مجلة العلوم البحثة والتطبيقية
Journal of Pure & Applied Sciences
www.Suj.sebhau.edu.ly ISSN 2521-9200
Received 19/11/2017 Revised 24/01/2018 Published online 30/06/2018

Effect of toxicity zinc chloride, bulk zinc oxide and zinc oxide nanoparticles on survive and life cycle the Amphipod *Corophium volutator* (Pallas)

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Abstract The amphipod, *Corophium volutator* was exposed to zinc chloride, bulk zinc oxide and zinc oxide nanoparticles to assess toxicity on the survive, growth and life cycle. The results showed that waterborne exposure of zinc oxide nanoparticles was significantly more toxic to *C.volutator* than when organisms were exposed via sediment. The survival decreased with increasing zinc chloride concentration after 4 weeks of exposure. However for zinc oxide nanoparticles survival was not affected. Following 14 weeks, organisms were more affected at concentrations of 0.5 and 1 mg/L, the percentage of survival declined to less than 70% for all experimental samples. The average length of *C. volutator*after 4 weeks was lower than the control at the concentrations of 0.2, 0.5, and 1 mg/L for animals exposed to zinc chloride and bulk, but not for zinc oxide nanoparticles. After 9 weeks, zinc chloride and bulk zinc oxide had a more significant effect on*C. volutator* growth compared to zinc oxide nanoparticles. However, at the end of the exposure to bulk zinc oxide organisms were slightly smaller.

Keywords: Amphipod, zinc oxide, *Corophium volutotor*, zinc chloride،Zinc oxidenanoparticles.

اثر سمية كلوريد الزنك و أكسيد الزنك الكتلي و جسيمات أكسيد الزنك النانوية على معدل البقاء ودورة حياة مزدوجة الأرجل *Corophium volutator* (Pallas)

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الملخص عرضت مزدوجة الارجل لتراكيز من كلوريد الزنك و اكسيد الزنك الكتلي و لجسيمات أكسيد الزنك النانوية لتحديد سمتها وتأثيرها على النمو و دورة حياة الكائن. اظهرت النتائج ان تعرض هذه الكائنات لجسيمات أكسيد الزنك النانوية في الماء اكثر سمية عن ما هو في التربة. معدلات البقاء تناقصت مع تزايد تراكيز كلوريد الزنك في حين المعرضة لجسيمات أكسيد الزنك النانونية لم يظهر عليها أي تأثير بعد أربع أسابيع. ولكن بعد 14 اسبوعاً كانت التراكيز 0.5 ، 1 مليجرام/ لتر أكثر تأثيراً فتدنت معدلات البقاء الى أقل من 70% لكل عينات التجربة. متوسط الطول للحيوانات المعرضة لتراكيز 0.2 ، 0.5 ، 1 مليجرام/ لتر من كلوريد الزنك كانت أقل طولاً من مجموعة السيطرة و أكسيد الزنك الحجمي بعد 4 أسابيع في حين لم يظهر أي تأثير على متوسط الطول للحيوانات المعرضة لجسيمات أكسيد الزنك النانونية. أما أثر كلوريد الزنك و اكسيد الزنك الكتلي فقد ظهر معنوياً بعد مضي 9 اسابيع مقارناً بالحيوانات المعرضة لجسيمات اكسيد الزنك النانوية ، أما الحيوانات التي عرضت لأكسيد الزنك الكتلي فقد كانت الاصغر حجماً بعد مضي 14 اسبوعاً من الدراسة.

الكلمات المفتاحية: مزدوجات الارجل ، اكسيد الزنك ، الجسيمات النانوية لأكسيد الزنك ، اكسيد الزنك الكتلي ، كلوريد الزنك.

