Enhanced bioaccumulation of cadmium in carp in the presence of titanium dioxide nanoparticles

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Abstract

In this study adsorption of Cd onto TiO2 nanoparticles and natural sediment particles (SP) were studied and the facilitated transports of Cd into carp by TiO2 nanoparticles and SP were assessed by bioaccumulation tests exposing carp (Cyprinus carpio) to Cd contaminated water in the presence of TiO2 and SP respectively. The results show that TiO2 nanoparticles had a significantly stronger adsorption capacity for Cd than SP. The presence of SP did not have significant influence on the accumulation of Cd in carp during the 25 d of exposure. However, the presence of TiO2 nanoparticles greatly enhanced the accumulation of Cd in carp. After 25 d of exposure Cd concentration in carp increased by 146%, and the value was 22.3 and 9.07 \( \mu \text{g/g} \), respectively. And there is a positive correlation between Cd and TiO2 concentration. Considerable Cd and TiO2 accumulated in viscera and gills of the fish.

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1. Introduction

Nanometer-scale materials possibly exhibit different physical, chemical, and biological properties that may not be predictable from observations on larger-sized materials extensive researches on the applications of nanomaterials have been carried out recently (Kong et al., 2000; Králik and Biffis, 2001; Long and Yang, 2001; Kipp, 2004). As common nanomaterials, TiO2 nanoparticles are now becoming increasingly popular. The opacity and whitening properties of TiO2 nanoparticles have made this pigment desirable for a variety of industrial applications, including the manufacture of paints, textiles, papers, plastics, sunscreens, cosmetics, and food products (Levine et al., 2003). For environmental applications, suspended TiO2 nanoparticles have been largely used as efficient catalyst for the decomposition of organic contaminants present in water and aqueous wastes (Centi et al., 2002; Pirkanniemi and Sillanpää, 2002). Due to their widespread use, potential occupational exposure to TiO2 nanoparticles is of concern. This concern has fueled several investigations to evaluate the potential health consequences associated with chronic inhalation of TiO2 nanoparticles. Numerous epidemiological, toxicological, and medical case studies related to TiO2 exposure are described in the literature (Chen and Fayerweather, 1988; Driscoll et al., 1991; Warheit et al., 1997). Although bulk TiO2 has generally been regarded as a compound of low toxicity, recent researches now show that when normally harmless bulk materials are made into nanoparticles they tend to become toxic (Howard and Maynard, 1999). The size effect is considerably more important to nanoparticles toxicity than the actual composition of the material (Donaldson et al., 2000; Öberdörster, 2000; Gumbleton, 2001; Tinkle et al., 2003). The study of Tan et al. (1996) show that TiO2 nanoparticles used in sunscreen, can get deep enough into the skin to be taken up into the lymphatic
system, while larger particles (greater than 1 µm in diameter) can not.

Furthermore, in the near future the release of TiO$_2$ nanoparticles into aquatic systems is inevitable as more and more industrial applications are found for this nanomaterial. However, little is currently known about the transfer and fate of nanosized materials and as well as their potential influence on the transfer and fate of other coexisting pollutant once they enter the aquatic environment. Contamination with heavy metal in natural environments has always been a great concern because they are toxic and nonbiodegradable. The colloid-facilitated transport of heavy metal in the environment (McCarthy and Zachara, 1989; Amrhein and nonbiodegradable. The colloidal-facilitated transport of heavy metal contaminants has been implicated in a number of studies (McCarthy and Zachara, 1989; Amrhein et al., 1993; Grohmann et al., 1996; Honeyman, 1999). The large surface area, crystalline structure, and reactivity of some nanoparticles may facilitate transport of the toxic pollutants in the environment (Zhang and Masciangioli, 2003). However, there is little to no data indicating how and to what extent the emerging nanoparticles may facilitate the transport of heavy metal in the environment.

In this study, the adsorption of Cd onto TiO$_2$ nanoparticles and the potential of TiO$_2$ nanoparticles to facilitate the transport of Cd into carp (Cyprinus carpio) were examined. To compare the different facilitated transport abilities between TiO$_2$ nanoparticles and natural particles, bioaccumulation of Cd in the presence of natural sediment particles (SP) sieved through 400 meshes were studied.

