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The effect of various nanoparticles towards *Artemia Salina* cyst

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Abstract

This work demonstrated the biocompatibility of various nanoparticles with *Artemia salina* cysts. The cyst of *Artemia salina* was exposed to a variety of nanoparticles including iron oxide, graphene oxide, reduced graphene oxide, zinc oxide, and titanium dioxide. *Artemia salina* is used as a model organism to investigate the effect of various types of nanoparticles on the hatching rate of *Artemia salina* cysts, as *Artemia salina* are the primary food organism in the marine ecosystem. The effect of the nanoparticle on *Artemia salina* could have a significant impact on the marine ecosystem. Typically, the *Artemia salina* cyst is incubated for 24 hours in artificial seawater. Thus, by using identical incubation conditions, it is possible to correlate the biocompatibility of different types of nanoparticles with the hatching percentages of *Artemia salina* cysts. Iron oxide, reduced graphene oxide, and titanium dioxide were found to be biocompatible with *Artemia salina* cysts at concentrations less than 1 mg/ml.

Keywords: *Artemia salina*, iron (III) oxide, graphene oxide, reduced graphene oxide, titanium dioxide, zinc oxide

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1. Introduction

Due to their favourable physical and chemical properties, nanoparticles (NPs) are constantly evolving and have enormous potential in the aquatic and terrestrial environments [1]. NPs such as Iron (III) Oxide (IO), Graphene Oxide (GO), Reduced Graphene Oxide (rGO), Zinc Oxide (ZnO), and Titanium Dioxide (TiO_2) are of particular interest in this regard due to their exceptional physicochemical properties, which make them highly suitable and desirable for a wide variety of consumer products and industrial technologies. NPs are currently used in a wide variety of commercial applications and disciplines, including technology, industry, and medicine.

As a result of the manufacture, distribution, application, and disposal of associated NPs are unavoidably released into the environment. All NPs will eventually make their way into aquatic ecosystems, particularly marine ecologies. Aquatic creatures are largely vulnerable to the harmful effects of NPs due to their direct or indirect effects.

Artemia Salina (*A. Salina*) is an invertebrate zooplankton that can be found in a variety of saltwater environments, from streams and rivers to seas. As one of the most popular live feeds in saltwater environments, *A. Salina* is critical to the energy flow of the food chain [2]. *A. Salina* is a non-selective filter feeder that can filter a significant amount of water per hour. As a result, it is more vulnerable than other marine species [3]. Additionally, it has the following physiological characteristics: a short life cycle, a high tolerance for harsh environmental conditions, a small body structure, a high rate of offspring production, multiple life cycles, is abundant, and is easy to cultivate [2].

Additionally, the US Environmental Protection Agency has designated *A. Salina* as a test organism for acute toxicity testing [4]. When taken together, these characteristics make *A. Salina* as one of the most desirable organisms for ecotoxicological testing. Ecotoxicological studies have been conducted in recent years using the *A. Salina* life cycle to determine the toxicity of NPs and their potential effects on the aquatic ecosystem.

Owing to the inevitable release of NPs into the environment as a result of their increasing use in a wide variety of applications, it is critical to understand their biological impact on the ecosystem. Aquatic species are thus more vulnerable to the direct and indirect effects of NPs. The purpose of this study is to examine the ecological imbalance potential of NPs (IO, GO, RGO, ZnO, and TiO_2) in top-of-

the-food-chain crustaceans such as *A. Salina* for alimentary (trophic) transfer between various primary consumer species.

We examined the hatchability of capsulated cysts following NPs exposure in order to determine the adverse reactions that occur after prolonged acute exposure. Additionally, *A. salina* was examined under a microscope to determine its NPs intake and distribution. The combined findings contribute to a more complete understanding of the detrimental effects of NPs on marine ecosystems and lay the groundwork for future NPs use.

2. Materials and methods

2.1. Preparation of various nanoparticles

This study employs a variety of NPs, including IO, GO, rGO, ZnO and TiO_2, which were synthesized in Biophysics Laboratory by our colleague except for one of the GO. GO was purchased from GO advance. IO was synthesised using the co-precipitation method described in previous work [5]. Our colleague synthesised GO by using carbonized tea waste followed by modified Hummer's technique [6]. Another NPs, ZnO, was successfully prepared by our colleague using green method [7]. The green approach employed two types of plants: palm oil leaves and roselle flower. Palm oil leaves and roselle flowers were collected from Universiti Putra Malaysia's estate. TiO_2 was synthesised using calcination method according to our colleague's technique [8].

