2nd Marine NanoEcoSafety Workshop

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PROGRAMME

PROGRAMME

Monday, 17 November

14:00 **Registration**

14:30 **Welcome of the Organisers**

Ilaria Corsi & Valeria Matranga

EU Nanosafety Cluster - Marine Ecotox Focus Group Chairs

14:40-15:00 **Opening Remarks**

Authorities' Speeches with the participation of:
Rappresentative of the Dept of Earth System Science and Environmental Technologies of CNR
Rappresentative of the Assessorato al Verde Pubblico ed Ambiente (Comune di Palermo)
Giovanni Viegi, Director of the Institute of Biomedicine and Molecular Immunology "A. Monroy"

15:00-15:20 Niall McDonough (Oostende, Belgium)

Unravelling the complex links between the oceans and human well-being: developing an interdisciplinary research capacity in Europe

Session 1

Nanomaterials interaction at marine ecosystem level

Chairs: Gary N. Cherr & Cristina Nasci

15:20-16:00 **Keynote Lecture**

Gary N. Cherr (Davis, CA, USA)

Metal oxide nanomaterials induce oxidative stress and act as chemosensitizers in sea urchin embryos

16:00-16:15 Cristina Nasci (Venice, Italy)

NM in marine environment: a stakeholder's point of view

16:15-16:30 Miren P. Cajaraville (Leioa, Spain)

Classification of the toxic potential of metal-bearing nanoparticles and the corresponding bulk and ionic forms based on *in vitro* tests with mussel cells: a contribution to the risk assessment of nanomaterials in the marine environment

16:30-16:45 Antonio Marcomini (Venice, Italy)

Species Sensitivity Weighted Distribution (SSWD) as screening tool for ecological risk assessment of engineered nanomaterials: the n-TiO_2 case study

Session 2

Nanomaterials fate and behaviour in salt water

Chairs: Jerome Labille & Antonio Marcomini

16:45-17:25 **Keynote Lecture**

Jerome Labille (Marseille, France)

Assessing the behavior and fate of manufactured nanoparticles in seawater. Case study for nano-TiO_2 used in sunscreen

17:25-17:40 Diana Antonio (Ispra, Italy)

Silver nanoparticles behavior in artificial sea water by mean of AF4

17:40-18.00 **Poster session**

18:00 ***Wine & Cheese***

Tuesday, 18 November

Session 3

Nanomaterials mechanisms of toxicity

Chairs: Julian Blasco & Francesco Regoli

9:00-9:45 **Keynote Lecture**

Richard Handy (Plymouth, UK)

Marine Nano Ecotoxicology: a review of progress on fish, crustaceans, bivalves and other species

9:45-10:00 Ambrogina Albergamo (Messina, Italy)

Investigation of protein expression signatures associated with long-term exposure to nanosized CuO and Cu^{2+} ions in the clam *Ruditapes decussatus*

10:00-10:15 Ilaria Marisa (Padua, Italy)

Effects of zinc oxide and titanium dioxide nanoparticles on haemocyte parameters of the marine bivalve *Ruditapes philippinarum*

10:15-10:30 Tiziana Cappello (Messina, Italy)

Effects of CuO NPs on first developmental stages of the sea urchin *Arbacia lixula*

10:30-10:45 Isabella Buttino (Livorno, Italy)

Toxicity of nickel on the marine calanoid copepod *Acartia tonsa*: nickel chloride versus nanoparticles

10:45-11:00 Thiago Rocha (Faro, Portugal)

Tissue-specific accumulation and metallothionein induction in mussels *Mytilus galloprovincialis* exposed to quantum dots and soluble cadmium

11:00-11:30 ***Coffee break***

Session 4

Model/Target marine organisms from invertebrates to fish

Chairs: Laura Canesi & Richard Handy

11:30-12:15 **Keynote Lecture**

Laura Canesi (Genoa, Italy)

Interactive effects of nanoparticles with other contaminants in aquatic organisms: friend or foe?

12:15-12:30 Catherine Mouneyrac (Angers, France)

The use of two marine invertebrate species; the bivalve mollusk *Scrobicularia plana* and the ragworm *Nereis diversicolor*, to assess ecotoxicity of metal-based engineered nanoparticles

12:30-12:45 Annalisa Pinsino (Palermo, Italy)

Effects of titanium dioxide nanoparticles on sea urchin immune defence

13:00-14:00 ***Lunch***

14:00-14:15 Julian Blasco (Puerto Real, Spain)

Uptake, elimination and oxidative stress response to 0.75μg L^{-1} citrate gold nanoparticle exposure in the marine clam *R. philippinarum*

14:15-14:30 Marianna Santonastaso (Caserta, Italy)

Marine environmental contamination by titanium dioxide nanoparticles (n-TiO_2): a genotoxicological study in two edible species

14:30-14:45 Maria Luisa Vannuccini (Siena, Italy)

Combination effects of nano-TiO_2 and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on biotransformation gene expression in the liver of European sea bass (*Dicentrarchus labrax*)

14:45-15:00 Filomena Mottola (Caserta, Italy)

Co-exposure to titanium dioxide nanoparticles and cadmium: Genomic, DNA and chromosomal damage evaluation in the marine mussel (*Mytilus galloprovincialis*) and in the fish European sea bass (*Dicentrachus labrax*)

Session 5

Nano frontiers in the marine environment: from nanoplastics in marine litter to nanoremediation

Chairs: Heather Leslie & Giovanni Libralato

15:00-15:45 **Keynote Lecture**

Heather Leslie (Amsterdam, Netherlands)

The challenges of understanding nano-sized plastic particles in the sea

15:45-16:00 Elisa Bergami (Siena, Italy)

Nanoplastics impact on marine organisms: accumulation and toxicity of polystyrene nanoparticles in three model species

16:00-16:15 Camilla Della Torre (Siena, Italy)

Effects of amino polystyrene nanoparticles on sea urchin embryo development and stress response

16:15-16:30 Caterina Ciacci (Urbino, Italy)

Preliminary results on the effects of cationic polystyrene nanoparticles in the mussel *Mytilus galloprovincialis*

16:30-16:45 Giovanni Libralato (Venice, Italy)

Embryotoxicological effects of nFe, nCo and nNi on *Mytilus galloprovincialis* Lamark: preliminary results

16:45-17:00 Elena Bocci (Siena, Italy)

Titania mesoporous cleaning up in the marine environment: ecotoxicological study for the definition of eco-friendliness