2. Materials and methods

2.1. Reagents and solutions

All reagents used were of analytical-reagent grade except for acids, which were of trace metal analysis grade. MiniQ water was used for preparation of stock solution. Laboratory equipment and containers were dipped in 25% (v/v) HNO$_3$ solution for at least 12 h prior to each use. Cd stock solution (1000 mg/l) was prepared by dissolving 1.000 g Cd metal in 5 ml HNO$_3$ (1 + 1) and then diluted to 1 l. Further working solution were freshly prepared from the stock solution for each experimental run. The stock solution for hydride generation was 0.4% (w/v) NaBH$_4$ dissolved in 0.5% (w/v) NaOH solution, which was prepared immediately prior to use.

Surficial sediment was collected from a non-contaminated reservoir. After collection the sample was dried and sieved through 400 meshes. Degussa P25 TiO$_2$, with an average BET surface area of 50 m$^2$/g and an average particle size of 21 nm, was used for all experiments. Standard titanium (IV) stock solution (1.0 g/l) was prepared by heating 0.4170 g TiO$_2$ nanoparticles in 30 ml sulphuric acid–ammonium sulphate solution (400 g ammonium sulphate in 700 ml concentrated sulphuric acid) and finally diluted to 250 ml with MiniQ water. Low-concentration standards were prepared daily by diluting of the stock solution with sulphuric acid (10% v/v).

2.2. Instrumentation

Particle size distribution of SP was analyzed using a laser particle analyzer (Mastersizer, 2000, Malvern). Zeta potential of SP and P25 were determined using a zeta potential analyzer (Zetasizer 3000, Malvern). A microwave digestion system with temperature and pressure control (WX-3000 plus, EU Chemical Instruments Co., Ltd, Shanghai, China) was used for sample digestion. An ICP-OES (IRIS Intrepid II, Thermo Electron) was used to determine titanium concentration in digested samples. Cd was measured using an atomic fluorescence spectrometry equipped with hydride generation (HG-AFS 2201, Haiguang Co., Beijing, China).

2.3. Adsorption of Cd onto TiO$_2$ nanoparticles and SP

Sorption kinetics and isotherm were performed using batch experiments. Dechlorinated tap water was used in the experiment in order to keep the similar water circumstances with those in the bioaccumulation tests. In each experiment, 100 ml 10 mg/l suspension of TiO$_2$ or SP were prepared and added to a series of 250 ml Pyrex glass Erlenmeyer flasks. Required amount of Cd standard solution was added to initiate the adsorption. The flasks were shaken at 150 rpm in a reciprocating shaker and kept in dark at 25 ± 1°C. Kinetic data were collected with a nominal initial Cd concentration of 100 µg/l. Then 10 ml of the suspensions were taken out and centrifuged twice for 10 min at 12000 rpm using a high speed centrifuge (Hermle Z323, Germany) at 0, 10, 20, 30, 60, 120, 180, and 360 min, and residual aqueous Cd concentrations were analyzed. Adsorption isotherms were studied by varying initial Cd concentration (10, 25, 50, 75 and 100 µg/l) under a fixed TiO$_2$ or SP suspensions of 10 mg/l. Isotherm tests were conducted for 2 h and then residual aqueous Cd concentration was analyzed. The amount of Cd adsorbed was calculated by mass balance between the initial and final solution concentrations. In order to correct for any loss of Cd due to adsorption to the containers, control experiments were carried out without the adsorbent and there was negligible adsorption by the container walls (<5%).

2.4. Accumulation experiment

A group of carp (Cyprinus carpio) was purchased from a local pet shop. The initial body weight and length of the fish were 6.1 ± 2.2 g and 4.0 ± 0.7 cm respectively. All fish were acclimatized in dechlorinated tap water with a natural light/dark cycle for ten days before experiment. For the accumulation tests, 16.0 ml 100 mg/l Cd solutions were added into three glass tanks containing 161 dechlorinated tap water respectively, and the initial concentration of Cd was 97.3 ± 6.9 µg/l. In two of the above three tanks, 0.160 g TiO$_2$ nanoparticles and 0.160 g SP were added respectively. Two h later, 30 carp were placed into the
and 4.0 ml concentrated HNO₃ was added into each of 6 ground into powder. Approximately 0.20 g dried sample was achieved. Then the dried fish were dissected into skin and scales, muscle, gills, and viscera. After pretreatment, Cd and TiO₂ concentrations in carp or different parts of the four treatments were not available, laboratory controls were prepared. Fish samples were pooled together and mechanically homogenized to form a uniform control tissue matrix. The pH in the exposure water was 7.8. A control test without the contaminants was conducted under the same conditions. Three fish were removed and sacrificed at 2, 5, 10, 15, 20 and 25th day, and on the 20th day, 6 carp from each of the four treatments were dissected into skin and scales, muscle, gills, and viscera. After pretreatment, Cd and TiO₂ concentrations in carp or different parts of carp were analyzed respectively.