2.2. Brine Shrimp Hatching Assay

The hatchability of *A. Salina* cysts was investigated using variety of NPs at various concentrations. The 96-well plates were filled with 200uL of test solution containing twenty (20) *A. Salina* cysts in each well. All plates were incubated under continuous illumination at a temperature of 28 °C. Using a microscope, the number of hatched cysts exposed to NPs at various concentrations and times was determined (Motic, BS2010BD, Hong Kong).

3. Results

3.1. Hatching rate of A. Salina following exposure to Iron (III) Oxide Nanoparticles

As shown in Fig. 1, the concentrations of IO NPs (Fe_3O_4) increased from 0 mg/mL to 1 mg/mL over a 24 h, 48 h, and 72 h period. As the concentrations of *A. Salina* cysts increased from 0 to 1 mg/ml, hatching rates decreased. At 24 h and 48 h after encasement, there were no changes in the percentage of cysts that hatched. After 72 h, the number of hatched cysts decreased by approximately 25 % across all concentrations.

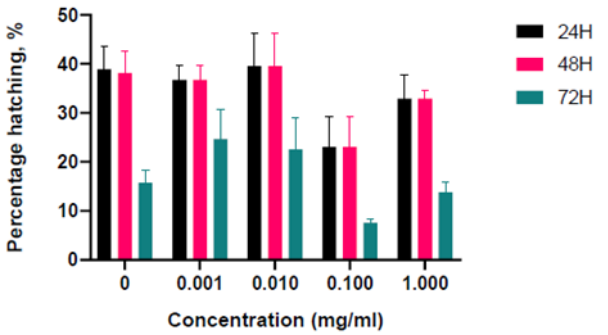

Fig. 1 The hatching percentage of cyst vs concentration of the IO

3.2. Hatching rate of A. Salina following exposure to Graphene Oxide and Reduced Graphene oxide Nanoparticles

Fig. 2 (a) and (b) showed the hatchability of *A. Salina* cysts is affected by the GO bought and synthesis using carbonized tea waste (b) respectively. At 24 h, 48 h, and 72 h, GO showed dose-dependent reductions in hatching rates for *A. Salina* cysts when compared to controls. At a relatively low dosage (0.001 mg/ml), the hatching percentage is comparable to the control after 24 h. At 1 mg/ml, however, significant changes occurred, with no *A. Salina* cysts hatching. After 72 h, less than 10% of cysts hatched, and the majority of hatched cysts starved to death, possibly due to a lack of nourishment. Next, the GO produced from carbonized tea waste was exposed to the *A. Salina* cyst display in Fig 2 (b). The range of the concentration were changed to become wider which are from 0.001 mg/ml, 0.01 mg/ml, 0.1 mg/ml, 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml and 1 mg/ml. The number of hatched cyst are increasing from 18 h to 48 h. When the concentration of the GO commercialize were more than 0.5 mg/ml to 1 mg/ml hatching percentage of *A. Salina* cyst is less than 10 %. The hatching rate of *A. Salina* cyst when exposed to the rGO was depicted in Fig 2 (c). The hatching percentage appears to be comparable to the control at the rGO concentrations ranging from 0.001mg/ml to 0.5 mg/ml. Indeed, when the cyst is treated with rGO at a concentration of 0.75 mg/ml to 1 mg/ml, the hatching percentage is greater than when the cyst is treated with rGO at a concentration of less than 0.5 mg/ml.

