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# Toxicity Evaluation of Reduced Graphene Oxide on First Larval Stages of Artemia sp.

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**Abstract :** The focus of this work was to investigate the potential toxic effect of titanium dioxide-reduced graphene oxide ($TiO_2$-rGO) nanocomposites on nauplii of microcrustacean Artemia sp. In order to assess the nanocomposite's toxicity, a short-term test was performed by exposing nauplii to solutions containing $TiO_2$-rGO. To prepare titanium dioxide-reduced graphene oxide ($TiO_2$-rGO) nanocomposites, a green procedure based on solar photoreduction was proposed; it allows to obtain the photocatalysts by exploiting the photocatalytic properties of titania activated by the solar irradiation in order to avoid the high temperatures and pressures required for the standard hydrothermal synthesis. Powders of $TiO_2$-rGO supplied by the Department of Chemical Sciences (University of Catania) are indicated as $TiO_2$-rGO at 1% and $TiO_2$-rGO at 2%. Starting from a stock solution (1mg rGO-$TiO_2$/10 ml ASPM water) of each type, we tested four different concentrations (serial dilutions ranging from $10^{-1}$ to $10^{-4}$ mg/ml). All the solutions have been sonicated for 12 min prior to use. Artificial seawater (called ASPM water) was prepared to guarantee the hatching of the cysts and to maintain nauplii; the durable cysts used in this study, marketed by JBL (JBL GmbH & Co. KG, Germany), were hydrated with ASPM water to obtain nauplii (instar II-III larvae). The hatching of the cysts was carried out in the laboratory by immersing them in ASPM water inside a 500 ml beaker and keeping them constantly oxygenated thanks to an aerator for the insufflation of microbubble air: after 24-48 hours, the cysts hatched, and the nauplii appeared. The nauplii in the second and third stages of development were collected one-to-one, using stereomicroscopes, and transferred into 96-well microplates where one nauplius per well was added. The wells quickly have been filled with 300 µl of each specific concentration of the solution used, and control samples were incubated only with ASPM water. Replication was performed for each concentration. Finally, the microplates were placed on an orbital shaker, and the tests were read after 24 and 48 hours from inoculating the solutions to assess the endpoint (immobility/death) for the larvae. Nauplii that appeared motionless were counted as dead, and the percentages of mortality were calculated for each treatment. The results showed a low percentage of immobilization both for $TiO_2$-rGO at 1% and $TiO_2$-rGO at 2% for all concentrations tested: for $TiO_2$-rGO at 1% was below 12% after 24h and below 15% after 48h; for $TiO_2$-rGO at 2% was below 8% after 24h and below 12% after 48h. According to other studies in the literature, the results have not shown mortality nor toxic effects on the development of larvae after exposure to rGO. Finally, it is important to highlight that the $TiO_2$-rGO catalysts were tested in the solar photodegradation of a toxic herbicide (2,4-Dichlorophenoxyacetic acid, 2,4-D), obtaining a high percentage of degradation; therefore, this alternative approach could be considered a good strategy to obtain performing photocatalysts.

**Keywords :** Nauplii, photocatalytic properties, reduced GO, short-term toxicity test, titanium dioxide
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