2.5. Analytical procedures

Determination of dissolved Cd in water. After centrifugation, 1.0 ml concentrated HCl, 5.0 ml thiourea solution (5%) and 0.5 ml CoCl₂ solution (100 mg/l) were added to 2.0 ml of the supernatant. Then the solution was transferred quantitatively to a 50-ml volumetric flask by MiniQ water and Cd concentrations were measured by HG-AFS. The following instrumental parameters were used: current of lamp 40 mA; high voltage of PMT 300; carrier solution 1% v/v hydrochloric acid; carrier gas (Ar) flow rate 0.3 l/min; shielded Sheath (Ar) flow rate 0.5 min/l; peristaltic pump rate 130 rpm; and observation height 8 mm.

Cd and TiO₂ in fish analysis. After rinsed with dechlorinated tap water, the fish were dried at 105 °C until a constant weight was achieved. Then the dried fish were ground into powder. Approximately 0.20 g dried sample and 4.0 ml concentrated HNO₃ was added into each of 6 PTFE digestion tubes. After 10 min, the vessels were sealed and put in the microwave. Then the samples were digested using a three-stage digestion protocol (5 min at 150 °C, 5 min at 180 °C and 5 min at 190 °C). Afterwards the vessel was cooled down. Filtration of the samples was not required since the dissolution was complete.

For Cd analysis, the excess acid was removed from the digested solution by heating the digested solution to near dryness at 90 °C using an electric furnace. After pretreatment as mentioned above, Cd was determined. Triplicate analyses were performed for each sample. The accuracy of the measurement was tested by the analysis of a certified reference material, GBW 08571 (mussel sample, National Research Center for CRM’s, Beijing), which has a certified value of 4.5 ± 0.5 µg/g. The value obtained was 4.3 ± 0.3 µg/g.

For TiO₂ analysis, the digests were transferred to triangular flasks and evaporated to dryness. TiO₂ nanoparticles released by digestion were decomposed into titanium (IV) ion by heating with 5 ml of the sulphuric acid – ammonium sulphate solution. After cooling down, the above solution was transferred quantitatively to a 25 ml volumetric flask. TiO₂ concentration in digested samples was determined by ICP-OES. The instrumental parameters were: RF power 1150 W; Nebulizer pressure 15.2 MPa; carrier gas (Ar) flow rate 0.5 l/min; peristaltic pump rate 130 rpm; Integration time High WL Range 5 s, Low WL Range 30 s; and wavelength 336.12 nm.

Several quality control (QC) measures were taken during the analysis of samples. Firstly, the calibration blank and a calibration standard (10.0 mg/l) were run before and after each group of 5 samples analyzed. The determined concentration in the standard was required to be within 5% of its nominal concentration for the analysis of bracketed samples to be considered valid. Secondly, a calibration check standard was also prepared at a concentration within the calibration range using a titanium stock solution purchased from another vendor (GSB G 65014-90 (2001), Central Iron & Steel Research Institute, Beijing). This check was then analyzed as a sample to verify analyte concentration and instrument calibration. Thirdly, triplicate digestions and analyses were performed for each sample.

Because biological standard reference materials containing TiO₂ were not available, laboratory controls were prepared. Fish samples were pooled together and mechanically homogenized to form a uniform control tissue matrix. About 0.20 g fish simple aliquots were transferred directly into each of six acid cleaned PTFE digestion tubes. Four of the matrix aliquots were fortified with 0.020 g TiO₂ nanoparticles, while the remaining 2 were not fortified and served as matrix blanks. These samples were digested, evaporated, decomposed and analyzed as described above. The TiO₂ recovery in these samples ranged from 90% to 105%.