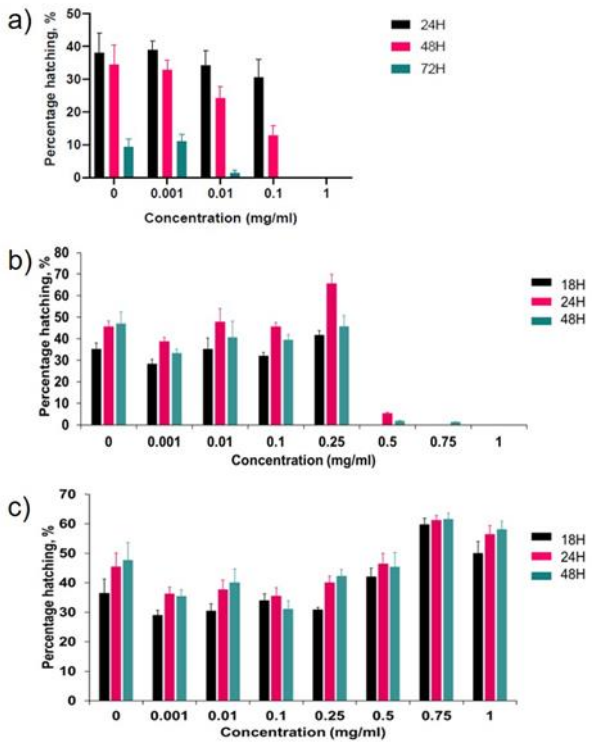

Fig. 2 The hatching percentage of cyst vs concentration of the a) GO (commercialize) b) GO (tea waste) c) rGO (palm oil leaves)

3.3. Hatching rate of A. Salina following exposure to Titanium Dioxide Nanoparticles

The hatching rates of the remaining NPs (ZnO and TiO_2) were increased to cover a broader concentration range (0, 0.001, 0.01, 0.1, 0.25, 0.5, 0.75, and 1 mg/ml). While the time period was reduced to less than 72 h because *A. Salina* cannot survive longer than 72 h without food. A suspension of TiO_2 was used to treat A. salina cysts. As demonstrated in Fig. 3, the hatching percentage is negligible at concentrations less than 0.1 mg/ml. Hatching rate decreases from 0.001 to 0.25 mg/ml. There were no cysts hatching at concentrations greater than 0.75 mg/ml. As a result of this, we know that *A. Salina* can survive in small amounts of TiO_2, but in a small amounts. After 48 h, as the TiO_2 concentration increases, the percentage of cysts gradually decreases.

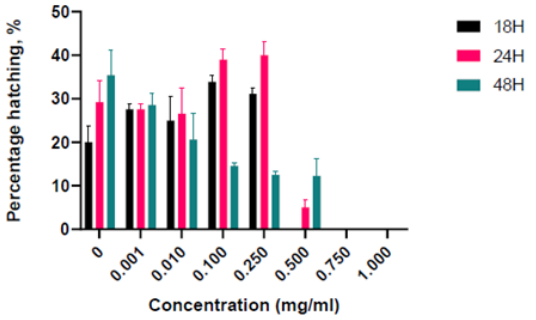

Fig. 4 The hatching percentage of cyst vs concentration of the TiO_2

3.4. Hatching rate of A. Salina following exposure to Zinc Oxide Nanoparticles

Fig. 4 shows the hatching rate of *A. Salina* after exposure to various concentrations of ZnO NPs for 18, 24, and 48 h. As illustrated in Fig. 4 (a), when palm oil leaves are used as the reducing agent, the hatching rate for ZnO synthesis is nearly negligible when compared to the control. *A. Salina* was also exposed to another ZnO produced using roselle in Fig. 4 (b), and it was discovered that the cysts can hatch at a dosage of 0.001 mg/ml, but the hatching rate was extremely low, less than 10% for the entire time period tested, at concentrations greater than 0.001 mg/ml.

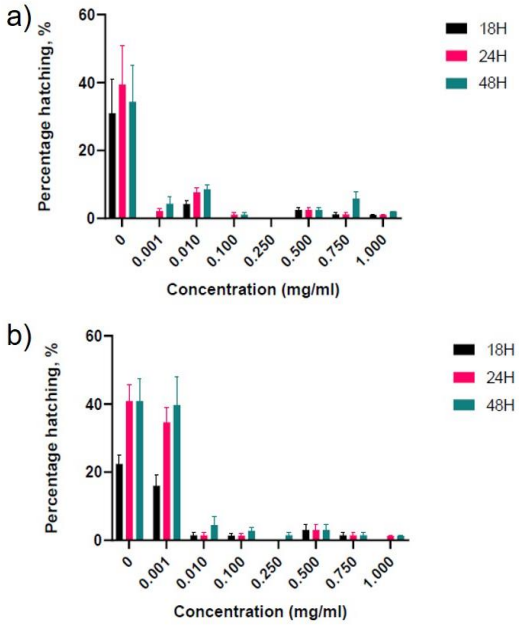

Fig. 4 The hatching percentage of cyst vs concentration of the ZnO a) Palm oil leaves b) Roselle