Introduction

The mud shrimp *Corophium volutator* Amphipoda (Pallas1766)is a cosmopolitan genus that includes more than 50 species which are distributed along the North Atlantic, the American and the European coasts, the Black sea and the Azov Sea [1]. There are three different types of feeding modes shown by the benthic amphipod*C. volutator*.,(i) deposit feeding – via scraping microorganisms and surface detritus., (ii) epipsammic browsing – done by scraping the sediment grains [2-5] . Amphipods are euryhaline organisms, thus they are able to tolerate large variations in salinity of the surrounding environment. Salinity controls the distribution and abundance of the organisms; they can live in water saltier than seawater (around 34‰) as well as in water slightly saltier 5‰ than freshwater [1]and [6]. Mc lusky [7] pointed outthat salinities higher than 7.5‰ are the most suitable to breed *C.volutator*. It is easy to handle and to culture in the laboratory, and because of this the species is now a standard European test organism for acute and chronic toxicity testing [8-9], since it also reproduces heavily and it is a major food item for many estuarine fish, shorebirds as well as macroinvertebrates. Furthermore, it has a high sensitivity to environmental condition as well as a lack of larval stages and wide diversity in coastal waters. These animals can survive for more than three months in laboratory conditions [10-13]. Benthic invertebrates represent an important link in the transfer of contaminants to higher trophic levels [11]. In particular, trace metals may be taken up by marine invertebrates from seawater across the permeable body surface or via food and sediment [14-15] . These metals can bind with enzymes, structural protein, and nucleic acids [16]. Zinc (Zn) is an essential trace element for plants and animals, but it is toxic in high concentrations [17]. Most Zn contaminated areas are associated with human activity and mining wastes. Zinc oxide is being used in care products (e.g. sunscreens), coating, and paints, on account of its UV absorption [18]. Zinc, copper and cobalt, for example, are necessary for normal growth and development of marine organisms. Food chains transfer trace metals, but such transfer is controlled not just by the amount of metals accumulated in the prey but also by the physicochemical form [15]and[19]. Nanoparticles(NPs) are materials defined as ultrafine particles, with one dimension between 1 and 100 nm. They occur naturally or can be manmade [20]. Currently, the extent of research on NPs has increased considerably, and in particular there is a great deal of interest in the way in which the properties of the surface area are changed when particles are generated at the nanoscale. The number of atoms in NPs ranged from 50 to 200,000 atoms; and a greater surface area increases chemical reactivity [21]. Some NPsdissolved easily whereas other NPs do not degrade nor dissolve. Instead, they may accumulate and persist for a long time. However, some nanomaterials (NMs) such as quantum dots and carbon nanotube are able to interact with proteins, nucleic acids and cell membrane and also generate reactive oxygen [20]. There is a large amount of research on the environmental impacts of NPs due to the wide variety of scientific potential applications of this technology. For instance, biomedical, optical, and electronic field use various kinds of NPs. Moreover, NPs can also be favourable in catalytic and remediation applications. Nonetheless, due to their unknown toxicity their release into the environment might become a threat to ecosystems [22]. Zinc oxide nanoparticles (ZnO NPs) are widely used in optoelectronics, cosmetics, catalysis, ceramics, and pigments, as well as a fungicide. Recent studies have shown the toxicity of nano zinc oxide to some living organisms such as bacteria [23], freshwater microalgae [5], ciliated protozoa [24], Daphnia [25], earthworms [22], and several marine organisms [26]. Living organisms require trace amounts of some heavy metals, but excessive levels of essential metals can be highly toxic. Metals are also entering our waterways in the form of NMs. Uptake of these metals in aquatic biota may occur via direct ingestion or across epithelial boundaries such as the gills or the body wall [27]. The aims of the present study were: (i) to study the influence of metals and metal NPs on the amphipod *C. volutator* when exposed for short (acute) and long (chronic) term exposure to estimate the range of concentrations which cause mortality to the amphipod via dosing of the sediment or the water.,(ii) to investigate the fate and effects of NPs exposure to the amphipod *C. volutator* via the water and the sediment, and to compare their effects to bulk (microscale metal particles) and to soluble metal. The acute exposures are investigated over a period of 10 days, while chronic tests cover the life cycle of the animal (about 3 months).