2.6. Statistical analysis

For both Cd and TiO₂ concentrations, the mean values were calculated from the three replicates and expressed with standard deviation (n = 3). The homogeneity of variance was checked out and a one-way analysis of variance (ANOVA) was then performed to assess the significance of differences observed between Cd concentrations in fish exposed to Cd, Cd + TiO₂ nanoparticles and Cd + SP. All statistical analyses were conducted at a significance level of 0.05.

3. Results and discussion

3.1. Adsorption characteristics of Cd on TiO₂ nanoparticles and SP

Sorption kinetics was observed for six h and the results are presented in Fig. 1. The adsorption process of Cd onto TiO₂ nanoparticles and SP were fast, reaching equilibrium within 30 min. After equilibrium, the amount of Cd adsorbed by TiO₂ nanoparticles was approximately 65%, which is four times higher than that adsorbed onto SP.
(12%), indicating that TiO$_2$ nanoparticles has a stronger adsorption capability for Cd than SP.

Isotherm was determined using 2 h of equilibrium time. Experimental data of Cd adsorption onto TiO$_2$ nanoparticles and SP fit Freundlich isotherm well and the correlation coefficients were 0.959 and 0.956. The plots are shown in Fig. 2. The constants $K_F$ and $n$ were found to be 250 mg/g and 0.962 for TiO$_2$ nanoparticles and 23.5 mg/g and 0.856 for SP. The average particle size of Degussa P25 nanoparticles is 21 nm, which is much smaller than that of SP (19 µm), thus the specific surface area of Degussa P25 nanoparticles is much larger than that of SP, and the value is 50 and 30 m$^2$/g respectively. The small particle size and large specific surface area of P25 TiO$_2$ nanoparticles may account for their stronger adsorption ability. Furthermore, besides the small particles size and large surface area, the electrostatic attraction also account for their stronger adsorption ability of P25 TiO$_2$ nanoparticles (Nguyen et al., 2003). The zeta potential of a TiO$_2$ suspension at the experimental pH (8.2) was found to be about $-24.2$ mV. At this level of pH, Cd remains as Cd$^{2+}$ cations in the solution. Cd could be readily adsorbed onto the catalyst surface due to electrostatic attraction. However, the zeta potential of SP at this level of pH was less negative ($\zeta = -13.4$ mV).

3.2. The enhanced accumulation of Cd in carp in the presence of TiO$_2$

When TiO$_2$ nanoparticles and SP were added into the tanks, dissolved Cd concentration decreased due to the adsorption onto the particles. As a result, dissolved Cd concentrations were 34.4 ± 4.8 µg/l for the tank in the presence of TiO$_2$ and 81.3 ± 7.3 µg/l for the tank in the presence of SP. For the tank spiked with Cd only, the soluble Cd concentration were 97.3 ± 6.9 µg/l. TiO$_2$ concentrations were 10.0 ± 1.3 mg/l when TiO$_2$ nanoparticles were added into the tank.

The accumulations of Cd in carp exposed to Cd, Cd + TiO$_2$ nanoparticles and Cd + SP as a function of exposure time are shown in Fig. 3. In the figure, the accumulation of Cd was described using standard exponential equation as follows (Pendleton et al., 1995):

$$C_t = A \cdot (1 - e^{-Bt})$$  \hspace{1cm} (1)

where $C_t$ is the Cd concentration in whole fish (µg/g dry weight), $A$ is the Cd concentration at equilibrium (µg/g dry weight), $B$ is the first-order rate constant (d$^{-1}$), which give an insight at how rapidly the element is accumulated, and $t$ is the exposure time (d).

Cd concentration in carp of control was undetected. Cd concentrations in carp exposed to Cd contaminated water increased gradually and after 25 d of exposure it reached 9.07 µg/g. When exposed to Cd-contaminated water in
the presence of TiO$_2$ nanoparticles, the carp accumulated considerably more Cd. As can be seen from Fig. 3, Cd concentration in the carp increased sharply, and reached 22.3 µg/g at the 25th day, which increased by 146% than that without TiO$_2$ nanoparticles, suggesting that the presence of TiO$_2$ nanoparticles greatly enhanced the accumulation of Cd in carp. However, Cd concentrations in carp exposed to Cd + SP increased slowly and it was not so much different from those in carp exposed to Cd only. The presence of SP did not have much influence on the accumulation of Cd in carp during the 25 d of exposure.

