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Heavy Metal Ions on Titanium Dioxide Nano-Particle: Biomagnification in an Experimental Aquatic Food Chain

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ABSTRACT: Heavy metal metals are non-biodegradable, have a remarkable ability to transfer through food chains and are potentially toxic for organisms.They are introduced tomarine environment via different anthropogenic sources. In this study, the ability of titanium dioxide nano-particle in transfer of Cr, Cu, Pb and Se metal through an aquatic food chain involving Ceratium tripos as the phytoplankton Daphnia hyaline as the zooplankton and Liza abu as the fish was investigated. The phytoplankton specie Ceratium tripos was exposed to 0, 0.2 and 0.5 of TiO_2. Subsequently, each species was fed to the zooplankton Daphnia hyaline, which was then used as food for the fish Liza abu. There were significant differences between the level of Cr, Pb and Se among the groups. The results showed that Cr and Se are biomagnified through the food chain. Whereas, Cu, which is essential element and could be regulated by organisms body, and Pb were not biomagnified through the chain.

Keywords: *Heavy Metal; Titanium dioxide; Nano-particle; Biomagnification; Food Chain*

INTRODUCTION

Some heavy metals are potentially harmful to most organisms at some level of exposure and some are essential for their biological mechanisms. Mercury, cadmium and arsenic are toxic elements that have poisonous properties (Munoz-Olivas and Camara, 2001; Tuzen, 2009). Previous studies have described that this kind of heavy metals have acute and chronic effects on different systems of body including central nervous system (Thronhill and Pemberton, 2003). Heavy metal naturally occur in the environment in very low levels, however their concentrations have increased due to anthropogenic contaminants over time. Industrial activities such as tankers traffic and petrochemical activities as well as mining and agriculture create a potential source of heavy metals in the marine environment (Mooraki *et al.*, 2009; Safahieh *et al.*, 2011; Abdolahpur Monikh *et al.*, 2012b; Hosseini *et al.*, 2013, 2015).

Titanium dioxide is produced either in the anatase or rutile crystal form. Most titanium dioxide in the anatase form is produced as a white powder. It may be coated with small amounts of alumina and silica to improve technological properties.Titanium dioxide is the most widely used white pigment in products such as paints, coatings, plastics, paper, inks, fibres, and food and cosmetics because of its brightness and high refractive index, which determines the degree of opacity that a material confers to the host matrix. When compared with bulk particles, nano-sized TiO_2 possess unique properties such as increased specific surface area, an increased number of surface activation sites, and therefore, high sorption capacity for other ions such as metals (Chena *et al.*, 2012).In

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previous studies, the adsorption of nano-sized TiO_2 with metals has been investigated. For example, in an investigation conducted by Engates and Shipley (2011), the adsorption of some heavy metals such as Pb, Cr, Cu, Zn, and Ni to nano-TiO_2 (anatase) and bulk TiO_2 (anatase) particles have been considered. Their results showed that nanoparticles had higher efficiency and faster rates of adsorption than bulk particles.Adsorption of metal ions onto nano-TiO_2 may not only increase the toxicity of this particle but also influence the mobility and bioavailability of toxic metals (Chena *et al*., 2012)

Bioaccumulation is the uptake and retention of a bioavailable metal from all possible external sources. For bioaccumulation to take place, the rate of uptake must be greater than the rate of loss of the metal from the organism's body. Aquatic organisms are able to bioaccumulate most bioavailable ions of heavy metals from their food that called trophic transfer (Dalman *et al*., 2006; Demirak *et al*., 2006; Abdolahpur Monikh *et al*., 2012a). The accumulation of a metal through successive trophic levels, which happens if it is not eliminated or excreted or controlled, is called biomagnification.

Among marine organisms, fish are at the top of the aquatic food chain and may accumulate large quantities of certain metals from water, food or sediment (Henry *et al*., 2004). However, different fish species have different capacity to accumulate heavy metal from their surrounding (Abdolahpur Monikh *et al*., 2012a, b). Previous studies from laboratory experiments indicated that accumulation of heavy metals in fish organs depends mainly on metal concentrations in water as well as exposure period (Ruangsomboon and Wongrat, 2006).