2. Materials and Methods Stocks of *C. volutator* were collected from the mudflat of the Otter estuary, Exmouth, UK during low tide, and transported to the laboratory. Sediment for the toxicity testing was obtained at the same sampling site (upper 5 mm). Sediment was gentle sieved with seawater (salinity 25‰) through a 500 µm mesh to eliminate the larger particles and debris, and then washed again through a 300 µm mesh sieve to ensure a standard particle size for the sediment in all experiments and the lack of neonate *C.volutator*. The amphipods were acclimated in a temperature controlled room at 12 ± 1 °C for about a week before the beginning of the exposures. The photoperiod was maintained at 12 h light: 12h dark . Artificial seawater was prepared by dissolved 7.5 Kg of Tropic Marin Neu (Tropicarium Buchshlag, Driech, and F. R. Germany) in 200 L deionized water (DIW) to give salinity 35‰. Artificial seawater was prepared at least 24h before use and stored in a container, diluted to 25‰, and was oxygenated by air-bubbling. Salinity, dissolved oxygen and temperature of artificial seawater were measured weekly using a salinometer(28).

2.1. Acute exposure The amphipod *C.volutator* wasexposed to different concentrations of ZnO NPs for 10 days directly in seawater or mixed with the sediment, in order to investigate their effects and uptake when dosed via water and sediment. Three replicates were done per treatment. The exposure was conducted in 250 ml beakers containing 50 ml of sediment and 100 ml seawater (25‰). Each beaker contained 10 adult amphipods that were transferred to the beakers from the stock cultures using a wide –mouthed pipette. The amphipods were not given any food during the course of the experiment and the test solution was not changed. After 10 days, the sediment from each beaker was sieved through a 500 µm screen to collect the remaining amphipods. Live, missing and dead amphipods were counted (28,29).

2.2. Chronic exposure Neonates of *C.volutator* were collected by sieving the sediment though a 500 µm mesh and consequently with a 300 µm sieve. The concentrations of ZnO NPs, bulk ZnO and zinc ions were 0, 0.2, 0.5, and 1 mg/L. Bulk and NPs were dispersed in ultrapure water by probe sonication for 10 seconds to increase dispersability and disaggregation. The length of experiment was 100 days in order to allow for assessment of changes to growth, reproduction and survival. The treatment was conducted in 2000 ml beakers containing 2 cm of sediment and 500 ml of overlying seawater; each exposure consisted of nine replicates, each of them containing 20 neonates. The animals were fed with 'Liquifry Marine' (Inter-prêt, Prod. 0308) mixed with seawater. Water changes and dosing of ZnO NPs, bulk zinc and zinc ions were done weekly 24 h after feeding. Beakers were constantly aerated avoiding disturbing the sediment surface. Every 4-5 weeks three beakers from each exposure were sampled. The number of surviving organisms was counted, and they were preserved in ethanol 70% with glycerin to measure the total body length (measured along the dorsal side, from rostrum to the posterior of the telson by using ocular micrometer). Throughout the experiment, the number of moults in each beaker was counted(29).

2.3.Statistical analyses Differences between samples and the control were evaluated using the statistical analysis package SPSS 16.0, data were analysed by one way analysis of variance (ANOVA). The significance was tested at the $p < 0.05$ level. The data were further analysed using Microsoft® Excel values are reported±S.D.

3. Results

3.1. Acute exposure of zinc oxide nanoparticles form via sediment and water The mortality of *C. volutator* increased significantly ($p<0.05$) with increasing ZnO NPs in sediment, with concentrations of 300 mg/L to 500 mg/L, causing the death of about 50 and 90% of the population, respectively. A concentration of 1000 mg/L caused 100% mortality (Figs.1).

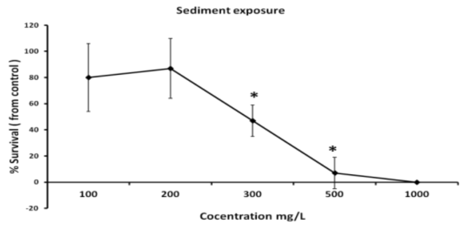

Fig.1: The effect of concentrations ZnO NPs on survival *C.volutator* (10 day), star (*) a significant difference ($P < 0.05$)

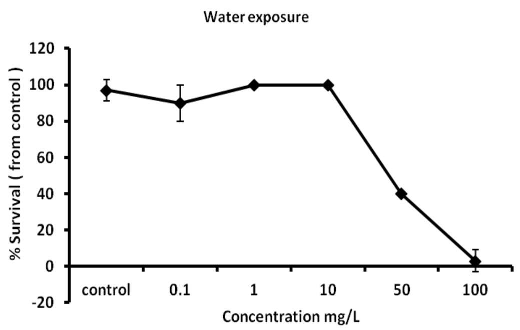

Fig.2: The effect of concentrations ZnO NPs on survival *C.volutator* (10 day).