The regression analysis results of the experimental data using Eq. (1) are presented in Table 1. Cd concentration in carp at equilibrium (A value) in the presence of SP is a little higher than that exposed to Cd only. However, in the presence of TiO$_2$ nanoparticles, carp accumulated more Cd, A value is 3-fold higher than that exposed to Cd without TiO$_2$ nanoparticles.

TiO$_2$ concentration in carp was analyzed simultaneously and the results are given in Fig. 4. It can be seen from Fig. 4 that strong accumulation of TiO$_2$ nanoparticles in carp was observed. TiO$_2$ concentration in carp reached 3.39 mg/g on the 25th day. Experimental data of TiO$_2$ nanoparticles accumulation in carp gave a better fit using the exponential equation ($R^2 = 0.971$), where $A$ and $B$ is 11.4 µg/g and 0.0152 d$^{-1}$, respectively.

Cd concentration in carp comes from the accumulation of soluble Cd ions and TiO$_2$ nanoparticle bound Cd, which can be calculated from the following equation:

$$C_t = k \times C_Ti + C_d$$  \hspace{1cm} (2)

where $C_t$ is the Cd concentration in carp (µg/g dry weight), $C_Ti$ is the TiO$_2$ concentration in carp (mg/g dry weight), $k$ is facilitated transport coefficient, which gives an insight of the facilitated transport ability of TiO$_2$ nanoparticles, and $C_d$ is Cd concentration accumulated as dissolved Cd ions (µg/g dry weight) during the exposure period (d).

In our study, a positive correlation between Cd concentration ($C_t$) and TiO$_2$ concentration ($C_Ti$) during the exposure period existed with a correlation coefficient ($R$) greater than 0.975, and the regression equation is $C_t = 6.45C_Ti + 1.74$. Facilitated transport coefficient $k$ is 6.45, which is very close to the amount of Cd adsorbed onto TiO$_2$ in water ($\chi/m$), where the value is 6.29, indicating that Cd can be adsorbed onto TiO$_2$ nanoparticles and accumulated into carp with the accumulation of TiO$_2$ nanoparticles.

Grolimund et al. (1996) demonstrated that suspended in situ mobilized colloids can provide a pathway for rapid transport of Pb. Flury et al. (2002) found that colloids mobilized in flow experiments with packed sediments carried Cs along. In another study, Maia et al. (2000) reported the great potential of solid lipid nanoparticles (SLN) to improve drug absorption by the skin. In their study, penetration of prednicarbate incorporated into SLN into human skin increased by 30% as compared to prednicarbate cream. This study provides evidence of facilitated bioaccumulation of the toxic contaminant by TiO$_2$ nanoparticles in the aquatic organisms. TiO$_2$ nanoparticles have a strong adsorption capacity for Cd and accumulated in carp fast, so carp accumulated much more Cd in the presence of TiO$_2$ nanoparticles due to the facilitated transport. Hence, research should not be addressed only on the fate and toxicity of nanoparticles themselves, but also to the potential of the facilitated transport of other trace toxic pollutants when they co-exist, which is a significant step in better understanding of the potential exposure risks that nanoparticles might cause.

<table>
<thead>
<tr>
<th>Exposed media</th>
<th>$A$ (µg/g)</th>
<th>$B$ (d$^{-1}$)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>6.98</td>
<td>0.143</td>
<td>0.631</td>
</tr>
<tr>
<td>Cd and SP</td>
<td>8.25</td>
<td>0.0810</td>
<td>0.960</td>
</tr>
<tr>
<td>Cd and TiO$_2$</td>
<td>29.3</td>
<td>0.0630</td>
<td>0.942</td>
</tr>
</tbody>
</table>

Table 1
The exponential accumulation parameters

Fig. 4. The accumulation of TiO$_2$ nanoparticles in carp.

