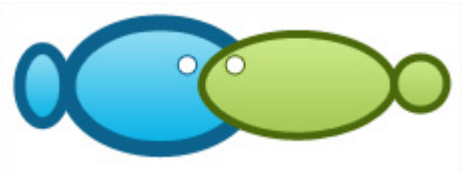

Trophic transfer potential of silver nanoparticles from *Artemia salina* to *Danio rerio*

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Abstract. This study was conducted to measure the bioconcentration of silver nanoparticles (AgNPs) in artemia brine shrimp (*Artemia salina*) and its transfer to zebrafish (*Danio rerio*), in order to: (1) determine the uptake of AgNPs by artemia from water, and (2) evaluate trophic transfer potential of AgNPs from artemia to zebrafish under controlled conditions. The artemia exposed to 0.5, 1.0, and 2.0 mgL^{-1} AgNPs for 24 h and then were administrated to zebrafish for 14 days. Silver body burden was assayed using a Phoenix 886 flame atomic absorption spectrophotometer both in artemia and zebrafish. The results of this study showed that the uptake of AgNPs in artemia was higher than zebrafish ($p < 0.05$). The accumulation of AgNPs in zebrafish was dose dependent, with greater accumulation observed at higher AgNPs concentrations. Moreover, the results of this study indicated that the trophic transfer factor (BMF) of AgNPs was lower than 1 (< 1), and this nanoparticle was not potential of trophic transfer from artemia to zebrafish.

Key Words: biomagnification, uptake, brine shrimp, zebrafish, nanoparticle.

Introduction. In recent years, the use of nanoparticles (NPs) has been a growing trend in various industries. Silver nanoparticles (AgNPs) are one of the most extensively used nanoparticles in consumer products including biosensor, nanocomposite films, biocide, cosmetics, food packaging, as well as in medical products (Sotiriou & Pratsinis 2010; Sun et al 2009; Rhim et al 2006). Woodrow Wilson Database (2013) has listed 1,317 nanoparticle based consumer products on the market in 30 countries (USA: 587; Europe: 367; East Asia: 261; and elsewhere around the world: 73). Of these products 313 contain AgNPs. The annual production of AgNPs by 500 tons, and predicted this figure to raise in subsequent years. The widespread application of nanomaterials in recent years has risen a worldwide concern about its potential threat to the animals and aquatic organisms due to bioaccumulation, toxicity, non-degradable in environment, and bioavailability in the food chain (Salari Joo et al 2013; Wu & Zhou 2013; Wang & Wang 2014). Therefore, carrying out comprehensive studies in order to assess the levels of nanoparticles and their potential hazards to our aquatic environment are called for.

The uptake potential of nanoparticles by aquatic organisms is one of the most important factors in assessing the toxicity of nanoparticles. Uptake of nanomaterials in the body of aquatic organisms depends on several parameters such as NPs' size and shape, species, organs, dietary, environmental conditions, and exposure duration and concentration (Hao et al 2013; Salari Joo et al 2013; Tavana et al 2014). Zhao & Wang (2010) found more than 70% of AgNPs are accumulated in *Daphnia magna* through digestion of algae-associated AgNPs, suggesting the importance of dietary uptake route of NPs for bioaccumulation. Moreover, Salari Joo et al (2013) demonstrated that AgNPs uptake in organs of rainbow trout depends on salinity, exposure concentrations, and duration. In recently years, some studies on the trophic transfer potential of

nanoparticles in the food chain are condcted to a few reports on simplified food chains. Information about the potential of nanoparticles transferring from lower to higher trophic levels in food chain is little. Some study examples are zinc oxide nanoparticles transferring from artemia to goldfish (Ates et al 2014), aluminum oxide nanoparticles transferring from primary producer, *Chlorella ellipsoidea* to a primary consumer, *Ceriodaphnia dubia,* (Pakrashi et al 2014), zinc oxide nanoparticles transferring from *D. magna*, to zebrafish - *Danio rerio* (Skjolding et al 2014), and titanium dioxide nanoparticles transferring from daphnia to zebrafish (Zhu et al 2010).