On the other hand, a waterborne exposure of ZnONPs wassignificantly more toxic to *C.volutator* than when organisms were exposed via sediment (Fig.2), with 50 mg/L causing about 50% mortality and 100 mg/L 100% mortality. This indicates that the bioavailability of ZnO NPs or soluble zinc from the nanoparticles is more bioavailable to *C. volutator* when exposed via water.

3.2. Chronic exposure The mean temperature during the experimental period was 12^{0}C± 0.2, dissolved oxygen was 7.28±0.6 ppm, salinity 24.78± 0.1‰, and pH was 8.2±0.2. The result of this experiment indicates that after 4 weeks of exposure the survival decreased with increasing zinc chloride concentration (Fig. 3A). However for ZnO NPs survival was not affected(Fig. 3B). After 14 weeks, organisms were more affected at concentrations of 0.5 and 1 mg/L, and the percentage of survival declined to less than 70% for all experimental samples (Fig.3C). The average length of *C.volutator* was measured throughout the exposure. After 4 weeks, average length was lower than the control at the concentrations of 0.2, 0.5, and 1 mg/L for animals exposed to Zn Cl_2 and bulk, but not for ZnO NPs(Fig. 4A). After 9 weeks, Zn Cl_2 and bulk ZnO had a more significant effect on *C. volutator* growth compared to ZnO NPs (ANOVA $p<0.05$) (Fig. 4B). However, at the end of the exposure there wasn't a significant difference on *C. volutator* length, except for bulk ZnO, where organisms were slightly smaller (ANOVA, $p>0.05$) (Fig. 4C).

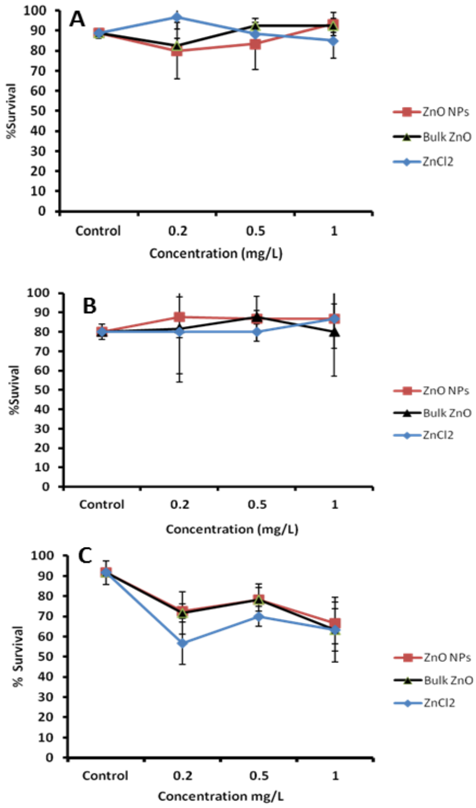

Fig. 3: Effect of concentrations ZnO NPs, bulk ZnO and $ZnCl_2$ on survival *C.volutator*. A(4 weeks), B(9 weeks) and C (14 weeks).

