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Toxicity Test of Ag^+, Nano-Ag^0 and Nano-Ag_2O using Green Algae (*Chlorella* sp.) and Water Flea (*Moina macrocopa*)

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***Abstract*—**The research objective was to study the toxicity of silver nanoparticles in aquatic organisms. Three forms of free silver ion nanoparticles (Ag^+), silver nanoparticles (nano-Ag^0) and silver oxide nanoparticles (nano Ag_2O) were examined for toxic effects with *Chlorella* sp. and *Moina macrocopa*. The results showed that the toxicity of three silver ion forms to both organisms was examined with the following toxicity ranking: Ag^+ > nano-Ag^0 > nano-Ag_2O. A test using Ag^+ with *Chlorella* sp. yielded an EC_{50} (Effective Concentration) of 0.58±0.17 mg/L and a test using nano-Ag^+ with *M. macrocopa* yielded an LC_{50} (Lethal Concentration) of 0.03±0.43 mg/L. For toxicity test of nano-Ag^0, the yield of EC_{50} was 30.52±0.70 mg/L with *Chlorella* sp. and the yield of LC_{50} with *M. macrocopa* was 5.77±0.82 mg/L. The EC_{50} for *Chlorella* sp. was 46.92±0.44 mg/L and the LC_{50} for *M. macrocopa* was 13.21±1.52 mg/L when testing with nano-Ag_2O toxicants.

Keywords*—Chlorella* sp., *Moina macrocopa*, Silver nanoparticles, Toxicity

I. Introduction

SILVER nanoparticles (AgNPs) are one of the most widely used nanomaterials in consumer products. AgNPs are used in industrial products, applications in cosmetics and as bacteriocides in fabrics mainly because of their anti-bacterial properties [1]. AgNPs in water have high mobility and can be easily transported to aquatic environment [2]. They have been shown to be toxic to microbes and invertebrates although somewhat less so to fish and humans. However, their environmental impact on aquatic ecosystem is still unknown [3]. The freshwater invertebrate is a well established test organism in ecotoxicology, and recognized by the OECD due to ease of culture, short life span and ecological importance [3].

Algae play an important role in aquatic ecosystem, not only by producing biomass that forms the basic nourishment for food webs, but also by contributing to the self-purification of polluted water [4]. In toxicity examination of toxicant and nanoparticles, algae especially *Chlorella* sp. (a species of unicellular green organism) is one of normally used model organisms [1], [4]. Zooplankton is also commonly used in aquatic toxicity testing. A species of *Dapnia magna* in particular has normally been used during the last several decades worldwide. However, this species is not common in Thailand. On the contrary, *M. macrocopa* is a world-wide distributed cladoceran and belongs to a group of large-bodied *Moina* species. It inhabits in small and large and usually ephemeral water bodies in both temperate and tropical zones.

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In Thailand *M. macrocopa* is indigenous and abundant. It is suggested that a native species, *M. macrocopa* rather than the international standard test species can also be an ecologically representative zooplankton species in tocixity test [5], [6], [7].

The objective of this research was to study the toxicity effect of silver nanoparticles on aquatic organisms. Three forms of free silver ion nanoparticles (Ag^+), free silver nanoparticles (nano-Ag^0) and silver oxide nanoparticles (nano Ag_2O) were investigated for ecotoxicity assessment with *Chlorella* sp. and *M. macrocopa*.

II. Materials and Methods

A. Preparation of silver nanoparticles

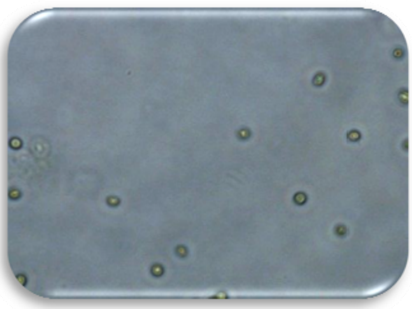
Nanoparticle powder used in this study was purchased from Dongyang (HK) International group limited, Hongkong, China.