The main purpose of this study was to evaluate the ability of nano-sized titanium dioxide in transfer of heavy metals in an aquatic food chain including phytoplankton, zooplankton and fish species, in order todetermine their biomagnification in the chain.

MATERIALS AND METHODS

Ceratium tripos was streaked on agar plates and after a few days a colony of this phytoplankton species was selected and transferred to a liquid medium. The colony was immersed in an antibiotic solution (streptomycin and penicillin) for 48 hours. Subsequently, the species was transferred and cultured in a glass bottle with suitable medium under illumination of 300 µE m^{-2} s^{-1} at 23 ºC. An electric pump was applied to bubble air into the bottle. After two weeks the cells transferred to the laboratory and used in the accumulation experiments. The species were washed with deionized water and separated by centrifugation.

Commercially prepared titanium dioxide nanoparticles (anatase) from Sigma-Aldrich (St. Louis, MO, USA) were used in this study. All other solutions were prepared using analytical grade chemicals and Milli-Q element ultrapure water (18.2X, Millipore, Billerica, MA, USA).

Approximately ten gram wet weight of *C. tripos* was added to 5 treatmentsof 0, 0.2 and 0.5 µg/l nano-TiO_2solutions in conical flasks. The conical flasks were placed in a shaker for 48 hours. Centrifuge system was applied to separate the cells and the aqueous phase. The concentrations of the metals in the phytoplankton cells and water were measured. The concentration of Ti metal was not measured, because the level of uptaken metallic Ti is not within the aim of this study. Then the cells were used to feed *Daphnia hyaline* (zooplankton) in the next stage.

In order to culture *D. hyaline* an aquarium (1m × 1m × 1m) filled with deionized water was used. This aquarium was kept in room temperature under 12h light and 12 h dark period. After harvesting, 10 gram wet weight of adult *D. hyaline* were transferred to other aquariums (1m × 1m × 1m). Then *D. hyaline* of each aquarium was fed on *C. tripos* from each group. The *D. hyaline*was kept for 96 h and was harvested by filtration. The levels of heavy metals in *D. hyaline* were determined and this species was used to feed fish in the next stage.

Fish species, *L. abu*, with a body weight of 195-230 g and of mixed gender, were fed *D. hyaline* that had accumulated metals from the previous stage. At first, the fish were kept in holding tanks (10 fish in each tank) with a temperature of 20 ºC and fed on non-contaminated *D. hyaline* for a week to allow them acclimatize to laboratory conditions. A 12:12 h light/dark cycle was used and water of tanks was changed every 5 days. Every 12 h, the fish were fed *D. hyaline* at approximately 5% of their fresh body weight. After acclimation, on day 0 and 50, three *L. abu* were randomly chosen from each tank and killed by a blow on the head. Livers were dissected out, weighed and immediately stored at -20 ºC until use.

The concentrations of the studied element in the background solution for each step were determined before starting the experiment (Table 1). The samples, based on wet weight, were digested in mixture of 6 ml concentrated HNO_3 and 2 ml H_2O_2 in microwave digestion system. Digested samples were subsequently diluted in 10 ml deionized water. A graphite furnace atomic absorption spectrometry (SHIMADSO AA680) was employed to determine the levels of Cr, Pb, Seand Cu in the samples. Standard reference material DORM 2 (National Research Council of Canada: dogfish muscle) was used to check the accuracy and precision of analytical procedures. Percent recovery means was between 91% and112%. The statistical analysis was done using the one way ANOVA, followed by Duncan multiple comparison test. The significance level was set at a = 0.05.

RESULTS AND DISCUSSION

Titanium only reacts with water after its protective titanium oxide surface layer is destroyed. It is therefore water insoluble. Titanium compounds generally are not very water soluble. It means that the concentration of nano-TiO_2 related metals in the water of experiment is relatively near zero (Table 1). However, the structure of this particle can be completely destroyed under physiological activities of organism's body. This phenomenon can result in the release of compoundsof the particle surface, such as metal ions, in the organism's body.