17:00	**Concluding remarks** *Ilaria Corsi & Valeria Matranga*
18:00-18:30	**Cocktail to welcome the 6th BSIJ National Experts**
20:30	***Social dinner at "Casena dei Colli"***

ABSTRACTS

Oral communications
in chronological order of presentation

Unravelling the complex links between the oceans and human well-being: developing an interdisciplinary research capacity in Europe

N. McDonough
European Marine Board, Oostende, Belgium

The European Marine Board facilitates enhanced cooperation between European organizations involved in marine science towards development of a common vision on the research priorities and strategies for marine science in Europe. In 2013 the EMB published a position paper entitled, *Linking Oceans and Human Health: A Strategic Research Priority for Europe*. This paper identified an urgent need for a more holistic and coherent approach to understanding the complex links between the seas and oceans on one hand, and human health and well-being on the other.
Extreme weather events such as coastal storms and flooding, and human exposure to marine-borne pathogens and to chemical and material pollution, pose significant threats to human health. At the same time, the seas provide numerous benefits to human well-being in the form of ecosystem services. Biotechnology is opening opportunities to exploit marine genetic resources with potential for new drugs and nutraceuticals. Research is also beginning to identify health-promoting effects of interacting with the coastal environment. Understanding this complexity can only be achieved with an interdisciplinary approach, drawing from expertise across a diverse range of disciplines within natural, social and economic sciences, including public health and medicine. This presentation provides a high-level summary of the key recommendation of the EMB paper, focusing in particular on material pollution impacts. It will include an update on recent activities towards developing an Oceans and Human Health research capacity in Europe.

Metal oxide nanomaterials induce oxidative stress and act as chemosensitizers in sea urchin embryos

G.N. Cherr[1,2], B. Wu[1,3], C. Torres-Duarte[1], E. Fairbairn[1], B. Cole[4]
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[3]School of the Environment, Nanjing Univ., Nanjing, Jiangsu, P.R. China
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Copper and zinc nanomaterials and ions were studied with respect to toxicity (development and reactive oxygen species, ROS) in the White sea urchin (*Lytechinus pictus*). With respect to developmental toxicity (based on Cu concentrations), $CuSO_4$ was the most toxic (EC50 33ppb), with synthesized, purified nano-CuO (~1-2% soluble) being next with an EC50 of 463ppb, and commercial nano-CuO (~0.1-0.2% soluble) being least toxic, with an EC50 of 5565ppb. Toxic effects to embryos in seawater were directly related to copper solubility. Embryos exposed to both types of nano-CuO showed increased protein oxidation and oxidative stress (reduced total antioxidant capacity) as compared to control seawater. Finally, nano-CuO was found to increase intracellular copper in embryos and to decrease their total antioxidant capacity. Nano-ZnO was shown to be toxic to sea urchin embryos down to 50ppb (based on total Zn) and this level was not significantly different than the toxicity of $ZnSO_4$[1]. Nano-ZnO induced apoptosis (100ppb Zn) in both White and Purple sea urchin embryos, specifically at the blastula stage, and this may explain developmental delays and abnormalities with early developmental exposures.
We have also investigated if metal oxide nanomaterials impact multidrug resistance transporters (ABC efflux transporters), since the Multidrug Resistance-Associated Protein (MRP) is known to efflux metal-glutathione conjugates in sea urchin embryos. Nano-CuO (as well as nano-ZnO for comparison) increased calcein-AM (CAM; a fluorescent efflux transporter activity marker) accumulation in sea urchin embryos, indicating ABC efflux transporters were inhibited by metal oxide nanomaterials (at non-toxic concentrations). CAM is normally effluxed from cells by MDR transporters but the dye accumulates when efflux transporters are inhibited. Nano-CuO (insoluble) and nano-ZnO (soluble) showed differential efflux transporter inhibition at different developmental stages of sea urchin embryos based on their solubility differences. After the first 30 min post-fertilization, crosslinking of the fertilization envelope reduced the inhibition of nano-CuO of ABC transporters. At >30 min. post-fertilization, MDR inhibition was greater with nano-ZnO than nano-CuO since the former undergoes rapid dissolution in seawater while the latter is mostly insoluble. This directly indicates that the crosslinked fertilization envelope (>30 min post-insemination) protects embryos from exposure to nanomaterials, but not soluble metals ions. The MRP inhibitor MK571 caused an increase in intracellular copper in embryos exposed to nano-CuO and $CuSO_4$. Furthermore, nano-CuO and nano-ZnO, at non-toxic concentrations (500ppb), significantly increased another chemical's toxicity, vinblastine, a cell division inhibitor that is an MDR substrate, indicating that nano-metal oxides and metal ions can act as chemosensitizers in sea urchin embryos. This suggests that metal oxide nanomaterials can make organisms more susceptible to other contaminants that are substrates of efflux pumps and would normally be removed from the cell. This is a potential new source of indirect metal oxide nanomaterial toxicity.

[1]Fairbairn, E.A., Keller, A.A., Mädler, L., Zhou, D., Pokhrel, S., and G.N. Cherr. 2011. Metal oxide nanomaterials in seawater: Linking physical characteristics with biological response in sea urchin development. *Journal of Hazardous Materials*, 192:1565-1571.

NM in marine environment: a stakeholder's point of view

C. Nasci
Thetis spa, Venice, Italy

Improving the scientific knowledge on the fate, behavior and effects of NanoMaterial (NM) in marine environment still remain a priority for an eco-safe use of NM and the base for the development of European legislation (still lacking) to regulate and promote a wide use of so promising technologies (for example in the remediation of contaminated sites).
Introducing this Session, as representative of a stakeholder sector, working in Thetis, an environmental engineering company, involved in sustainable management of coastal urban areas, I would like to confirm our interest for this field of research and in particular from our experience, to highlight the need of developing the framework for a permanent connection between science and stakeholder as fundamental and effective way to provide robust innovative solutions and strategies for environmental issues. On the other hand the development of integrated methodologies to assess the environmental risk of NM is important also in relation to the emerging issues of nano-litter in aquatic environment and marine and coastal areas considering the need of Action Plans addressed to the definitions of ecosystem safety measures.
These two days of discussion offer the occasion to improve and create opportunities for bridging science and stakeholder to contribute to the development of sustainable environmental strategies. To promote the dissemination and sharing of workshop outcomes a white paper or declaration will summarize the main results and the step forward.

