Ecotoxicology and Environmental Safety 128 (2016) 213–221

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effect of subcellular distribution on nC_{60} uptake and transfer efficiency from *Scenedesmus obliquus* to *Daphnia magna*

Qiqing Chen [a,b], Xialin Hu [a,b], Daqiang Yin [a,b,*], Rui Wang [a,b]

[a] Key Laboratory of Yangtze River Water Environment, Ministry of Education, College of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Shanghai 200092, China

[b] State Key Laboratory of Pollution Control and Resources Reuse, College of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Shanghai 200092, China

ARTICLE INFO

Article history:
Received 26 November 2015
Received in revised form
21 February 2016
Accepted 24 February 2016
Available online 3 March 2016

Keywords:
Fullerene
Subcellular fraction
Uptake
Trophic transfer
Humic acid

ABSTRACT

The potential uptake and trophic transfer ability of nanoparticles (NPs) in aquatic organisms have not been well understood yet. There has been an increasing awareness of the subcellular fate of NPs in organisms, but how the subcellular distribution of NPs subsequently affects the trophic transfer to predator remains to be answered. In the present study, the food chain from *Scenedesmus obliquus* to *Daphnia magna* was established to simulate the trophic transfer of fullerene aqueous suspension (nC_{60}). The nC_{60} contaminated algae were separated into three fractions: cell wall (CW), cell organelle (CO), and cell membrane (CM) fractions, and we investigated the nC_{60} uptake amounts and trophic transfer efficiency to the predator through dietary exposure to algae or algal subcellular fractions. The nC_{60} distribution in CW fraction of *S. obliquus* was the highest, following by CO and CM fractions. nC_{60} uptake amounts in *D. magna* were found to be mainly relative to the NPs' distribution in CW fraction and daphnia uptake ability from CW fraction, whereas the nC_{60} trophic transfer efficiency (TE) were mainly in accordance with the transfer ability of NPs from the CO fraction. CW fed group possessed the highest uptake amount, followed by CO and CM fed groups, but the presence of humic acid (HA) significantly decreased the nC_{60} uptake from CW fed group. The CO fed groups acquired high TE values for nC_{60}, while CM fed groups had low TE values. Moreover, even though CW fed group had a high TE value; it decreased significantly with the presence of HA. This study contributes to the understanding of fullerene NPs' dietary exposure to aquatic organisms, suggesting that NPs in different food forms are not necessarily equally trophically available to the predator.

1. Introduction

The fullerene family, especially fullerene (C_{60}), has delighted the scientific community during the last thirty years with their unique properties and wide applications. Especially, the synthesis of fullerene aqueous suspension (nC_{60}), has allowed their use in various biological and biomedical fields. Earlier studies demonstrated that nC_{60} could be accumulated by aquatic organisms through aqueous exposure (Oberdorster et al., 2006; Tao et al., 2009). Wang et al. (2012) also reported that C_{60} will be ultimately partitioned into bio-components and pose potential ecological risks. The accumulation and intracellular distribution in prey will influence their trophic availability to their predators. The significance of nanoparticles (NPs) dietary ingestion as a predominant way for accumulation and assimilation in aquatic organisms has been well documented (Bouldin et al., 2008; Skjolding et al., 2014; Zhu et al., 2010). NPs accumulated in prey can also be transferred and potentially cause secondary toxic effects in their predators, such as causing alterations in the feeding behavior (Pakrashi et al., 2014). Subcellular partitioning of contaminants in prey is suspected to be a strong indicator of their trophic availability to a predator (Cheung and Wang, 2005; Goto and Wallace, 2009; Wallace and Luoma, 2003). Scholars have made a deep study on metals in this aspect. They have traced the metals' subcellular distribution in aquatic organisms (Pan and Wang, 2008; Rosabal et al., 2015), and found that the soluble metals distributed in organelles were considered to be more trophically available than those in insoluble fractions (Dang and Wang, 2010; Zhang and Wang, 2006). Also, Chang and Reinfelder (2000) concluded that metals associated with cytoplasm of phytoplankton cells were more easily to be assimilated by herbivorous consumers than metals adsorbed on cell membranes. Nevertheless, most of the above work has been done using metals, with

