase and glutathione peroxidase. High and continuous ROS dosages can eventually lead to cell death. The increased activity of antioxidant enzymes when samples are exposed to toxicants is utilised as a biomarker indicating the induction of oxidative stress.

Antioxidant enzymes activity

- **Superoxide dismutase (SOD):**

$2\ O_2^{\cdot -} + 2\ H^+$ SOD $H_2O_2 + O_2$

- **Catalase (CAT):**

$2\ H_2O_2$ CAT $2\ H_2O + O_2$

- **Ascorbate peroxidase (APX):**

$H_2O_2 + \text{ascorbate} \rightarrow H_2O + \text{monodehydroascorbate}$

- **Glutathione peroxidase (GPX):**

$H_2O_2 + 2GSH \rightarrow 2\ H_2O + GSSG$

Oxidative damage: lipid peroxidation

Malondialdehyde (MDA) and carbonyl groups are end products of lipid peroxidation caused by oxidative stress. In this study the quantity estimation of these end products on exposed samples is used as a biomarker indicating oxidative damage.

Structural cellular integrity

Chloroplasts and mitochondria are cellular energy generation/conversion centres and are therefore prone to ROS and oxidative stress induction. A comparative microscopic analysis of chloroplasts and mitochondria of ENMs exposed samples relative to controls will be undertaken.

Photosynthesis: pigments quantification

Chlorophyll pigments are used to assimilate photo energy and carotenoids to reduce oxidative damage. The change of the chlorophyll:carotenoid ratio from "normal state" indicates some form of stress; in this study the chlorophyll pigment ratio will be quantified as a biomarker to investigate stress due to exposure to ENMs.

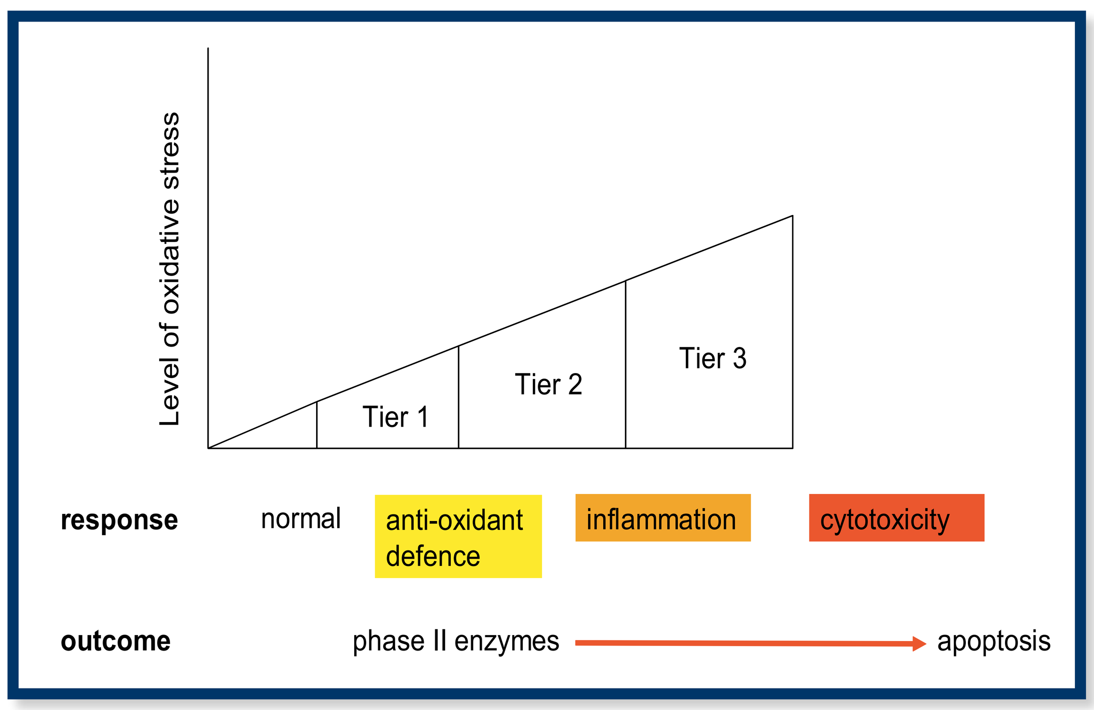

Figure 2: Diagram indicating increasing levels of cellular oxidative stress. The current study's focus begins at Tier 1 whilst Tier 3 investigates structural cellular integrity and photosynthetic effects.

CONCLUSION

This study is according to current literature the first to investigate the toxicity effects of ENMs on higher aquatic plants. Such information is vital for aquatic ecosystem protection information from the relatively less understood ENMs. The various levels of effects assessment planned in this study are applicable to product safety data sheet and ecological risk assessment requirements. The ENMs will be various suspension materials to evaluate the safety of each suspension method; such information is vital for "safer" product development. The type of data to be generated by the current study is aimed to sufficiently feed into the global data requirements and also assist in the general nanotechnology safety and application debate.

ACKNOWLEDGEMENTS

The Department of Science and Technology, South Africa for funding the Nanotechnology Environmental Health and Safety research project.

The CSIR for awarding the Young Researcher Establishment Fund and a bursary for PhD studies.

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Nanomaterials are increasingly being used in consumer products, especially sunscreens and beauty products, with limited research about the environmental effect of these nanomaterials once they are released into the environment.