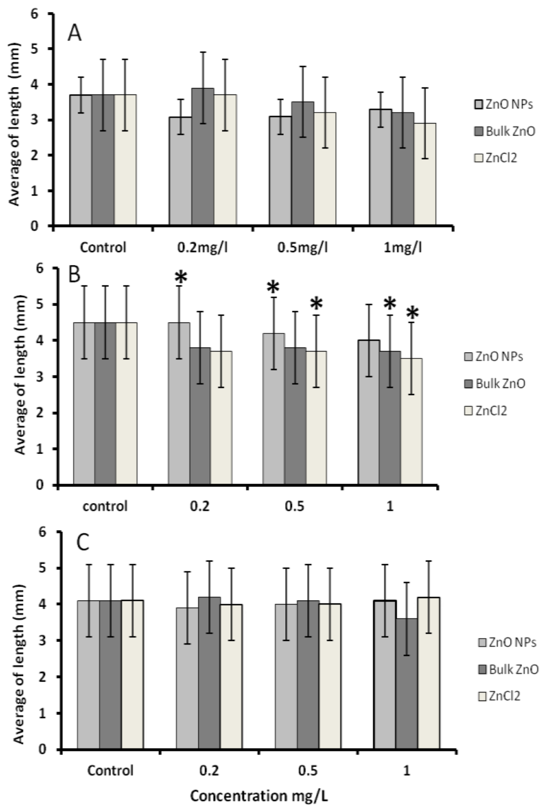

Fig.4: Effect concentrations on the average length of *C.volutator*. A (4 week), B(9 weeks) and C (14 week). All results are expressed as mean± SD.P< 0.05

4. Discussion The present study shows that survival and the life cycle of *C.volutator* are affected by the presence of high concentrations of zinc. It has been reported that both ZnO NPs and ZnO bulk aqueous suspensions are capable of delaying zebrafish embryo and larvae development, their hatching rates and reduced their survival, moreover, caused tissue damage [30]. Also, metal oxide NPs are toxic to the nematode *Caenorhabditis elegans*, affecting in particular its reproductive capability[31]. The toxicity of ZnO NPs has been attributed to the dissolved Zn^{2+} ions [18]. Bat *et al*, [32] indicated that when amphipods were exposed to zinc and iron in the sediments there was more chance for survival than when they were exposed to the same material but without sediment. Rainbow and White[31]pointed out that the young amphipods had higher zinc residues than older stages. The present study shows that the average length of *C. volutator* was decreased; in spite of the maximum length reaches in normal cases was more than 8mm. Both ZnO NPs and ZnO bulk aqueous suspensions are delayed Zebra fish embryo and larva development, hatching rates and reduced their survival, moreover, caused tissue damage [30]. Metal oxide NPs are toxic to nematode *Caenorhabditis. elegans*, especially to its reproductive capability [30]. The toxicity of ZnO NPs attributed to the dissolved Zn^{2+} ions. While, NPs are more toxic than bulk due to their greater surface reactivity [33]. Zinc also impaired growth owing to a reduction in the amount of metabolic energy available in the animal [34], and increasing survival via postponing the production of broods through absorption of eggs or dead[34]. This was also observes inthe present study, the vigorous females disappeared that correspond to the results [35].The rate of uptake of zinc by decapods *Palaemon elegans* and *Palamoneters varians* rises with decreased salinity at constant zinc concentration [36]. The process of uptake, excretion of heavy metals by *C.volutator* depended on the detoxification of accumulated metals in the cells of ventral caeca [36]. ZnO NPswere more toxic towards algae than bulk, but less toxicity towards crustacean and fish and this was attributed to dissolved zinc ions [26]. All zinc formulations were very toxic (bulk ZnO, nano ZnO and $ZnSo_4 .7H_2O$ to bacteria *Vibrio fisheri,* Crustacea *Daphnia magna, Thamnocephalus platyurus* due to solubilised Zn^{2+} ions [37]. Although, the toxicity of bulk and ZnO particles were both similar to that of $ZnSo_4$ to microalgae *Pseudokircheriella subcapitata*[18] and [38].

Acknowledgements We thank ministry of Libyan Higher Education for providing financial support for this study.

Abbreviations Zinc oxide nanoparticles(ZnO NPs), Deionized water (DIW),Nanomateriales (NMs). Ultraviolate (UV).

References

[1]- Lincoln, R. J.(1979). British marine Amphipoda: Gammaridae. Trustee of the British Museum. London.

[2]- Hughes, R. G., (1988). Dispersal by benthic invertebrates the in situ swimming behaviour of the Amphipod *Corophium volutatorJournal of Marine Biology* Association of the United Kingdom. **68**:565-579.

