SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 35, No. 7, pp. 1852–1859, 2016

Printed in the USA

IMPACT OF CARBON NANOTUBES ON THE TOXICITY OF INORGANIC ARSENIC [AS(III) AND AS(V)] TO *DAPHNIA MAGNA*: THE ROLE OF CERTAIN ARSENIC SPECIES

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(Submitted 11 June 2015; Returned for Revision 6 September 2015; Accepted 13 December 2015)

Abstract: As a type of emerging nanomaterial, hydroxylated multiwalled carbon nanotubes (OH-MWCNTs) may interact with other pollutants in the aquatic environments and further influence their toxicity, transport, and fate. Thus, evaluation of toxicity to arsenic in the presence of CNTs needs to receive much more attention. The present study was conducted to explore the underlying mechanisms of OH-MWCNT-induced arsenic (As[III] and As[V]) toxicity changes in the aquatic organism *Daphnia magna* at different pH levels. The most toxic species for As(III) and As(V) to *D. magna* were found to be $H_2AsO_3^-$ and $H_2AsO_4^-$. It appeared that the pH values were of greatest importance when the biological toxicity of As(III) and As(V) was compared. Furthermore, the effects of OH-MWCNTs on arsenic toxicity to *D. magna* indicated that the presence of OH-MWCNTs could enhance the toxicity of arsenic. The interactions of arsenic with OH-MWCNTs were further investigated by conducting adsorption experiments. The adsorption capacity of As(V) by OH-MWCNTs was found to be higher than that of As(III). To conclude, adsorption of certain arsenic species onto OH-MWCNTs is crucial for a reliable interpretation of enhanced toxicity. *Environ Toxicol Chem* 2016;35:1852–1859. © 2015 SETAC

Keywords: Hydroxylated multiwalled carbon nanotubes Arsenic species Toxicity Adsorption *Daphnia magna*

INTRODUCTION

With the development of nanotechnology, carbon nanotubes (CNTs) have been widely used in a variety of fields, including drug delivery, material strengthening, energy conversion, and environmental management [1,2]. Because of current and tremendous potential uses of CNTs, manufactured nanoparticles are likely to enter ecological environments and aquatic and terrestrial food chains [3,4]. Carbon nanotubes can easily be functionalized with both covalent and noncovalent moieties by using chemistry derivatization techniques to graft hydrophilic functional groups (such as COO, COOH, OH, NH, etc.) to the ends or sidewalls of the nanotubes [5,6]. Because of these surface functional groups, CNTs exhibit a very strong dispersivity and mobility in the aquatic environment [7]. The potential toxicity of surface functionalized CNTs to different aquatic organisms (algae, daphnids, zebrafish, trout, etc.) has attracted much research interest [1,8–14].

Because of their large specific surface area, CNTs exhibit a very strong adsorption affinity for various environmental contaminants and may act as adsorbents, concentrators, and carriers of contaminants [15,16]. It is reasonable to expect that CNTs will interact with heavy metal contaminants in aquatic environments, and therefore affect the bioavailability and fate of heavy metals. At present, there have been some studies focusing on the effects of CNTs on the transport, bioaccumulation, and toxicity of heavy metal contaminants [17,18]. Yu and Wang [19] demonstrated that nonfunctionalized CNTs decreased the Cd and Zn uptake rate, whereas functionalized CNTs increased metal accumulation in *Daphnia magna*. Kim et al. [18] found that the addition of surface-modified single-walled CNTs enhanced the bioavailability and toxicity of copper to *D. magna*. In addition, Wang et al. [20] demonstrated that hydroxylated multiwalled carbon nanotubes (OH-MWCNTs) increased Ni toxicity to *D. magna* at different pH levels and that a lower pH level contributed to a higher overall toxicity. However, to the best of our knowledge, no research has been conducted to systematically investigate the changes in arsenic [As(III) and As(V)] toxicity to *D. magna* in the presence of functionalized CNTs at different pH values.

Arsenic is a ubiquitous and controversial metalloid that has been used in various fields, including medicine [21], agriculture [22], livestock [23], and metallurgy [24]. Trace levels of arsenic can be detected in natural water in the forms of arsenate or arsenite—As(V) and As(III), respectively [22,25]. The toxicity of As to *D. magna* has been well documented in the literature; inorganic arsenic has been shown to be generally more toxic than organic forms, and As(III) has been demonstrated to be more toxic than As(V) [26–29]. In the aqueous environment, many factors (such as oxidation state, hardness, adsorbing surfaces, biological mediation, dissolved organic carbon, and pH) can influence arsenic bioavailability and toxicity to exposed organisms [28,30,31]. Therefore, it is important to investigate arsenic toxicity to aquatic organisms as a function of pH.