Classification of the toxic potential of metal-bearing nanoparticles and the corresponding bulk and ionic forms based on *in vitro* tests with mussel cells: a contribution to the risk assessment of nanomaterials in the marine environment

A. Katsumiti, M.P. Cajaraville
CBET Research Group, Dept. Zoology and Animal Cell Biology, Faculty of Science and Technology and Research Centre for Experimental Marine Biology and Biotechnology PIE, Univ. of the Basque Country UPV/EHU, Basque Country, Spain

Given the large number and diverse types of nanoparticles (NPs) present in the market and the huge variety of exposure media, the use of *in vitro* techniques as a tool for environmental risk assessment has been recommended. In the present work, hemocytes and gill cells of mussels *Mytilus galloprovincialis* were used in order to assess the cytotoxicity and mechanisms of action of different metal-bearing NPs: Au, ZnO, SiO_2, TiO_2, CdS, Ag and CuO. Heat maps representing the dose/effect response of NPs and the corresponding ionic and bulk forms on mussel hemocytes and gill cells were constructed using an arbitrary scale common for all tested NPs. Ag, CuO, CdS and TiO_2 NPs and the corresponding ionic and bulk forms were the most toxic materials tested, cytotoxicity ranging from moderate to very high. ZnO NPs and the corresponding ionic and bulk forms were moderately toxic, and Au and SiO_2 NPs and the corresponding ionic and bulk forms were in general not cytotoxic to mussel cells. Even at sublethal concentrations, CdS, Ag and CuO NPs were toxic to mussel cells, varying between moderately toxic to very toxic based on parameters such as ROS generation. Overall, physico-chemical properties and behavior in exposure media of metal-bearing NPs strongly influence their cytotoxicity to mussel cells. Data generated may contribute to define "safe" levels of NPs in the marine environment.

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Species Sensitivity Weighted Distribution (SSWD) as screening tool for ecological risk assessment of engineered nanomaterials: The n-TiO_2 case stud

E. Semenzin, E. Lanzellotto, D. Hristozov, A. Critto, A. Marcomini
Dept Environmental Sciences, Informatics and Statistics, Univ. Ca' Foscari Venice, Venice, Italy

The growing use of nanoscale titanium dioxide (n-TiO_2) in consumer products has raised societal concerns regarding its potential environmental and health risks, which call for robust risk analysis. However, the quantitative Ecological Risk Assessment (ERA) of n-TiO_2 has been constrained by significant uncertainties and methodological limitations. In order to address some of these challenges we propose a methodology that further develops the Species Sensitivity Weighted Distribution (SSWD) approach by including three weighting criteria (i.e. species relevance, trophic level aboundance and data quality) to address nano-specific needs. This nano-SSWD model was tested with ecotoxicological data for n-TiO_2 including 189 ecotoxicological endpoints (e.g. LCx) for species representative of the freshwater, seawater and soil compartments. Environmental quality criteria and ecological risk were estimated. These results were then compared to similar results from applying the conventional SSD approach to the same dataset. Unfortunately, there were insufficient data in the literature to apply the tool for ERA in the marine environment. There is a need to exploit "grey" datasets and/or generate more hazard data to allow the qualitative estimation of the risks n-TiO_2 poses to marine organisms.

Assessing the behavior and fate of manufactured nanoparticles in seawater. Case study for nano-TiO_2 used in sunscreen

J. Labille
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International Consortium for the Environmental Implications of Nanotechnology, iCEINT, Aix en Provence, France

Among the risk posed by engineered nanomaterials (ENMs), predicting the exposure aspect implies better understanding their fate and behavior in marine ecosystem. Their dispersion stability is a key factor that determines their residence time in the water column, and thus their concentration profiles in the benthic versus pelagic systems. It is influenced by a variety of parameters, including the intrinsic characteristics of the ENMs, the solution chemistry, and the interaction with surrounding components. We studied the respective roles of these parameters for the case of nano-TiO_2 used as UV-filter in sunscreens. The intrinsic properties of the nanoparticles were varied according to the different forms encountered through the value chain and life cycle of the nano-products. Bare nano-TiO_2, functionalized UV-filters, sunscreens, and alteration byproducts were characterized and compared.
The composition of the aquatic system studied was complexified progressively so as to fulfill that of the environment. The effects of salt, pH, natural organic matter, and naturally occurring suspended colloids were assessed.
Finally, a relevant environmental concentration of ENMs, falling in the µg/L range was used. This is likely to favor the heteroaggregation of ENMs with naturally occurring colloids rather than ENM homoaggregation, and to modify the fate scenarios usually proposed based on higher working ENM concentration.

Current funding program: ERA-NET SIINN Call 2012 NANOHETER

Silver nanoparticles behavior in artificial sea water by mean of AF4

D.C. António, C. Cascio, L. Calzolai, F. Rossi
European Commission – DG Joint Research Centre, Inst. for Health and Consumer Protection, Ispra (VA), Italy

The availability of nanomaterials-containing wastes, coming from consumer products (i.e. food, cosmetics, paints, drugs), represents a route of entrance in the aquatic ecosystems. Therefore, the detection and fate of nanomaterials in the environment has become a topic of increasing interest. Considering the interaction of nanoparticles with charged material and the predominance of organic matter in marine systems, we evaluated the effect of such molecules on the nanoparticles stability. Temperature, presence of organic matter (represented in this study by alginate), or oxygenation are key factors for nanoparticles fate prediction on natural systems.
Our experimental approach consists in the combination of size-separation techniques (Asymmetric Flow Field Flow Fractionation - AF4) with size measurement techniques (Dynamic Light Scattering - DLS) and chemical identification (Inductively Coupled Plasma – Mass Spectrometry - ICP-MS) to detect the adsorption or organic matter on NP and the evolution of the formed complexes with time. Silver (60 nm) nanoparticles where incubated for 2 days, at room temperature and 14°C, in presence and absence of alginate. Low temperature and presence of alginate revealed to be extremely important on the maintenance of the nanoparticles in solution.