* Corresponding author at: Key Laboratory of Yangtze River Water Environment, Ministry of Education, College of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Shanghai 200092, China.
E-mail address: yindq@tongji.edu.cn (D. Yin).

http://dx.doi.org/10.1016/j.ecoenv.2016.02.026
0147-6513/

few studies on NPs. Moreover, even though people found that the subcellular distribution can affect the contaminants' trophic transfer availability, according to our knowledge, there is no research focusing on the actual transfer ability of NPs from different subcellular fractions to the predator yet, which could indicate the transfer efficiency more directly.

Several studies have shown that the form of the contaminants within the prey can influence the assimilation by consumers. The relevance of contaminant subcellular distribution within prey to their trophic transfer availabilities has been widely strengthened (Wallace et al., 1998; Zhang and Wang, 2006). As Wallace and Zhang have proved that metals bound to organelles and cytosolic proteins are readily solubilized by the digestive processes of a predator (Wallace et al., 1998), and will be more available to predator than those with other components, and the metals associated with organelles were compartmentalized as "trophically available". Even though the partitioning varies with species, animal size, these trophically available metals are similarly influenced (Wallace and Luoma, 2003). In contrast, metals partitioned to insoluble components such as metal-rich granules within prey are not readily available to many predators, as most granules are not easily solubilized by the digestive processes (Goto and Wallace, 2009). Nevertheless, the relationship between subcellular distribution and trophic availability of NP remains limitedly reported and needs to be explored further. Moreover, NPs are able to enter into the cell through endocytosis or diffusion across the cell membrane in case of small particle size and positive ions on the surface (Navarro et al., 2008; Verma et al., 2008). Aggregated NPs can absorb onto algal cells (Sadiq et al., 2011) and NPs can pass through the semipermeable barrier (Navarro et al., 2008). Once inside the cell, NPs would interact with organelles such as mitochondria (Yang et al., 2009; Zhang and Gutterman, 2007).

Natural organic matter may influence the bioavailability of NPs to predators, and is therefore deserving of study. Several studies have focused on the effect of natural organic matter (NOM) on the physicochemical properties of C_{60}. For example, the solubilization of C_{60} could be increased with the presence of either fulvic acid (FA) or humic acid (HA) (Terashima and Nagao, 2007). NOM could effectively increase the stability of nC_{60} (Chen and Elimelech, 2007), and decrease the NPs' size (Xie et al., 2008). Pakarinen et al. (2013) have also evaluated nC_{60} water stability with NOM from different lakes, and indicated that increasing NOM molecular sizes with high aromatic content can enhance nC_{60} water stability, and subsequently influence nC_{60} uptake in *D. magna*. Moreover, NOM could increase the release of C_{60} from living systems (e.g., biosolids) (Navarro et al., 2013; Xie et al., 2008), due to the lowered particle size and enhanced dispersion (Mashayekhi et al., 2012). Therefore, the presence of natural organic matter may induce alterations in the NP intracellular distribution in prey, relating to nC_{60} assimilation by a predator.

In the present study, we hypothesized that nC_{60} NPs bound to different subcellular fractions could correspondingly differ in bioavailability, and we will determine the subcellular partitioning of nC_{60} within prey (*Scenedesmus obliquus*) and the influence of subcellular forms on the trophic transfer ability to the predator (*Daphnia magna*). The results will be used to understand the nC_{60} NPs uptake and the potential trophic transfer efficiency when assessing ecological impacts associated with nC_{60} pollution.