Fig. 5. Cd and TiO$_2$ concentrations in different parts of carp (µg/g for Cd concentration and mg/g for TiO$_2$ concentration).
3.3. The distribution of Cd and TiO₂ in different parts of carp

Heavy metals enter the aquatic organism through direct consumption of water or food and through nondietary routes such as uptake through absorbing epithelia. The gills, skin, and digestive tract are potential sites of adsorption of water borne chemicals (Pedlar and Klaverkamp, 2002). Carp taken out on the 20th day were dissected. Skin and scales, muscle, gills and viscera were analyzed for Cd and TiO₂ content and the results are shown in Fig. 5. After 20 d of exposure, Considerable Cd and TiO₂ accumulated in viscera and gills of the fish, and the lowest level of accumulation was found in muscle. The order of Cd and TiO₂ accumulation in different parts of carp was viscera > gills > skin and scales > muscle.

Bioconcentration factor (BCF) are typically used to reflect the relation between chemical concentrations in water and in the target organism. BCFs of Cd and TiO₂ in different parts and whole body of carp after 20 d of exposure are presented in Table 2.

The study of Tan et al. (1996) shows that TiO₂ nanoparticles used in sunscreen, can get deep enough into the skin to be taken up into the lymphatic system, while larger particles (greater than 1 μm in diameter) can not. In our study there was no significant enhancement of Cd concentration in the skin and scales of carp in the presence of TiO₂ nanoparticle, however, BCFs increased by 1.5 fold (Table 2).

Gills are in direct contact with aquatic environment and are a physiologically complex and vulnerable structure, making them target organs for waterborne toxicants (Reid and McDonald, 1991). Moreover, the amount of mucus on the gill surface increases during metal exposure (Handy and Eddy, 1991), which may contribute to the adsorption of TiO₂ nanoparticles onto gill. TiO₂ concentration in gills was much higher than those in the skin and scales, and muscle, (0.74, 0.17 and 0.09 μg/g, respectively), suggesting that direct uptake of TiO₂ nanoparticles from water via the gills may have occurred, and subsequently TiO₂ nanoparticles were rapidly transferred, distributed and accumulated in internal organs. Thus, the BCF of TiO₂ nanoparticles in viscera was very high (1065). Facilitated transport of bound Cd occurred when TiO₂ nanoparticles transported from water via the gills. As a result, Cd concentrations in gills of carp exposed to Cd-contaminated water in the presence of TiO₂ nanoparticles increased by 60%, as compared to that in the absence of TiO₂ nanoparticles. Although TiO₂ nanoparticles have stronger facilitated transport ability for Cd than that of SP, the much lower concentration of soluble Cd may reduce their accumulation in gills. Thus the Cd concentration in gills of carp exposed to Cd + TiO₂ and Cd + SP were not significantly different.

Consumption of particles may be another way for TiO₂ and SP uptake, and with this process particle bound Cd was transported to carp. SP have a weak adsorbing capability for Cd (K_P = 23.5 mg/g) so that a relatively small amount of Cd could enter the alimentary tract through bound on the surface of these particles. TiO₂ nanoparticles bind a higher proportion of Cd (K_P = 250 mg/g) so that a larger dose is provided when these particles are ingested. Then the bound Cd on the surface of these particles may be released, distributed and accumulated in liver, kidney or other organs, resulting in the high concentration of Cd in viscera. As shown in Fig. 5, Cd concentration in viscera of carp exposed to Cd + TiO₂ nanoparticles and Cd + SP were increased by 179% and 43.0% comparing with that exposed to Cd contaminated media without particles, and the value was 57.7, 29.6 and 20.7 μg/g respectively.

4. Conclusions

Due to their small particle size, large specific surface area and strong electrostatic attraction, TiO₂ nanoparticles have a stronger adsorption capacity for Cd than SP. The presence of SP did not have significant influence on the accumulation of Cd in carp during the 25 d of exposure. However, the presence of TiO₂ nanoparticles greatly enhanced the accumulation of Cd in carp. After 25 d of exposure Cd concentration in carp increased by 146%. And there is a positive correlation between Cd concentration and TiO₂ concentration with a correlation coefficient greater than 0.975. Considerable Cd and TiO₂ accumulated in viscera and gills of the fish. Facilitated transport of adsorbed Cd may have happened when TiO₂ nanoparticles transported from water onto the gill surface. And also the consumption of particles contaminated food may be another way for TiO₂ uptake, and with this process particle bound Cd was transported to carp. As a result, Cd concentrations in viscera of carp exposed to Cd contaminated water in the presence of TiO₂ nanoparticles were significant higher than that exposed to Cd contaminated water.

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