As a kind of functionalized MWCNT with hydrophilic oxygen-containing groups [32], OH-MWCNTs are of particular environmental interest [33]. It would be expected that the interaction of As(III) and As(V) with OH-MWCNTs would lead to different effects on aquatic organisms than effects observed in the presence of the individual chemical. Thus, the aim of the present study was to explore the underlying mechanisms for the As toxicity change in the presence of OH-MWCNTs at different pH levels. First, the OH-MWCNTs were characterized in detail and the metal impurities were determined. Then the

This article includes online-only Supplemental Data.

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Published online 18 December 2015 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.3340

combined toxicity of As(III) or As(V) and OH-MWCNTs to *D. magna* was measured at pH 6.0, 7.0, and 8.0. Furthermore, the effects of pH on arsenic species, adsorption, and accumulation were explored, to characterize the interactions of As with OH-MWCNTs. The results of the present study add to our current understanding of the potential environmental risk of CNTs.

MATERIALS AND METHODS

Preparation of CNTs and arsenic stock

The OH-MWCNTs were obtained from Shenzhen Nanotech Port. According to the provider, OH-MWCNTs were synthesized using chemical vapor deposition with a CH_4 and H_2 mixture at 700 °C. The OH-MWCNT samples were thoroughly characterized, including metal impurities, diameters, point of 0 charge, and hydroxyl group content (see details in Supplemental Data, Appendix 1). The physicochemical properties are given in Table 1.

Dispersions of CNTs were prepared according to the methods described by Alpatova et al. [34]. Briefly, CNTs (10.0 mg) were added to 100 mL of Milli-Q water (Millipore) in a 100-mL glass centrifuge tube with sonication (100 W, 40 kHz, 25 °C) for 24 h. Then the suspension was left to settle for 24 h, and the supernatant was collected and used as stock. The amount of undispersed CNTs was determined gravimetrically, and this weight was subtracted from the initial weight of CNTs to get the stable stock concentration. The concentration of CNTs in the supernatant was approximately 90 mg/L. Different concentrations of OH-MWCNTs were obtained by directly diluting the stock with aerated tap water. The dispersion and stability of OH-MWCNTs in suspension are described in detail in Supplemental Data, Appendix 2.

Sodium arsenate ($Na_3AsO_4 \cdot 12H_2O$), arsenic trioxide (As_2O_3), hydrochloric acid (HCl), and sodium hydroxide (NaOH; Sinopharm Chemical Reagent) were of analytical grade with a purity of 99.7%. The As(V) or As(III) stock solutions were prepared by dissolving the sodium arsenate or arsenic trioxide in a sodium hydroxide solution, and the solutions were stored in a refrigerator at 4 °C. To check the arsenic concentrations actually present, these arsenic stock solutions were measured by an inductively coupled plasma mass spectrometer (ICP-MS; NexION 300x ICP-MS Spectrometers). The actual concentration of arsenic stock solution was found to be 1.19 g As(V)/L and 6.94 g As(III)/L.

Culturing of D. magna

Daphnia magna were supplied by the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (Beijing, China). The culturing of *D. magna* was performed using the method detailed in our previous study [35]. The culturing water was tap water that had been purified using activated carbon and aerated for more than 48 h. The composition of the aerated tap water was 1.03 ± 0.02 mM Ca^{2+}, 0.319 ± 0.022 mM Mg^{2+}, 0.064 ± 0.011 mM K^+, 0.523 ± 0.025 mM HCO_3^-, 0.023 ± 0.009 mM SO_4^{2-}, 0.019 ± 0.007 mM NO_3^-, 0.012 ± 0.008 mM HPO_4^{2-}, and 0.822 ± 0.024 mg/L dissolved organic carbon. The pH was 7.8. The daphnids were cultured at 22 ± 1 °C, with a 14:10-h light:dark photoperiod in an intelligent artificial climate chamber (Safe). The fresh green algae *Scenedesmus obliquus* was cultured under the same conditions and fed to *D. magna* daily. After 3 generations of parthenogenesis, juvenile fleas (6–24 h old) were used in the toxicity experiments, and 7-d-old *Daphnia* were used in the accumulation tests.

As toxicity tests at different pH levels

The acute toxicity of the As-spiked samples to *D. magna* was determined in accordance with the Organisation for Economic Co-operation and Development guideline [36]. Ten neonates were placed in each glass beaker that contained 50 mL of test solution, and each treatment was replicated 5 times. A series of As test solutions was prepared to measure the As toxicity. No food was provided for the *Daphnia* during the experimental period. After a 48-h exposure, the number of immobilized individuals was recorded. Median lethal concentration (LC50) values were estimated using the logistic response model according to Heugens et al. [37]. Several additional experiments were performed to verify the validity of the immobilization experiments, as detailed in the Supplemental Data, Appendix 3.