Marine Nano Ecotoxicology: a review of progress on fish, crustaceans, bivalves and other species

R.D. Handy
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Early work on nano-ecotoxicology focussed on the freshwater organisms used for regulatory testing. Less is known about the ecotoxicity of engineered nanomaterials (ENMs) to marine species, and the consequences for ecosystem functions. This presentation summarises the state of knowledge across different species. For microbes, some lethal concentrations are known, but effects on microbial functions in marine sediment and inside host biota (e.g., the gut of fishes) is poorly understood. The data set on environmentally-relevant microbes is limited compared to model organisms like *E. coli* or *Streptococcus spp.* For freshwater fishes at least, there is some understanding emerging on target organs and the ADME processes for ENMs. However, there are concerns that data from standardised regulatory tests with freshwater species may not be protective of marine organisms for ENMs. The brine shrimp, *Artemia salina*, shows different acute toxicity to ENMs than the equivalent test with freshwater, *Daphnia magna*. Data is emerging on the uptake and accumulation of ENMs in some fishes, bivalves and decapod crustaceans. The shore crab, *Carcinus maenas*, shows a similar low toxicity, and target organs for TiO_2, as freshwater trout. However, crabs also show toxicity to Cu NPs that is only partly explained by the free ion activity model, or the effects of salinity on metal toxicity. The accumulation of ENMs by marine shellfish raises concerns about inflammation and immunotoxicity. However, translating such responses to the field to predict the health of wild populations and the incidence of disease or parasitic infection is unclear at present.

Investigation of protein expression signatures associated with long-term exposure to nanosized CuO and Cu2+ ions in the clam *Ruditapes decussatus*

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Copper (Cu) is an essential micronutrient that beyond certain threshold levels becomes highly toxic to aquatic biota, primarily in the form of Cu2+ ions. Copper oxide (CuO) is the simplest member in the family of Cu compounds and exhibits, at the nanometre scale, unique physicochemical properties, such as size, structure and surface area that account for its wide application in several industrial products and commodities. However, the same characteristics responsible for beneficial uses suggest novel environmental impacts and interactions with the aquatic biota. So far, the toxicity mechanisms of CuO nanoparticles (NPs) remain relatively unknown, especially in aquatic invertebrates. Nevertheless, proteomics could elucidate the mode of action of nano CuO and differentiate from its ionic counterpart. Within this context, our work aims to investigate the protein expression signatures (PESs) of clam *Ruditapes decussatus* exposed to an environmentally relevant dose of CuO NPs and Cu2+ during 15 days. PESs were obtained through two-dimensional electrophoresis performed on protein extracts of gills and analyzed with PDQuest software (Bio-Rad, Hercules, CA). Software mediated-analysis included spot detection and quantitation, gel comparison and statistical analysis. Preliminary results show that CuO NPs and Cu2+ exposures induced relevant changes of PESs in the gills of *R. decussatus*. Qualitative and quantitative analysis suggested that proteome alterations are probably related to the intrinsic CuO NP properties in comparison to its ionic counterpart and, therefore, to the different modes of action by which both copper forms inflict toxicity to clams.

Effects of zinc oxide and titanium dioxide nanoparticles on haemocyte parameters of the marine bivalve *Ruditapes philippinarum*

I. Marisa, V. Matozzo, M.G. Marin
Dept of Biology, Univ. of Padova, Padova, Italy

The increased production and use of nanoparticles (NPs) in many consumer products raise concerns for environmental release and potential impacts in marine coastal ecosystems. As the immune system is considered a sensitive target for the effects of NPs in bivalves, cellular and biochemical responses were evaluated in haemocytes of the clam *Ruditapes philippinarum* during a 7-days *in vivo* exposure to sublethal concentrations of i) nZnO, $ZnCl_2$ and ii) $nTiO_2$ and bulk titanium dioxide. nZnO and $nTiO_2$ were chosen because widespread, and commonly present in sunscreens. $ZnCl_2$ was used to investigate possible contributions of Zn^{2+} release to nZnO toxicity and bulk TiO_2 was used to understand the potential differing action of metal oxide compared with the respective NP. Results showed that $nTiO_2$ more than nZnO can promote immune modulation in haemocyte parameters of clams. Indeed, in nZnO-exposed clams only a significantly increased haemocyte proliferation and a low level of DNA damage were observed, whereas under $nTiO_2$-exposure significant increases were found in total haemocyte count, diameter and volume of haemocytes, haemocyte proliferation and DNA damage. A modulation of both haemocyte pinocytotic activity and lysozyme activity in cell free haemolymph was also observed in $nTiO_2$-exposed clams. NP toxicity was suggested to depend not only on release of zinc ions or on metal oxide bulk effects, but also on the specific characteristics of NPs (e.g., size, surface, shape).

Effects of CuO NPs on first developmental stages of the sea urchin *Arbacia lixula*

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Nanotechnology is an emerging and fast-growing field, and today scientists are focusing on the potential risks to the environment and human health posed by the release of NPs. In this study, the embryotoxicity of CuO nanoparticles (NPs) was evaluated in *Arbacia lixula* embryos, a Mediterranean benthic sea urchin species. After collection of *A. lixula* adult specimens from the Mediterranean littoral coasts of Messina, southern Italy, fertilized eggs were exposed to artificial sea water containing suspensions of CuO NPs ranging from 0.009 to 2.9 μM, until the pluteus larva stage. Copper uptake, as measured by atomic absorption spectrometry, was up to 2.5 and 2.0 fold respect to control in larvae exposed to 2.9 and 1.9 mM, respectively. Developmental delay and morphological abnormalities due to a delay or block in the skeletal rods formation were observed, and the expression of genes involved in the skeletogenesis process was also investigated. In addition, evidences of serotoninergic and cholinergic systems affection were provided by a reduction in serotonin (5-HT) and inhibition of AChE enzymatic activity, suggesting the potential of CuO NPs to interfere with neurotransmission, larval morphogenesis and swimming behaviour. These results demonstrate that CuO NPs may cause a direct impact on the health of aquatic organisms, and the embryo-toxicity tests are highly sensitive and effective for the evaluation of embryonal abnormalities and monitoring of aquatic ecosystems.