In this study our aim was to investigate the potential of transferring silver nanoparticles in a simulated food chain. For this purpose, two aquatic species, brine shrimp (*Artemia salina*) and *D. rerio*, were chosen as model aquatic organisms. *A. salina* is a zooplanktonic crustacean selected as an invaluable organism for ecotoxicological examinations. *A. salina* is known for its adaptability to a wide range of salinity, high fecundity, small body size, short life cycle, high degree of tolerance to adverse environmental conditions such as temperature fluctuation, drought and changes in aeration (Ates et al 2013; Gambardella et al 2014; Libralato 2014). Moreover, this zooplankton is widely used as live food in the larviculture of aquatic organisms (Ates et al 2013). To our knowledge, this is the first study on the assessment of trophic transfer potential of AgNPs from *A. salina* to a fish under controlled condition. Therefore, equivalently, the aim of this study was to assess trophic transfer potential of AgNPs from *A. salina* to *D. rerio*.

Material and Method. This cross-sectional study was conducted at the Department of Health, Kurdistan University of Medical Scinences in July 2015. This study used a colloidal AgNPs (Nanocid®) which is commercially available in the Iran Market. For more information about this product readers are advices to refer to Johari et al (2013) and Salari Joo et al (2013). In this study, exposure analysis was carried out at two phases. In the first phase, one gram of *A. salina* cysts was hatched in 1000 mL conical flasks containing 800 mL 35 ‰ of water. Newly hatched nauplii were cultured in 500 L fiberglass tanks and fed up with *Nannochloropsis oculata* for three weeks when they reached adultness. Adult *A. salina* (300 individuals) were exposed to three concentrations of AgNPs colloids including 0.5, 1, and 2 mg L^{-1} as well as a control in triplicate. After 24 h exposure time to AgNPs, the *A. salina* were sampled from each group and washed out two times with deionized water to ensure complete removal of any extra AgNPs attached to the surface of body of *A. salina*. Samples were then digested using concentrated nitric acid (HNO_3). Finally, the Ag concentrations were measured using a Phoenix 886 flame furnace atomic absorption spectrophotometer.

In the second phase, the adult *D. rerio* (n = 120) were obtained from local aquarium shop, and prior to beginning of the experiments were acclimatized in 30 L tanks for a two weeks supplied with continuously aerated tap water under a 16 hr daylight and 8 hr darkness. Water changes were renewed every day. After this period, the *D. rerio* randomly assigned into three experimental (n = 90) and one control groups (n = 30) in triplicate. The *D. rerio* were fed with *A. salina* at a daily rate of approximately 5% of the fish wet weight. For the 14 days feeding trials, the *D. rerio* were fed daily with adult *A. salina* exposed to AgNPs at a concentration of 0.5, 1.0, and 2.0 mg L^{-1}. After 14 day feeding trials, the *D. rerio* were digested in concentrated nitric acid and the amount of accumulated Ag in fish's body was determined by Phoenix 886 flame furnace atomic absorption spectrophotometer.

Biomagnification factor of AgNPs from artemia to *D. rerio* was determined using Wang and Wang (2014):

Trophic transfer factor (TTF): TTF= Cfish/CArtemia

where Cfish (µg g^{-1}, dry wt.) is the Ag concentration in fish after 14-day chronic exposure and CArtemia (µg g^{-1}, dry wt.) is the Ag concentration in prepared artemia as fish food (Pakrashi et al 2014). According to this formula, a value higher than 1 is indicative of biomagnification trend of AgNPs from one trophic to the higher level in a food chain.

Results and Discussion. Bioaccumulations AgNPs in *A. salina* and *D. rerio* are shown in Table 1. The results of this study showed that the uptake of AgNPs was higher in *A. salina* than *D. rerio* ($p < 0.05$). Moreover, approximately uptake of AgNPs in *D. rerio* was dose dependent that higher uptake (0.312 mg L^{-1}) observed at higher concentration of AgNPs (2 mg L^{-1}), in contrast, this condition was not dose-dependent in *A. salina*.