The mean, standard deviation and comparison of heavy metal concentrations (µg g^{-1} w. w) among the selected species were given in Table 2, 3 and 4. According to these data, copper had the highest concentration, followed by lead, mercury and cadmium. As can be seen from the table, as the concentration of titanium dioxide nano-particle in the medium increase, the level of the metal rises significantly, except for Cu which showed no significant variation among the treatments. Its shows well that titanium dioxide can cause metal accumulation in the phytoplankton even though it is not soluble in water.

The concentrations of Cr, Se and Pb in control group (0) were below the limits of detection. Generally, Cu had the highest concentration and Cr the lowest level in the phytoplankton, zooplankton and fish samples.

Table 1: the concentration of Cr, Pb, Se and Cu in the background water before and after the experiment (µg/g)

Metal	Before	After	Variation (Sig)
Cr	Not detected	-	-
Pb	0.01 ± 0.002	0.01 ± 0.001	Not significant
Se	Not detected	-	-
Cu	0.13 ± 0.02	0.15 ± 0.01	Not significant

Table 2: Mean and standard deviation of metal (µg/g wet weight) in the phytoplankton species of different groups

Metal	Titanium dioxide (µg/l)		
	0	0.2	0.5
Cu	0.21 ± 0.01	0.18 ± 0.01	0.20 ± 0.01
Cr	ND[a]	ND[a]	0.02 ± 0.01[b]
Pb	ND[a]	0.04 ± 0.01[b]	0.16 ± 0.04[c]
Se	ND[a]	ND[a]	0.05 ± 0.02[b]

ND, Not detected. Different letters show significant differences of the metal concentrations between groups

Table 3: Mean and standard deviation of metal (µg/g wet weight) in the zooplanctonwhich was fed by phytoplancton of different groups

Metal	Titanium dioxide (µg/l)		
	0	0.2	0.5
Cu	0.86 ± 0.21	0.79 ± 0.36	0.81 ± 0.2
Cr	ND[a]	0.02 ± 0.01[a]	0.07 ± 0.01[b]
Pb	ND[a]	0.08 ± 0.02[b]	0.34 ± 0.06[c]
Se	ND[a]	0.04 ± 0.01[b]	0.08 ± 0.06[c]

ND, Not detected. Different letters show significant differences of the metal concentrations between groups

Table 4: Mean and standard deviation of metal (µg/g wet weight) in the liver of*Liza abu*which was fed by zooplankton of different groups

Metal	Titanium dioxide (µg/l)		
	0	0.2	0.5
Cu	34.62 ± 4.88	36.58 ± 2.16	34.93 ± 5.82
Cr	ND[a]	ND[a]	0.04 ± 0.03[b]
Pb	ND[a]	ND[a]	0.52 ± 0.23[b]
Se	ND[a]	ND[a]	0.12 ± 0.05[b]

ND, Not detected. Different letters show significant differences of the metal concentrations between groups

The concentrations of heavy metals in fish liver were determined at the beginning and the end of the experiment, day 0 and 50. The level of Cr, Pb and Se in the liver of the fish fed by zooplankton from control group (0) and second group (0.2) was below the detection limit.After 50 days of exposure, heavy metals concentrations in liver of fish were significantly higher than those in the control groups.

In general, different species showed different capacities for accumulating the metals. The comparison of Cr concentrations among the species indicated that the highest Cr levels in group 2 and 3 were found in *D. hyaline*.The distribution of Cr in group 2 and 3 follows the order: *D. hyaline*>*C. tripos* = *L. abu*. Whereas the distribution of Cu in the chain was completely different and follows the sequence: *L. abu*>*D. hyaline*>*C. tripos*. The concentration of Cu in *L. abu* of group 1 and 2 was significantly higher than *C. tripos* and *D. hyaline*.

Our results for Pb concentration in different trophic levels of the chain were surprising. The concentration of Pb in the fish of group 2was lower than those concentrations in the phytoplankton and zooplankton, while in the third group the distribution follows the order: *L. abu*>*D. hyaline*>*C. tripos*. The distribution of Se in group 2 follows the sequence: *D. hyaline* >*C. tripos* = *L. abu*, while in group 3 follows the order: *L. abu*>*D. hyaline*>*C. tripos*.