Toxicity of nickel on the marine calanoid copepod *Acartia tonsa*: nickel chloride versus nanoparticles

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Nickel (Ni) is an ubiquitous element naturally present in the water, however, Ni compounds have been introduced in the environment under different chemical forms. Particulate forms can also be generated by nanoparticle industries. In marine environment Ni toxicity, as dissolved salt metal, has been well investigated on planktonic organisms. More recently, attention has been focused on nanoparticle elements. Here we report the effect of two different chemical form of Ni: $NiCl_2$ and nanoparticles (NiNPs) on the reproductive physiology of the marine calanoid copepod *Acartia tonsa*. Acute test (48h) and 7-day semichronic tests were conducted exposing *A. tonsa* embryos to different concentrations of $NiCl_2$ and NiNPs solution. Egg hatching and naupliar viability were considered as end-point and the effective concentration (EC_{50}) was calculated. Moreover, two different incubation protocols have been compared to test if a previously sonicated nanoparticle solution induced different toxicity in *A. tonsa* embryos, with respect to a non-sonicated solution. Furthermore, we evaluated Ni toxicity on the fecundity of *A. tonsa*, during 4-days exposure of adult copepods in $NiCl_2$ and in NiNPs. At the best of our knowledge, this is the first study on the toxicity of Ni on the reproductive physiology of the copepod *A.tonsa* which compares two different chemical forms and methodological approaches to test nanoparticle toxicity in seawater.

Tissue-specific accumulation and metallothionein induction in mussels *Mytilus galloprovincialis* exposed to quantum dots and soluble cadmium

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Metallothioneins (MTs) are metal inducible proteins used as biomarkers of metal exposure; however its role in the homeostasis and detoxification of metal engineered nanoparticles (ENPs) in invertebrates is less known. Despite their technological application, the increased use of Cd based-Quantum Dots (QDs) leads to their release into aquatic environment but their mode of action in marine bivalves is unclear. So, the aim of this study was to investigate the tissue-specific accumulation and MT response of mussels *Mytilus galloprovincialis* exposed to CdTe QDs and its soluble counterpart. Mussels were exposed to 10 μgCd.L^{-1} of CdTe QDs and cadmium nitrate for 21 days (accumulation period) and transferred to clean seawater for 50 days (depuration period). QDs behavior and Cd speciation in seawater was analyzed using Stripping Chronopotentiometry (SCP) and Absence of Gradients and Nernstian Equilibrium Stripping (AGNES). Cd accumulation and MT levels were assessed in mussel´s gill and digestive gland by atomic absorption spectrophotometry and differential pulse polarography, respectively. The results showed that both CdTe QDs and soluble Cd accumulated with time in mussels' tissues in a Cd form and tissue dependent manner. MT levels in exposed mussels were induced during the 21 days of accumulation, also dependent on the Cd form and tissue. During the depuration period, mussels showed a higher Cd retention rate related with MT levels. This data suggest that MTs participate in the homeostasis and metabolism of Cd-based QDs in *M. galloprovincialis* and are potential biomarker of aquatic pollution by Cd-based ENPs.

Interactive effects of nanoparticles with other contaminants in aquatic organisms: friend or foe?

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The increasing production and use of manufactured nanoparticles-NPs in industrial applications and consumer products will lead to their release into the aquatic environment, posing a potential threat to the health of aquatic organisms. Both in the water phase and in the sediments NPs could mix and interact with other pollutants, such as organic xenobiotics and heavy metals; these interactions may lead to changes in bioavailability/bioconcentration/ toxicity of these contaminants. However, few studies have been carried out so far in the attempt to evaluate whether these interactive effects may lead to increased harmful effects in aquatic biota, with available data mainly on C60 fullerene and n-TiO_2, as examples of widespread carbon-based and metal-oxide NPs. Here data are summarized on the interactive effects of n-TiO_2 and Cd^{2+} and 2,3,7,8-TCDD, chosen as models of common and persistent inorganic and organic contaminants, respectively, in the model marine invertebrate, the bivalve *Mytilus*. The results reveal complex and often unexpected interactive responses of NPs with other pollutants from the molecular to the organism level, depending on type of contaminant and the endpoint measured, as well as differences in bioaccumulation. Overall, the available data obtained so far indicate that interactive effects of NPs with other contaminants do not necessarily lead to increased toxicity or harmful effects in aquatic organisms.

The use of two marine invertebrate species; the bivalve mollusk *Scrobicularia plana* and the ragworm *Nereis diversicolor,* to assess ecotoxicity of metal-based engineered nanoparticles

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Among engineered nanoparticles (ENPs), metal-based ENPs, such as silver, gold, copper oxide (CuO), zinc oxide (ZnO) and cadmium sulphide (CdS), are used in a wide range of products. In seawater, ENPs become rapidly agglomerated favoring their deposition onto the sediment surface; thus, benthic organisms may be particularly at risk of exposure. Two marine endobenthic species, the bivalve mollusc *Scrobicularia plana* and the ragworm *Hediste diversicolor* were selected to assess toxic effects of Ag NPs, Au NPs, CuO NPs, CdS NPs, ZnO NPs. The experiments were carried out under laboratory conditions (microcosms) or under environmentally realistic conditions in outdoor mesocosms. The following research questions were addressed: (1) How did the environment into which nanoparticles were released affect their physicochemical properties? (2) How did the route of exposure (seawater, food, sediment) influence bioaccumulation and effects ? (3) Which biomarkers were the most responsive? and (4) Which tools were the most efficient to evaluate the fate and effects of NPs in the marine environment? The results showed that metal-based NPs in general were highly agglomerated/aggregated in seawater. DGT (diffusive gradient in thin films) tools could be used to estimate the bioavailability of metals released from NPs in solution in the aquatic environment. Both metal forms (nanoparticulate, soluble) were generally bioaccumulated in both species. Among biochemical tools, glutathione S-transférase and catalase were the most sensitive revealing the enhancement of anti-oxidant defenses in both species exposed to sub-lethal concentrations of NPs. Apoptosis and genotoxicity were also frequently observed.

Effects of titanium dioxide nanoparticles on sea urchin immune defence

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The presence of Engineered Nanoparticles and their aggregates in many consumer products has attracted a growing scientific concern on the possible effects of these materials on the environment and biota. Here, we took advantage of an amenable laboratory model organism, the sea urchin *Paracentrotus lividus* (phylum Echinodermata), to elucidate a potential pathway that can be involved in the persistent Titanium dioxide (TiO_2) engineered nanoparticles-immune cell interaction. Sea urchins are phylogenetically related to humans (at least 70% of their proteins are shared with humankind), and have been proven to possess a complex and effective immune defence. In this study, TiO_2 nanoparticles were injected into the sea urchins body cavity to expose immune cells, that were harvested after 24 hours, and analyzed for their morphology, phagocytic ability, activation of proteins triggering immune defence (e.g. HSPs, TLRs, p38 MAPK) and expression of related immune response genes. We found that TiO_2 nanoparticles elicit a receptor-mediated endocytotic mechanism carried out by phagocytes, and affect the p38 MAPK signaling pathway. Our working hypothesis is based on the role of the TiO_2 nanoparticles-mediated p38 MAPK inactivation in the renewal and homeostasis of sea urchin immune "stem" cells. In conclusion, we strongly recommend sea urchin immune cells as a new powerful tool for nano-safety investigations.