[3]- Gerdol, V., Hughes, R. S., (1994a). Effect of *Corophiumvolutator* on the abundance of benthic diatoms, bacteria and sediment stability in two estuaries in south eastern England. *Marine Ecology Progress Series*. **114**:109-115.

[4]- Gerdol, V., Hughes, R. S., (1994). Feeding behavior and diet of *Corophium volutator* in an estuary in south eastern England. *Marine Ecology Progress Series*. **114**:103-108.

[5]- Limia, J., Raffaelli, D., (1997). The effect of burrowing by the amphipod *Corophium volutator* the ecology of intertidal sediments. *Journal of Marine Biology Association of the United Kingdom*. **77**:409-423.

[6]- Percy, J., (1999). Keystone *Corophium*. Master of the mudflats. Fundy Issue. 13: p: 10.

[7]- Mc Lusky, D. S., (1968). Some effects of salinity on the distribution and abundance of *Corophium volutator* in the Ythan estuary. *Journal of Marine Biology Association of the United Kingdom*. **48**:443-454.

[8]- USEPA (US Environment Protection Agency). Methods for assessing the toxicity of sediment-associated contaminants with estuarine and marine amphipods. EPA/600/R-94/025. Office of Research and Development, Washington, DC. 1994.

[9]- USEPA (US Environment Protection Agency). (2001).Method for Associated Contaminants

with the Amphipod *Leptocheius plumulosus*. EPA 600/R-01/020. Office of science and Technology.

[10]- Meadows, P. S., Reid, A., (1966). The behaviour of *Corophium volutator* (Crustacea: Amphipoda).Journal of Zoology. **150**:387-399.

[11]- Erdem, C., Meadows, P.S., (1980). The influence of mercury on the burrowing behaviour of *Corophium volutator*. *Marine Biology*.**56**:233-237.

[13]- ASTM(American Society for Testing and Materials). Standard test method for measuring the toxicity of sediment-associated contaminants with estuarine and marine invertebrates. ASTM Designation 1367-03. Philadelphia. 2003

[14]- Depledge M. H., Rainbow, P. S., (1990). Model of regulation and accumulation of trace metals in marine invertebrates. *Comparative Biochemistry and Physiology*. **97**C (1):1-7.

[15]- Rainbow, P.S. (1990). Heavy metal levels in marine invertebrates: Furness, R.W., P. S. (Eds), Heavy metals in marine environment.CRC Press, Boca Raton, Florida.67-79.

[16]- Landis. G., Yu, M.H. (2003). Introduction to environmental toxicology: Impact of chemicals upon ecological systems.3rd.ed. Lewis publishers.USA.

[17]- Taylor, M.C., Demayo, A. and Taylor, K.W. (1982). Effects of zinc on humans, laboratory and farm animals, terrestrial plants, and freshwater aquatic life. *Crit. Rev. Envir. Controls***12**:113–181.

[18]- Franklin,N.M., Rogers, N,J., Apte, S.C., Batley,G. E., Gadd, G. E., Casey ,P.S. (2007).Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and $ZnCl_2$ to a freshwater microalga (*Psudokirchneriella subcapitata*): The importance of particle solubility. *Environmental Science and Technology*.**41**:8484-8490.

[19]- Rainbow, P. S., (2002).Trace metal concentrations in aquatic invertebrates: why and so what? *Environmental pollution*.**120**:497-507.

[20]- Klaine, S. J., Alvarez, P. J. J., Batley, G. E., Fernandes, T. F., Handy, R. D., Lyon, D. Y., Mahendera, S., McLaughlin, M. J., Lead, J.R., (2008). Nanomaterials in environment: Behaviour, Fate,Bioavailability, and Effects. *Environmental Toxicology and Chemistry,* Vol. 27, **9**:1825-1851.

[21]- Williams, L., Adams, W. (2007). Nanotechnology Demystified. 1st.ed. The McGraw-Hill Companies. USA.

[22]- Hu, C.W., Li, M., Cui, Y.B., Li, D. S., Chen, J., Yan, L. Y., (2010). Toxicological effects of TiO_2 and ZnO Nanoparticles in soil on earthworm *Eisenia fetida. Soil biology and biochemistry*.**42**:586-591.