Free silver ion nanoparticles (Ag^+) were prepared by stock solution (1000 ppm) from silver nanoparticle powder with conc. HCl, 1.41 mg/L initial concentration. Free silver nanoparticles (nano-Ag^0) and silver oxide nanoparticles (nano-Ag_2O) were prepared by stock solution (5,000 ppm) of 2 g silver nanoparticle powder in DI water (400 ml) and then sonicated for 30 minutes by sonicator (Elma; TRANSSONIC 460/H) with stirrer. Subsequently, the solution was filtered by filter papers (whatman no.1) and 69% HCl was added.Initial concentration measured by Atomic Absorption Spectroscopy (AAS), initial concentration was 3.68 and 3.88 mg/L, respectively [4] ,[8].

B. Study the toxicity of silver nanoparticles with algae and water flea

Algae and water flea in control experiment are showed in Fig. 1 and followed by OECD: Freshwater Algae and Cyanobacteria, Growth Inhibition Test 201 [9]. *Chlorella* cells were counted by Hemacytometer counting chamber (maximum < 2- 5 x10^3 cells/mL). Water flea used in the experiment was *M. macrocopa* and was cultured based on OECD: *Daphnia* sp., Acute Immobilisation Test 202 [10]. The second generation of cultured *M. macrocopa* was used at the age of less than 24 hours. The concentrations used for all experiments and for both organisms were 0-100% from initial concentration.

(a)



(b)

Fig. 1 Algae and water flea in control experiment (a) *Chlorella* sp. 100X and (b) *M. macrocopa* 10X

III. Results and Discussion

A. Characteristics of AgNPs

Morphology of silver nanoparticles was studied by Transmission Electron Microscope (TEM). Diameter of AgNPs was less than100 nm. Most particles were spherical in shape and a few lengthy [11]. X-ray Diffraction (XRD) analysis indicated that AgNPs consisted of pure silver element, without contamination of other elements (Fig. 2).

B. Toxicity of AgNPs with *Chlorella* sp.

Toxicity test of Ag^+, nano-Ag^0 and nano-Ag_2O are showed in Table 1. The EC_{50} of Ag^+ was 0.58 mg/L which performed the highest toxicity. This is because Ag^+ can be easily uptaken into living cells and thus resulting in higher toxicity when compared with other solid forms. Normally EC_{50} of Ag^+ on general algae is between 24-190 nM. [12]. The minimum value of NOEC (No Observable Effect Concentration) for Ag^+ of freshwater and marine algae is between 0.002 -2 mg/L, depending on the type of algae [13]. Nano-Ag^0 and nano- Ag_2O had less toxic effect because solid forms of such nanoparticles were less uptaken than than Ag^+ solution. In addition, aggregrated forms of nano-Ag^0 and nano-Ag_2O were difficult to get into cells of green algae due to larger size particles. They were only found entrapping and wrapping on the cell walls of green algae [4].

In general, nano-Ag^0 particles were aggregated due to the magnetic characteristic. Most aggregrated forms of AgNPs floated above water surface and therefore causing light obstruction and reducing rate of photosynthesis of green algae. As a result the growth of green algae had decreased. The toxicity of AgNPs on algae and invertebrates may derive from the release of Ag^+ from AgNPs that affected cell growth process of photosynthesis and process of chlorophyll production [14]. Toxicity of three forms of Ag was significantly different at 95%. The toxicity of silver nano forms was indicated with the following deceasing sensitivity: Ag^+ > nano-Ag^0 > nano-Ag_2O.

(a)