Recently, many researches have been conducted on the synthesis and characterization of TiO2 because of their novel properties such as unique shape, size confinement in radial-direction, large specific surface area and large pores volumes (Chena *et al*., 2012). Due to its high surface to volume ratio, it can carry some ionic metals in the environment. The adsorption of metal ions onto engineered nanoparticles such as TiO_2 is an important processfor treatment applications, their toxicity and their environmentalrisks. Hu and Shipley (2012) investigated the desorption of Pb (II), Cu (II) and Zn (II) from commercially prepared nano-TiO_2. This work showed that the affinity of metals to nano-TiO_2 surface sites is different and could result in different concentration of metal in the environment. However, there is still an question about its ability to carry metals through food chains, because, based on the condition of surrounding area and the ability of the surrounding environment to decompose this particle, it release different levels of metal in organism's body.

It is well known that magnification of heavy metals through food webs to top predators depends on various factors such as the length of food chains, the species that existed in the chains, the quantity of the metals and physicochemical factors (Castro *et al.*, 2002). However in experimental conditions where the environmental factors and the levels of metals are controlled, the length of the chain and the metabolisms of the organism's body play the main role in magnification process (Ruangsomboon and Wongrat, 2006).

Among the studied metals Cu was stable among the groups. Copper is an essential metal and is regulated by physiological mechanisms in most organisms (Eisler, 1988) especially biota situated in the middle to higher part of the food chain. Similar to our finding, atrophic level-dependent accumulation of Cu was not reported in Mekong Delta marine food web (Ikemoto *et al*., 2008).

Cadmium is a non-essential and potentially toxic element (Munoz-Olivas and Camara, 2001). This metal was not biomagnified through the food chain in current study. First, it should be considered that organisms in different groups had different accumulation mechanisms for metals (Abdolahpur Monikh *et al*., 2012a). Second, the mechanisms of detoxification and metal elimination are various among the organisms (Al-Yousuf *et al*., 2000). Fish have a complicated and developed detoxification system and canreduce the concentration of toxic metals to certain level. However, previous studies have reported that phytoplankton have the ability to accumulate Cr metal to high concentration (Ruangsomboon and Wongrat, 2006). This can explain the trend observed for the distribution of Cr among the species. Barwick and Maher (2003) studied metal concentration in a food web in NSW Australia and found that there is no evidence of biomagnification of Cr in the food web. On the other hand, Campbell *et al.* (2005) studied the biomagnification of heavy metals in an Arctic marine food web from Baffin Bay. According to those results, Cr has the ability to biomagnified through the food chains. Yoshinaga *et al.* (1992) reported biomagnification of Cr in anaquatic food chain from Papua New Guinea. However, this study is, to our knowledge,

The first considered the ability of TiO_2 in metal transfer through aquatic food chain. Lead, one of the most toxic elements in the present study, was not biomagnified in the second group, while showed a direct biomagnifications in the third group. The results showed that the Pb accumulation in the species in group 3 was 4 to 5-fold higher than those in group 2. Campbell *et al.* (2005) concluded that Pb has the ability to biomagnified through aquatic food chains, and the same trends were also reported by Yoshinaga *et al.* (1992) in food webs from Papua New Guinea. Ruelas-Inzunza and Paez-Osuna (2006)reported a weak biomagnifications of Pb in a food web from Altata-Ensenada lagoon, SE Gulf Of California. Barwick and Maher (2003) also found the same results for a food web from NSW Australia. In contrast, Dietz *et al.* (2000) reported that Pb does not accumulate toward higher trophic levels in the marine ecosystem.

There are various reports about the ability of Se to be biomagnificated in aquatic food chains; however, there is no results report the efficiency of TiO_2 to carry Se through food chains. In this study, we observed that Se have higher concentration in zooplankton than phytoplankton and in fish than the other species. It means that it can be carried up by TiO_2 nano-particles in food chains; however its concentration, to high extent, based on the detoxification system of the organisms and other physiological activities.

CONCLUSION

Summarizing the finding from this study,titanium dioxide nano-particle has the ability to carry up the metal through food chain. Except for Cu, which is essential element and its high concentration in the organisms was expected,the concentration of Cr andSe increases toward higher trophic levels. The smoother trend observed between transfer of the metals from zooplankton to fish, in comparison to phytoplankton to zooplankton, could be attributed to the developed detoxification system in fish relative to the other organisms.

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