[23]- Reddy, K. M., Feris, K., Bell, J., Wingett, D.J., Hanley, C., and Punnoose, A. (2007). Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. *Applied Physics letter*.**90**, 21392.

[24]- Mortime, M., Kasemets, K., Kahru, A., (2009). Toxicity of ZnO and CuO nanoparticles to ciliated protozoa *Tetrahymena thermophila*. Toxicology, doi: 10.1016/j.tox.2009.07.007.

[25]- Zhu, X., Zhu, L., Y., Chen, Y., Tian. S., (2009). Acute toxicities of six manufactured nanomaterials suspensions to *Daphnia magna*. Journal *Nanoparticales and Research*. **11**:67-75.

[26]- Wong, S.W.Y., Leung, P.T.Y., Djurišić, A. B., Leung, M., (2010).Toxicities of nano zinc oxide to five marine organisms: influences of aggregate size and ion solubility. *Anal Bioanal Chem*. **396**:609-618.

[27]- Moore, M.N., (2006). Do nanoparticles present ecotoxicological risks for the aquatic environment? *Environmental international*. **23**:967-976.

[28]- Scarlett, A.; Rowland, S. J.; Canty, M.; Smith, E. L.; Galloway, T.S. Method for assessing the chronic toxicity of marine and estuarinsediment-associated contaminants using the amphipod *Corophiumvolutator*. *Mar. Environ. Res*. 2007, 63 (5), 457–470.

[29]- Conradi, M., Depledge, M.H., (1999). Effect of zinc on the life- cycle, growth and reproduction of the marine amphipod *C.volutator*. *Marine ecology progress series*. **176**:131-138.

[30]- Wang, H., Wick, R. L., Xing., (2009). Toxicity of nanoparticulate and bulk ZnO, $Al_2 O_3$ and TiO_3 to the nematode *Caenorhabditis elegans*. *Environmental pollution*. **157**: 1171-1177.

[31]- Zhu, X., Zhu, L., Li, Y, Qi. R, Duan, Z, Lang, Y. P., (2008). Comparative toxicity of several metal oxide nano-particle aqueous suspensions to zebrafish (*Daniorerio*) early developmental stage. *Journal Environmental Science and Health*. A **34**(3):278-284.

[32]- Bat, L., Raffaelli, Marr, I. L., (1998). The accumulation of copper, zinc and cadmium by the amphipod *C.volutator* (Pallas). *Journal of experimental marine biology and ecology*.**223**:167-184

[33]- Rainbow, P. S, and White, S. L., (1989). Comparative strategies of heavy metal accumulation by crustaceans: zinc, copper and cadmium in a decapod, an amphipod and a barnacle. *Hydrobiologia* .**174.**

[34]- Sovova, T., Kočī, V., Kochânková., (2009). Ecotoxicity of nano and bulk forms of metal oxides. *Nanocon*. **10**:20-22.

[35]- Weeks. J., (1993). Effect of dietary copper and zinc concentrations on feeding rate s of two species of Talitrid amphipods (Crustacea). *Bull Environ Toxico*l. **50**:883- 890.

[36]- Nugegoda, D., Rainbow, P.S., (1989). Effects of salinity changes on zinc uptake and regulation by the decapod crustaceans *Palaemon elegans* and *Palaemonetes varians*. *Mar. Ecol. Prog. Ser*.**51**, pp. 57–75.

[37]- Burgos, M. G., Rainbow, P. S., (2001). Availability of cadmium and zinc from sewage sludge to flounder, Platichthy flesus, via a marine food chain. *Marine environmental research*. **51**: 417- 439.

[38]- Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H., Kahru, A., (2008). Toxicity of nanosized and bulk ZnO, CuO and TiO to bacteria *Vibrio fisheri* and Crustacea *Daphnia magna* and *Thamnocephalus platyurus*. *Chemosphere.* **71**:1303-1316.

[39]- Aruoja, V., Dubourguier, H., Kasemets, H., Kahru, A., (2009). Toxicity of nanoparticles of CuO, ZnO, and TiO_2 to microalgae *Pseudokircheriella subcapitata. Science of the environment.* **407**:1461-1468.

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