Uptake, elimination and oxidative stress response to 0.75µg L^{-1} citrate gold nanoparticle exposure in the marine clam *R. philippinarum*

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Gold nanoparticles (AuNPs) have unique physical and chemical properties and are therefore used and developed for a wide range of novel applications in medicine, biology and chemistry. However in recent years concern has been raised over their ability to enter cells, organelles and nuclei, and provoke oxidative stress. In a laboratory-based experiment the non-target marine bivalve *Ruditapes philippinarum* was used as a model organism. Uptake, elimination and molecular effects of ~20 nm citrate AuNPs at 0.75 µg L^{-1} were studied over 7 days exposure and equal length elimination period. Our results demonstrate that the AuNPs were readily taken up into the digestive gland > gills. We further observed limited oxidative stress and inflammatory responses. The activity of several antioxidant enzymes was increased in an organ specific manner, with the digestive gland exhibiting the majority of its enzyme activity within 24 hours of exposure. Gene expression analysis highlighted the involvement of inflammatory response genes in digestive gland tissue, as measured by changes in TNF-α and Gadd45 expression. Suggesting that one mechanism of citrate AuNP toxicity is well described by the oxidative stress paradigm. Further a significant elimination of Au from the digestive tract within the purification period was observed, with excretion being an important pathway. In conclusion, while not producing significant oxidative damage, concern is raised regarding the effects of chronic AuNP exposure.

Marine environmental contamination by titanium dioxide nanoparticles (n-TiO_2): a genotoxicological study in two edible species

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The waste products from industrial process of n-TiO_2 discharged into waters, may cause harmful side effects to marine organisms. Our recent *in vitro* and *in vivo* studies have demonstrated the n-TiO_2 genotoxicity in marine edible species. The aim of the present study was to evaluate the genotoxic potential of the waste waters from a large-scale factory producing titanium dioxide, located along the coasts of Tuscany (Scarlino – GR). Specimens of *Mytilus galloprovincialis* were exposed in tanks for 4 and 14 days to channel waters collected *in situ* and to control waters taken from a reference site located far from the factory. *Dicentrarchus labrax* specimens were exposed for 7 days to sediments collected nearby the factory and to control sediments. Genotoxicity was evaluated by RAPD-PCR technique, Comet assay and Micronucleus test. RAPD-PCR detected a high reduction of Genomic Template Stability (GTS) in specimens exposed to industrial waste water (55% in mussel and 77% in sea bass). On the contrary, Comet assay and Micronucleus test did not detect any statistically significant increase of DNA strand breaks and micronucleated cells in the same specimens. The present study provide evidence of a genotoxic effects in two marine edible species exposed to waste waters from a n-TiO_2 factory. The results highlight the importance to investigate the mechanisms of n-TiO_2 toxicity and extend biomonitoring in order to protect ecosystems and human health.

Combination effects of nano-TiO_2 and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on biotransformation gene expression in the liver of European sea bass (*Dicentrarchus labrax*)

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The aim of present study was to investigate the influence of titanium dioxide nanoparticles (nano-TiO_2, Aeroxide©) on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) dependent biotransformation gene expression in juvenile European sea bass *Dicentrarchus labrax*. An *in vivo* 7 days of aqueous exposure was performed with nano-TiO_2 (1 mg/L), TCDD (46 pg/L), singly and in combination. The mRNA expression of aryl hydrocarbon receptor repressor (*Ahrr*), estrogen receptor ($Er_\beta 2$), ABC transport proteins as *Abcb1, Abcc1-c2-g2*, cytochrome P450 (*Cyp1a*), glutathione-s-tranferase (*Gst*), glutathione reductase (*Gr*) and engulfment and motility (ELMO) domain-containing protein 2 (*Elmod2*) were investigated in liver tissue. Ahrr, erβ2, abcc1 and abcg2were down-regulated with respect to controls in all experimental groups. Co-exposure to nano-TiO_2 and TCDD caused a further significant down regulation of *ahrr,*er_β*2,abcb1* and *abcc2* compared to single chemical exposure (nano-TiO_2 or TCDD alone). No effects were observed for TCDD and nano-TiO_2 alone in *abcb1* gene, while *abcc2* was down-regulated by nano-TiO_2 alone. *Cyp1a, gst* and *elmod2* genes were up-regulated by TCDD and to a similar extent after co-exposure to nano-TiO and TCDD. HR-TEM showed nano-TiO_2 (~27nm) uptake in liver which not affected by co-exposure by TCDD. Overall the results indicate that nano-TiO_2 is unlikely to interfere with TCDD-dependent biotransformation gene expression in the liver of European sea bass, although additive effects of co-exposure observed in ABC transport mRNAs might suggest effects on xenobiotic metabolite transport in liver.

Co-exposure to titanium dioxide nanoparticles and cadmium: Genomic, DNA and chromosomal damage evaluation in the marine mussel (*Mytilus galloprovincialis*) and in the fish European sea bass (*Dicentrachus labrax*)

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Due to the large production and growing use of titanium dioxide nanoparticles (n-TiO_2), their release in the marine environment and their potential interaction with conventional toxic contaminants represent a growing concern for biota. Different end-points of genotoxicity were investigated in the marine mussel and in the European sea bass exposed to n-TiO_2 either alone and combined with $CdCl_2$ for 4 and 7 days respectively. Genomic stability (Random Amplified Polymorphism DNA-PCR, RAPD assay), DNA primary damage (Comet assay), apoptotic cells (Diffusion assay) and micronuclei occurrence (Micronucleus test) were assessed. Results showed increased DNA strand breaks after $CdCl_2$ exposure in the two species accompanied by a decrease in genome template stability. Otherwise, increased micronucleated cell frequency associated with decreased genome template stability was again observed in specimens exposed to n-TiO_2 alone from the two species. These results suggest that n-TiO_2 and Cd^{++} have specific end point of genotoxicity and that n-TiO_2 might affect the mitotic machinery more than possessing a clastogenic potential in the present experimental conditions; however, further investigations are needed to verify this hypothesis. An antagonistic role of n-TiO_2 in abating Cd^{++} genotoxicity was observed only in mussel. Further research is thus strongly encouraged in order to clarify mechanisms underlying specific mixtures effects involving NPs and toxic contaminants in marine organisms.