4. Discussion

A. Salina is a nonselective filter feeder that can ingest particles as small as 50 µm in diameter. In comparison to other crustaceans, *A. Salina's* mechanism is extremely primitive; it is an obligate phagotrophic filter-feeder that feeds continuously and non-selectively. *A. Salina* consumes suspended particles of a suitable size on a continuous basis, regardless of their nature. According to previous research, NPs accumulate and are eliminated from microscopy observations following exposure to NPs [10].

Typically, cyst hatchability and larval mortality following exposure are chosen as endpoint criteria, and the sensitivity of both tests has been investigated in a variety of studies [2]. The various stages of *A. Salina* and the data that accompany them suggest that the hatching test is less sensitive than the 24 h and 72 h larval mortality assays. It has been suggested that the NPs' intrinsic properties contribute to their absorption by *A. Salina*. Table 1 summaries the inherent characteristics of NPs as compiled by our colleagues.

Table 1: The particle size and shape for variety of NPs

Types of NPs	Shape	Size
IO	Spherical	10 ± 2 nm
GO (commercialize)	One dimensional corrugated sheet	<5um
GO (tea waste)	Wrinkle multiple layers of carbon sheet	NA
RGO	Wrinkle multiple layers of carbon sheet	NA
TiO2	Irregular shapes	80 ± 23 nm
ZnO (Palm oil leaves)	spherical	11 ± 2 nm
ZnO (Roselle flower)	spherical	10 ± 2 nm

To determine the hatching rate of *A. Salina*, the ecotoxicity of the synthesised NPs was screened. Hatching tests on *A. Salina* exposed to IO confirmed the potential for ecotoxicity, as illustrated in Fig 1. The hatching rate of *A. Salina* exposed to IO at concentrations ranging from 0.001 to 1 mg/ml for 48 h is almost identical to that of the control. This indicated that IO had no discernible effect on *A. Salina* hatching ability.

A. Salina is a species that is resistant to heavy metal ions and can withstand a wide range of metal ion concentrations[10]. The particles of IO are 10 ± 2 nm in size and are easily ingested by larvae, as evidenced by microscopy observations. The IO NPs were absorbed into the guts of the *A. Salina* larvae, and it they did not cause mortality after 48 h. Similar to previous research, the accumulation of IO NPs within the stomach of *A. Salina* larvae and their inability to eliminate these ingested particles [11]. It is discovered that the presence of IO NPs impairs *A. Salina* ability to swim. Sensitivity of *A. Salina* to IO NPs has been determined to be in the following order: instar II > instar III > instar I > decapsulated cysts > capsulated cysts [10]. As a result, IO exposure to the cyst of *A. Salina* had no effect on the hatching rate. Cysts may be less susceptible to IO NPs due to their cortical membrane, which acts as a protective shield against the IO NPs.

Additionally, the *A. salina* cyst were exposed to commercialize GO and green GO using tea waste. Initially, the concentrations of NPs in GO (commercialise) were 0.001, 0.01, 0.1, and 1 mg/mL; however, after some consideration, the concentrations of NPs in GO (tea waste) were changed to 0.001, 0.01, 0.1, 0.25, 0.5, 0.75, and 1 mg/mL to cover a wider concentration range and identify the concentration at which the cyst are most affected by the NPs suspension.

The exposure period for GO (commercialise) has been changed to 24 h, 48 h, and 72 h, while the exposure period for GO (tea waste) and rGO (palm leaves oil) has been reduced to 18 h, 24 h, and 48 h only. The exposure period was reduced from 72 to 48 h because the majority of *A. Salina* larvae will die after 72 h due to a lack of food.

As stated in Table 1, GO (commercialise and tea waste) has a one-dimensional irregular corrugated or wrinkled sheet with particles smaller than 5 um. As a result of exposure to GO (commercialise and tea waste), the A. salina body surface suffers permanent damage, resulting in "holes" and "groove-like rifts" on the surface of the body [9]. This is because the GO (commercialise and tea waste) ridges are capable of rupturing cell membranes and causing cell death.