Table 1

Relation between concentration of AgNPs in the water and silver accumulation in the body of *Artemia salina* following 24 h water exposure; and silver accumulation in the body of *Danio rerio* following 14 days feeding with the brine shrimps, 24 h exposed to AgNPs

Concentrations of AgNPs in water (mg L^{-1})	*Silver concentration in Artemia (mg kg^{-1})*	*BCF**	*Silver concentration in fish (mg kg^{-1})*	*BMF***
0 (Control Group)	1.16	-	6.1	-
0.5	117.4	234.8	21.3	0.181
1	108.1	108.1	20.5	0.189
2	93.1	46.55	29.1	0.312
P value	0.3		0.02	
P value***		0.05		

*Bioconcentration factor; **Biomagnification factor; *** Between two species.

One of the most significant and not well-understood risks of nanoparticles is their potential transfer and magnification in food webs (Klaine et al 2008; Handy et al 2008). The trophic transfer potential and the subsequent bioaccumulation and magnification in the food web are the key factors of environmental toxicity (Liu et al 2002). The bioconcentration factor (BCF) is used as the criteria for bioaccumulation in the context of identifying and classifying substances that are hazardous to the aquatic environment (Mcgeer et al 2003). The results of this study showed that the trophic transfer factor (BMF) of AgNPs was lower than 1, and this nanoparticle was not potential of trophic transfer from *A. salina* to *D. rerio*. In contrast, these results provide the first direct evidence that AgNPs can transfer from *A. salina* to *D. rerio* by dietary exposure. Zhu et al (2010) reported that no biomagnification of $nTiO_2$ was observed in this simplified food chain from daphnia (*Daphnia magna*) to *D. rerio* because the values of the BMF (0.024 and 0.009) were all less than 1. Moreover, study results of Holbrook et al (2008) showed that no biomagnification of Quantum dots (QDs) NPs in a simplified invertebrate food web including bacteria (*Escherichia coli*), ciliate (*Tetrahymena pyriformis*) and rotifer species (*Brachionus calyciflorus*). In contrast, results of Judy et al (2011) demonstrated that the gold NPs have biomagnification within a terrestrial food chain in primary producer to a primary consumer. One of the main reasons for the AgNPs do not biomagnify during food chain transfer is not soluble in fat or lipophilic substances. Because lipophilic pollutant as DDT has high ability to be biomagnify in food chain transfer and can be retained in organs for long time (Zhu et al 2010).

AgNPs are one of the most beneficial products of nanotechnology that is widely used in many different sections (Chen & Schluesener 2008). Moreover, this nanoparticle can be toxic on aquatic organisms as fish (Mansouri & Johari 2016; Johari et al 2013). According to our results, body burden of AgNPs was 3.1 to 5.5-times higher in *A. salina* than *D. rerio*. *A. salina* is a nonselective filter feeder, and it can readily ingest AgNPs from water, but the efficiency of particle capture depends on the particle size (Hund-Rinke & Simon 2006; McEdward 1995). The research results of Wang & Wang (2014) showed that the *A. salina* can well-accumulate the AgNPs as small as 105 nm. Moreover, Wang & Wang (2014) reported that the body burden of AgNPs in *A. salina* was higher than marine medaka (*Oryzias melastigma*) under controlled condition. In addition, the results of this study indicated that the *A. salina* and *D. rerio* as aquatic organisms can uptake NPs via aqueous exposure.

Conclusions. In this study, we evaluated the trophic transfer of silver nanoparticles from *Artemia salina*, crustacean filter feeder, to *Danio rerio* under controlled condition. This

work shows that the accumulation of AgNPs in *A. salina* was higher than in *D. rerio*. Moreover, our results demonstrate that the AgNPs was not potential of trophic transfer from *A. salina* to *D. rerio*.

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