To check the effect of pH on As toxicity, pH values of 6.0, 7.0, and 8.0 were chosen to conduct the immobilization experiments. In these acute toxicity tests, the As test solutions and the pH control groups were adjusted to appropriate pH values with HCl (0.12 mol/L and 0.012 mol/L) and NaOH (0.05 mol/L and 0.005 mol/L), respectively, and the errors were within ±0.2. The buffer 3-morpholinopropanesulfonic acid was used at a concentration of 3.58 mM to ensure pH stability during assays according to previous research [38]. The final pH was checked with a pH Meter (Mettler-Toledo) for each solution. According to the preliminary experiments, 9 concentrations of As(V) (0.60 mg As/L, 1.19 mg As/L, 2.38 mg As/L, 4.77 mg As/L, 7.15 mg As/L, 9.53 mg As/L, 11.9 mg As/L, 14.3 mg As/L, and 16.7 mg As/L) and 9 concentrations of As(III) (0.69 mg As/L, 2.78 mg As/L, 4.86 mg As/L, 6.94 mg As/L, 9.02 mg As/L, 11.1 mg As/L, 13.2 mg As/L, 15.3 mg As/L, and 17.4 mg As/L) were used to determine LC50 values.

Table 1. Physicochemical properties of hydroxylated multiwalled carbon nanotubes[a]

OD[b] (nm)	Length[c] (μm)	Purity (%)	Ash[d] (%)	pH_{PZC}	SSA[e] (m^2/g)	Hydroxyl group content (% w/w)	Catalyst residue contents[d] (μg/g)		
							Co	Ni	Mo
36 ± 2	4 ± 1.5	>97	2 ± 0.3	6.1	115 ± 5	1.82	0.101	4.783	0.045

[a]The detection limit of the inductively coupled plasma mass spectrometer was 0.001 μg/L; the relative standard deviation was within ±5%.
[b]The outer diameters and lengths were measured using TEM.
[c]The ash contents were measured using a Thermo Gravimetric Analyzer by heating the carbon materials at 1000 °C for 10 h.
[d]The specific surface areas were calculated from the adsorption−desorption isotherm of N_2 at 77 K, using the multipoint BET (Brunauer-Emmett-Teller) method.
[e]Catalyst residue contents (μg/g) were determined using an inductively coupled plasma mass spectrometer.
OD = outer diameter; PZC = point of 0 charge; SSA = specific surface area.

As toxicity test with OH-MWCNTs at different pH levels

Petersen et al. [13] and Zhu et al. [39] demonstrated that the 48-h LC50 values of raw MWCNTs were 12.7 mg/L and 8.72 mg/L for *D. magna*, respectively. Hence, nonlethal concentrations of OH-MWCNTs (0.1 mg/L, 0.5 mg/L, 1.0 mg/L, 2.5 mg/L, and 5.0 mg/L) were used in the joint toxicity tests. Mixed solutions of As and OH-MWCNTs at pH 6.0, 7.0, and 8.0 were shaken thoroughly on an incubator shaker (HNY-200B, Tianjin Honour Instruments) at room temperature for 1 h. Then each of the test solutions with 10 daphnid neonates (6–24 h old) were transferred into the intelligent artificial climate chamber at 22 ± 1 °C during the exposure period. Mortality was recorded after 48 h of exposure. During the exposure period, the pH values were adjusted every 6 h. All the immobilization experiments were repeated, and reproducible results were obtained.

Adsorption experiments

In the batch adsorption experiments, As adsorption at a specific concentration of OH-MWCNTs (5.0 mg/L) was investigated at pH 6.0, 7.0, and 8.0 under a series of As concentrations (0.05 mg/L, 0.10 mg/L, 0.22 mg/L, 0.52 mg/L, 1.28 mg/L, 2.50 mg/L, 3.55 mg/L, 5.25 mg/L, 8.05 mg/L, and 10.65 mg/L), which covered the range between the sublethal and lethal As concentrations. Then these As solutions were left to settle for at least 24 h before use. The mixtures of As and OH-MWCNTs were mixed thoroughly by shaking at 25 °C for 24 h in an incubator shaker. Subsequently, the solutions were filtered through 0.22-μm membrane filters to separate the OH-MWCNT particles from the liquid phase. The filtrates were immediately analyzed using an ICP-MS to determine the As concentrations. The amounts of As adsorbed onto the CNTs were calculated by subtracting the equilibrium mass of ions in solution from the initial aqueous ion mass. All adsorption experiments were performed in quadruplicate.