2. Material and methods

2.1. Chemicals and organism culture

Fullerene (C_{60}, 99.9% pure) was purchased from Yongxin Sci & Tech Co., China. The nC_{60} stock suspension was prepared by the toluene-water exchange method as described in our previous study (Chen et al., 2014). Briefly, 200 mL of 1 g/L C_{60} toluene solution, 500 mL water, and 15 mL ethanol were mixed, and then stirring in ultrasonic bath at room temperature until all toluene evaporated. High performance liquid chromatography (1200, Agilent, USA) and inductively coupled plasma mass spectrometry (7700, Agilent, USA) were used to trace possible toluene residue and metals in the nC_{60} stock suspensions (Supplementary Information Table S1). The humic acid stock solution was prepared by introducing 100 mg of the dry humic acid sodium salt powder (Sigma-Aldrich, USA) into water. After complete dissolution, the black solution was filtrated through 0.45 μm cellulose membranes (Xingya Purification Material, China). Next, the filtrate was introduced into a volumetric flask, as stock HA solution. Through high-temperature oxidation, the total organic carbon (TOC) content of the stock HA solution was determined to be 348.6 mg/L by a TOC analyzer (V-CPN, Shimadzu Co., Japan). All other chemicals used throughout experiments were analytical grade.

The work suspension of nC_{60} was diluted by Brostol's solution (which is commonly termed as SE medium) (Atlas, 2004). The nC_{60}-HA work suspension was obtained by mixing 2 mg/L nC_{60} and 5 mg/L HA (described as dissolved organic carbon content) into SE medium with a glass rod, and the suspension was prepared 24 h before characterization. Both nC_{60} and nC_{60}-HA suspensions were very stable, which have been verified by monitoring the absorbance at 335 nm using a UV–vis spectrophotometer (Evolution 201, Thermo Scientific, USA).

Scenedesmus obliquus was obtained from the Institute of Hydrobiology (Chinese Academy of Sciences, China). The algae were cultured in SE medium, and the density of algae used for test is 10^6 cells/mL. *Daphnia magna* was cultured in the artificial fresh water (contents of macro-ions: 55.8 mg/L $CaCl_2 \cdot 2H_2O$, 24.7 mg/L $MgSO_4 \cdot 2H_2O$, 13.0 mg/L $NaHCO_3$, and 1.2 mg/L KCl) at a density of 1 individual/10 mL and fed with the green algae *S. obliquus* at a cell density of 10^5 cells/mL every day. In all trophic transfer experiments, the daphnids used were 7-d-old adults. A group of daphnia was used for the length and weight determination. A sub-group of daphnia (20 daphnids) was captured under microscope (SZX16, Olympus, Japan), and the length from head to capillus at the end was measured by a rule for each daphnid from the picture. Finally, the actual daphnid length was calculated according to the scale. Next, these daphnids were carefully dried with blotting paper and weighed their weight on a microbalance (Tervonen et al., 2010) and their length was measured by a rule. The average length was measured to be 2.0 ± 0.2 mm, and the wet weight was 12–15 mg/20 daphnids. Specific conditions of 23 ± 2 °C temperature and a light: dark cycle of 14 h: 10 h using fluorescent light at 3000 lx were maintained for both organisms.

2.2. Preparation of nC_{60} samples for characterization

Both nC_{60} and nC_{60}-HA work suspensions in SE medium were characterized by transmission electron microscope (TEM, H-7500, Hitachi, Japan) and Zetasizer (Malvern Instrument, UK). Also, the nC_{60} treated *S. obliquus* and algal subcellular fractions were collected, purified, and fixed in glutaraldehyde followed by repeated washing. The algal or daphnia samples were fixed in 2% glutaraldehyde, and the algal cells were centrifuged to obtain cell pellet. Then, specimens were rinsed in 0.1 M sodiumcacodylate and postfixed with 2% OsO_4, which contains 1.5% $K_3Fe(CN)_6$ for 1 h. After rinsing with pure water, a series of alcohol was used to dehydrate the specimens, and they were finally put in 100% alcohol and Epon 1:1 overnight. Next, the specimens were embedded in pure Epon, and cut into ultrathin sections by microtome for TEM observation (JEM-2010, JEOL, Japan).