When commercially available GO is compared to green GO derived from tea waste extract, the hatching rate is dose dependent. Additionally, because GO (commercialise) has a lower hatching rate than GO (tea waste), it was discovered that GO synthesised from tea waste has a higher biocompatibility for *A. Salina* cyst. The *A. Salina* cyst does not appear to exhibit any reduction in hatching rate when exposed to GO (tea waste) at concentrations greater than 0.25 mg/ml. As the GO (tea waste) concentration increased to 0.5 to 1 mg/ml, the hatching rate decreased.

The effect of rGO suspension on the hatchability of capsulated cysts is depicted in Fig 2 (c). When compared to controls, GO (commercialise and tea waste) produced dose-dependent hatching rates for both capsulated cysts that corresponded to the exposure period. The hatching rate decreases as the GO concentration increases. After 24 h, the hatching percentage is equivalent to the control at a relatively low dose (0.001 mg/ml). However, there were significant variations in cyst hatching at 0.5, 0.75, and 1 mg/mL, with very few cysts hatching due to the thick layer of GO (commercialise) obstructing light from reaching the *A. Salina*. After 72 h, less than 10% of cysts hatched, and the hatched cysts mostly died due to a lack of food, as the aggregated GO is too large for the *A. Salina* to consume.

TiO_2 particles are irregular in shape and range in size from 80 to 23 nm. In a simulated saltwater environment, previous research discovered that nano-TiO_2 aggregated at sizes ranging from 495 nm to 852 nm [12]. When *A. Salina* is exposed to TiO_2 at a concentration of 0.001 mg/ml to 0.25 mg/ml, the hatching rate increases slightly, as illustrated in Fig 3. Thus, it is confirmed that at low concentrations, TiO_2 increased hatching rate. This could be because the TiO_2 used in this study was prepared from a natural precursor in Malaysia, namely ilmenite. The hatching rate decreased from 0.5 mg/ml to 1 mg/ml TiO_2, indicating a strong resistance to TiO_2 toxicity. This finding is consistent with previous research indicating that TiO_2 is nontoxic to *A. Salina* due to the benign TiO_2 aggregates [13].

A. Salina was exposed to various concentrations of ZnO produced from Roselle and Palm oil leaves for 48 h. When *A. Salina* is exposed to ZnO suspension at a low concentration of 0.001 mg/ml as depicted in Fig 4, the hatching rate is less than 10 % of the hatching rate. This could

be explained by the high level of oxidative stress that the *A. Salina* cyst is subjected to during treatment with ZnO suspension. Previously published research has used lipid peroxidation assays to determine glutathione (GSH) and malondialdehyde (MDA) concentrations [14]. The levels of GSH and MDA were determined to serve as markers of oxidative stress and lipid peroxidation, respectively. When ZnO NPs with a particle size of less than 30 nm interact with *A. salina*, the GSH level increases in comparison to the control group [14]. Additionally, increased MDA levels indicate an increase in free oxygen radicals, which results in oxidative stress, which depletes antioxidant enzymes, promotes lipid peroxidation, and destroys the cyst's membrane structure, preventing it from hatching [15].

Both IO and ZnO are spherical in shape with particles less than 20 nm in diameter. However, the hatching rate of *A. Salina* cysts varies significantly. *A. Salina* cysts exposed to ZnO hatch at a lower rate than cysts exposed to IO, even at low NPs suspension concentrations. We proposed that shape and particle size cannot be the sole determinants of cyst hatching percentage. Additionally, the type of NPs has an effect on the hatching rate of cysts exposed to various types of NPs. This is due to the release of Zn ions (Zn^{2+}) from the ZnO into the solution, as previously suggested [3].

5. Conclusion

Nanomaterials can be made of any material, including metals and oxides, carbon, organic, and biomaterials, and can take on any shape, as long as at least one dimension is within the nanoscale size range. To summaries, nanomaterials, with their increasing diversity, unique properties, and seemingly limitless applications, may pose a risk to the environment and human health if released in large quantities. Increased utility would expose ecosystems to greater unknown risks. Their discharge into the environment can occur as a result of manufacturing, application, weathering, or wastes. The hatching rate of *A. Salina* cysts is found to be affected by the method used to synthesise the NPs, the precursor used to synthesise the NPs, the particle size and shape, and the type of NPs exposed to the *A. Salina* cyst.

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