The challenges of understanding nano-sized plastic particles in the sea

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We make a lot of stuff, we make a lot of waste, and we make marine litter. It's a symptom of our linear "take-make-waste" economy that a growing number of undaunted people are now trying to steer us away from. In the meantime, we are all still the chefs of the 'plastic soup', as it is often called in the Netherlands. Plastic is a major, highly persistent component of marine litter and it is known to break down in to microscopic sized particles. It's assumed to exist as particles <1 micrometer as well. We know that nano-sized plastic particles are currently being manufactured for various applications. The expectation is that like other contaminants, these too will be emitted to our marine environment. There is a growing body of evidence from laboratory experiments that nano-sized plastic particles have the potential to cause toxicity. We have no knowledge of nano-sized plastic particles in the field due to major analytical constraints at this time - a lot of development work is required. This presentation attempts to summarize what we know about plastic particle toxicity and distribution. It reflects on our challenges ahead and what we would need to know next in order to advance the science and ultimately offer relevant knowledge to policy makers and companies to inform their decision making.

Nanoplastics impact on marine organisms: accumulation and toxicity of polystyrene nanoparticles in three model species

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Nanoplastics debris represent an emerging concern for marine ecosystems. Nevertheless their fate and impact on marine biota is almost unknow. Polystyrene nanoparticles (PS NPs) can be considered as model for studying both fate and toxicity of nanoplastics in marine organisms. For this purpose, the present study evaluated the accumulation and toxicity of 40 nm anionic carboxylated (PS-COOH) and 50 nm cationic amine (PS-NH_2) NPs on three model species: brine shrimp *Artemia salina* larvae, mussel *Mytilus galloprovincialis* tissues and sea urchin *Paracentrotus lividus* early development. PS-COOH resulted massively sequestered inside the digestive trait of sea urchin embryos at 48 hours post-fertilization and brine shrimp after 48 h exposure. PS-COOH were exchanged between gills and circulatory system after 2 h of exposure and accumulate in lysome of mussel's hemocytes. No relevant toxicity was observed for PS-COOH in all three model species. PS-NH_2 resulted less clear disposed both in sea urchin embryos and brine shrimp larvae but caused severe alterations. In mussels, a high degree of vacuolization was found in hemocytes. The observed differences in accumulation and toxicity of PS-COOH and PS-NH_2 might be related to their surface charge and aggregation in sea water. Our findings suggest that these three species are vulnerable to PS NPs and further research is strongly encouraged on specific pathways of toxicity in marine organisms.

Effects of amino polystyrene nanoparticles on sea urchin embryo development and stress response

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Plastic waste has been identified as a widespread emerging concern for the marine ecosystems. Polystyrene nanoparticles in particular are representative nanoplastic type with well characterized toxicity in mammalian *in vitro* models due to their wide application in nanomedicine as drug carriers. The aim of this study is to evaluate the toxicity pathways of amino polystyrene nanoparticles (nPS-NH_2) in *Paracentrotus lividus* embryos. The nPS-NH_2 is well dispersed in NSW (35‰). The Z-potential confirmed the cationic nature of nPS-NH_2. Embriotoxicity was monitored at 6, 24 and 48 h post-fertilization (hpf) at concentrations ranging from 0 to 10 µg/ml. Two concentrations (3 and 4 µg/ml) were selected for investigating potential molecular mechanisms underlying the observed developmental abnormalities. At 48 hpf various striking larval alterations as incomplete/absent skeletal rods, ectoderm alteration, blocked embryo development at early stages were observed. Modulation of genes involved in stress response and embryo development (e.g. *abcb1, abcc5, cas8, 14-3-3e, p38 MAPK)* was measured by Q-PCR and levels of the related proteins (e.g. HSPs, LC3, Mn-SOD, p-*p38 MAPK, p-ERK*) were evaluated by immunoblotting, at gastrula (24 hpf) and pluteus (48 hpf) stages, respectively. Our preliminary findings indicate that embryos are vulnerable to nPS-NH_2 and suggest a subset of stress-sensing pathways that are involved in the regulation of a balance between survival and death.

Preliminary results on the effects of cationic polystyrene nanoparticles in the mussel *Mytilus galloprovincialis*

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Polymeric nanoparticles can reach the marine environment from different sources as weathering of plastic debris and nanowaste. Nevertheless, few data are available on their fate and impact on marine biota. Polystyrene nanoparticles (PS NPs) can be considered as model for studying both fate and toxicity of nanoplastics in marine organisms. Our recent findings on amine PS NPs (PS-NH_2) toxicity in sea urchin embryos underline that marine invertebrates can be biological targets of *nanoplastics*. The present study aims to investigate pathways of toxicity of 50 nm cationic PS-NH_2 in hemocytes of Mediterranean mussel *Mytilus galloprovincialis*. Hemocytes were exposed to different concentrations (1 – 5 – 50 μg/ml). of PS-NH_2 for different times (30 min- 2h),depending on the endpoint measured. Clear signs of cytoxicity were evident only at the highest concentrations (50 μg/ml). On the other hand, dose dependent decrease in phagocytic activity and increase in lysozyme activity were observed. PS-NH_2 NPs also induced an increase in extracellular ROS (reactive oxygen species) and NO (nitric oxide) production with maximal effect, respectively, at 1 and 5 μg/ml. Moreover, PS-NH_2 NPs induced signs of apoptotic process, as evaluated by TUNEL analysis and Annexin V binding. These preliminary results suggest that mussel hemocytes and their immune function are affected by PS-NH_2 NPs even at low concentrations. Therefore, further research is necessary on specific mechanisms of toxicity and cellular uptake in order to assess their impact on marine biota.