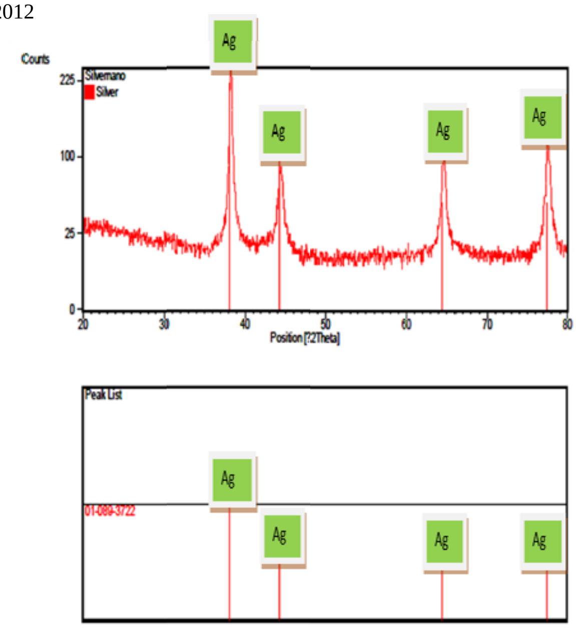


Fig. 2 Morphology and chemical composition of AgNPs by (a) TEM and (b) XRD

TABLE I
EC_{50} of AgNPs in Three Forms with *Chlorella* sp. at 48 Hours

Form of AgNPs	**EC_{50} (mg/L)**
nano-Ag+	0.58±0.17
nano-Ag^0	30.52±0.70
nano-Ag_2O	46.92±0.44

C. Toxicity of AgNPs with *M. macrocopa*

The LD_{50} of Ag^+, nano-Ag^0 and nano-Ag_2O is presented in table 2. The LD_{50} of Ag^+ was 0.026 mg/L indicating the highest toxicity as same as EC_{50} of *Chlorella* sp.

The uptake of free Ag^+ into the body of *M. macrocopa* was easier than solid forms through oral and dermal routes. Solution of Ag^+ was more toxic with *Daphnia pulex* than nano-Ag^0 particle size 30-20nm [15]. The minimum value of) NOECNo Observable Effect Concentration) of Ag^+ on water flea (*Daphnia*, spp.) wasµ 0.001 g/L.

In addition it was revealed that Ag^+ toxicity had direct effects on water flea survival and reproduction rate [16]. The LC_{50} of nano-Ag^0 on *D. magna* at 24hours was1 2 5 mg/L and it was found that nano-Ag^0 accumulated in gut and antennae [17]. Toxicity of nano-Ag^0 on invertebrates may come from the release of Ag^+ from AgNPs which affected cell growth [10].

Toxicity of nano Ag_2O on *Daphnia spp.* was higher than nano-Ag^0. A previous study on nano Ag_2O (particle size was 20-30 nm) [11] showed that LC_{50} of *D. magna*and *D. pulex* at 48 hours was 0.04 mg/L and L/mg 0.067, respectively.

However, our result of LC_{50} with *M. macrocopa* at 48 hours was average 13.21 mg/L. LC_{50} was higher possibly because of different types and wellness of water flea as well as the size of particle larger than nano-Ag_2O) < 100 nm.).

Statistical analysis showed that toxicity of Ag^+, nano-Ag^0 and nano Ag_2O was significantly different at95 %. The following toxicity ranking was Ag^+ > nano-Ag^0 > nano-Ag_2O

TABLE II
LC_{50} OF AgNPs IN THREE FORMS WITH *M. macrocopa* AT 48 HOURS

Form of AgNPs	**LC_{50} (mg/L)**
nano-Ag+	0.03±0.43
nano-Ag^0	5.77±0.82
nano-Ag_2O	13.21±1.52

IV. CONCLUSION

In conclusion the toxicity of some AgNPs forms including free silver ion nanoparticles (Ag^+), silver nanoparticles (nano-Ag^0) and silver oxide nanoparticles (nano-Ag_2O) with green algae (*Chlorella* sp.) and water flea (*M. macrocopa*) representing organisms of aquatic ecosystem was observed. From these results it can be concluded that AgNPs had negative effects on *Chlorella* sp. and *M. macrocopa*, as indicated by the decrease of growth rate and reduction of photosynthetic rate of green algae. In case of water flea, it was found that the AgNPs had accumulated in gut and antennae. Especially Ag^+, it demonstrsted the highest toxicity than the other forms. The results also showed the following decreasing sensitivities of toxicity with both organisms: Ag^+ > nano-Ag^0 > nano Ag_2O. The toxicity of free Ag^+ showed that the EC_{50} (Effective Concentration) was 0.58±0.17 mg/L with *Chlorella* sp. and the LC_{50} (Lethal Concentration) of *M. macrocopa* was 0.03±0.43 mg/L.

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