Embryotoxicological effects of nFe, nCo and nNi on *Mytilus galloprovincialis* Lamark: preliminary results

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The widespread use of engineered nanomaterials (ENMs) in a broad range of industrial and domestic sectors places them at the forefront of ecotoxicologist agendas. Indeed, year-by-year, ENMs are manufactured in increasing amounts. Nevertheless, few data exist on the ecotoxicological effects of uncoated nanosized iron (nFe) (*i.e.* contaminated water remediation), cobalt (nCo) (*i.e.* rechargeable batteries) and nickel (nNi) (*i.e.* heavy-oil recovery) towards marine species and their life stages. *Mytilus galloprovincialis* Lamarck was selected to assess the potential adverse effects of bulk ($FeCl_3$, $CoCl_2$ and $NiCl_2$) and nanosized forms of Fe, Co and Ni. Embryotoxicity tests were carried on looking at the effects on mussel early larval development stages (pre-D shell stage, malformed D-shell stage and normal D-shell stage larvae). The characterisation of nano-powders and solutions/suspensions is currently on going (TEM, DLS, BET, and ICP-OES/MS). Preliminary results evidenced that the effective median concentrations (EC50s) for ENMs fell within the following exposure ranges: 10^{-3}-10^{-2} g nFe/L, 10^{-5}-10^{-4} g nCo/L, and 10^{-5}-10^{-4} g nNi/L. The EC50s of bulk materials showed to be between 10^{-2}-10^{-3} times lower. Thus, the ionic forms appeared as more toxic in seawater than the relative nano-sized particles.

Titania mesoporous cleaning up in the marine environment: ecotoxicological study for the definition of eco-friendliness

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The use of nanomaterials (NMs) for *in situ* remediation referred as nanoremediation represents a promising and cutting-edge solution, by ensuring a quick and efficient pollutants removal. However, their use should not pose any additional risk for the environment and natural ecosystems. The aim of the present study is to validate and define ecotoxicological tools to plan and realize *ecosafe* remediation techniques in the marine environment based on the use of *eco-friendly* NMs. The study has been organized using ecotoxicological bioassays in selected marine organisms belonging to different trophic levels. Newly synthesized pure *mesoporous titania* (MT) for heavy metals remediation in sea water have been tested on algae (*Rhodomonas baltica*, *Rhinomonas reticolata),* zooplankton (*Artemia franciscana, Amphibalanus amphitrite, Acartia tonsa, Brachionus plicatilis*) and on primary consumer sea urchin (*Paracentrotus lividus*). MT have been tested for seawater remediation and lethal nor sub-lethal effects were observed, nevertheless, co-exposure of MT with Cadmium (Cd) as marine pollutant to be remediated, increased Cd toxicity. Preliminary data underline the need to define the *ecosafety* of NMs for environmental applications before any massive use in the natural environment in order to prevent any adverse environmental impact.

POSTERS

POSTERS

P.1W

Gold nanoparticles toxicity in marine crustaceans

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Gold nanoparticles (AuNPs) are emerging as promising agents for cancer therapy and are studied for numerous applications, such as biosensors, new paints, cosmetics, food industry, drug delivery and environmental remediation. The use of these nanoparticles, as most of nanomaterials, is rapidly increasing, while little is known about their possible ecotoxicological effect. Despite their potential advantages, nanomaterials may cause undesirable hazardous interactions with biological systems and the environment with potential to generate toxicity. In particular it has been reported that AuNPs can readily pass from the water column to the marine food web, therefore their toxicity in the marine ecosystem is becoming increasingly important.
The aim of this study was to investigate the potential toxicity of AuNPs in two marine crustacean larval stages: the larvae of brine shrimp *Artemia salina* and II stage nauplii of the barnacle *Amphibalanus amphitrite*. Three end-points having different sensitivity levels (mortality, immobilization and swimming speed alteration) were evaluated after 24 h and 48 h exposure to AuNPs (Plasma Diagnostics and Technologies srl, Italy) suspensions (from 0 to 3.8 mg/L). Results indicate that these NPs induced a toxic effect only towards *A. amphitrite* nauplii after 48 h of exposure. All end-points were able to underline this effect, that was quantified by means of LC_{50} and EC_{50} values. In conclusion, these findings provide new insights regarding the ecotoxicological effect of AuNPs in the marine environment.

P.2W

Toxicity of copper nanoparticles and copper soluble salt to the bivalve *Ruditapes decussatus* in different exposure matrices: water and sediment risk assessment

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Copper oxide nanoparticles (CuO NPs) have been released in aquatic environment by current manufactured products and have showed a high trend of partition to sediment after aggregation in the water column. Sediment represents a relevant sink for nanoparticles where a wide range of species and feeding sites for many predators, ecotoxicological assessment of an environmentally realistic concentration of CuO NPs in whole aquatic system provides the profile of exposure and effects to biota. In the present study, the filter-feeding clam *Ruditapes decussatus* was exposed, for 15 days, to sediment and seawater at a nominal concentration of CuO nanoparticles (10 μg/L) and the labile copper sulfate ($CuSO_4$) to assess the induced environmental risk of each contaminated matrix, by evaluating responses of metallothionein (MT) protein induction, membrane damage of lipid peroxidation (LPO) and antioxidant enzyme activities of CAT, GPx and SOD enzymes in gills and digestive gland of *R. decussatus.* Bioaccumulation of Cu was also followed for both organs, exposed to either dissolved and nanoparticulate forms. Biochemical biomarkers and Cu levels in tissues indicated a different uptake pattern of metal species in sediment and water systems, with digestive gland more susceptible to stress effects compared to gills.

P.3W

Alterations in proteomic pattern and gene expression in the marine microalga *P. tricornutum* exposed to CdSe/ZnS quantum dots

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Nanotechnology has a great potential to improve life and environmental quality, however the fate of nanomaterials in the ecosystems, their bioavailability and potential toxicity on living organisms are still largely unknown, mainly in the marine environment. Genomics and proteomics are powerful tools for understanding molecular mechanisms triggered by nanoparticle exposure. In this work we investigate the capability of the marine diatom *Phaeodactylum tricornutum* to adapt to the presence of CdSe/ZnS quantum dots (QDs) and we characterize differences both in gene expression and in the proteomic pattern induced by QDs. The results show that *P. tricornutum* cells, acclimated to the presence of 2.5 nM QDs, exhibited a growth rate higher than non-acclimated cells, suggesting the occurrence of a mechanism of adaptation. Transcriptional expression of stress responsive genes, i.e. Heat Shock Protein 101, catalase and glutathione reductase was investigated and the preliminary results showed alterations in their expression in exposed algae. In addition, the proteomes of exposed and unexposed cells were compared using 2-D gel electrophoresis. The processing of gel images detected 363 distinct spots, 127 of which showed significant changes in relative abundance between exposed and unexposed cells ($P<0.05$, based on *t*-test). Additional research is needed to identify the differentially expressed proteins and understand the biological significance of the observed alterations.

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