Accumulation of As in D. magna

In separate tests, *D. magna* were exposed to mixtures of As and OH-MWCNTs to determine the As content in *D. magna* using previous methods with some modifications [18,40]. Ten 7-d-old organisms were exposed to each 250-mL test solution for 48 h without water renewal. Three different sublethal As concentrations (100, 200, and 400 μg/L) and 5 OH-MWCNTs concentrations (0.1, 0.5, 1.0, 2.5, and 5.0 mg/L) were chosen to explore the accumulation of As in *D. magna*. After 48-h exposure, 10 animals were sampled to determine the accumulated As in *D. magna*. All of the organisms were rinsed with 10 g/L ethylenediaminetetraacetic acid disodium salt [EDTA-Na_2] for 20 s and with Milli-Q water for 10 s twice to remove any externally adsorbed As from the carapace. Subsequently, the daphnids were placed in aluminum weigh-boats, dried at 80 °C for 24 h, and weighed. Then the animals were transferred into conical polypropylene tubes and digested with 500 μL of 70% nitric acid in a water bath at 100 °C for 30 min. After the solution was diluted to 10.0 mL with Milli-Q water, the treated samples were analyzed for total As using an ICP-MS. The total As in *D. magna* was expressed as μg/g dry weight. Each treatment was performed in triplicate, and each sample was measured 3 times.

Calculations and statistical analysis

Arsenic speciation in natural water at different pH values was calculated by the chemical equilibrium model Visual MINEQL [41,42]. During computation, we assumed that the pH was fixed, and corresponding water quality parameters were used as input variables. The acute toxicity data were analyzed using the SPSS statistical package (Ver 16.0). One-way analysis of variance followed by Dunnett's test was performed to signal significant differences between the groups. Significance was denoted at $p < 0.05$ and $p < 0.01$.

RESULTS AND DISCUSSION

Characterization of OH-MWCNTs

From Table 1, we can see that the specific surface area of OH-MWCNTs is $115 \pm 5\ m^2/g$ with an outer diameter of 36 ± 2 nm. The outer diameters and lengths of OH-MWCNTs determined using transmission electron microscopy (Figure 1) were generally consistent with the values provided by the supplier. The metal residues analysis (Table 1) demonstrated the presence of Mo, Co, and Ni in the original CNT materials, which was further confirmed using X-ray diffraction (Figure 1). The

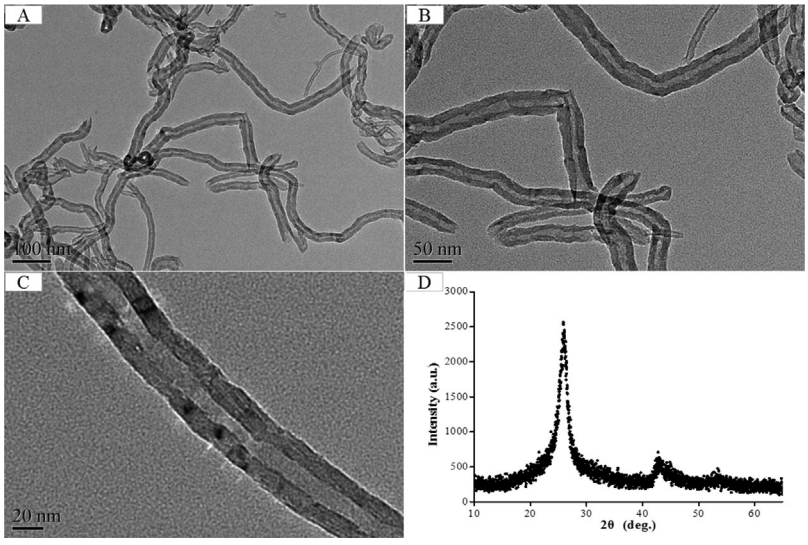

Figure 1. Transmission electron microscopy images (**A–C**) of hydroxylated multiwalled carbon nanotubes (OH-MWCNTs) at different magnifications (100 nm, 50 nm, and 20 nm, respectively), and X-ray diffraction (**D**) of OH-MWCNTs.

dominating peak at approximately 26° can be attributed to the amorphous quartz substrate, and the second peak at approximately 42.4° represents a typical crystalline state of Ni-matrix [43].

The hydroxyl content of the OH-MWCNTs was determined to be 1.82% [20], suggesting a high dispersion stability of OH-MWCNTs in an aquatic environment. Absorbance of the stable OH-MWCNTs solutions was measured at 255 nm (Supplemental Data, Figure S1), and the absorbance values have a highly linear correlation with the OH-MWCNTs concentrations (Supplemental Data, Figure S2). The stability of OH-MWCNT suspensions at 24 h (0.1 mg/L, 1 mg/L, and 5 mg/L) with and without daphnids was evaluated using the calibration curves (Supplemental Data, Figure S3). It was found that stability was reduced during the exposure period, and the CNT solutions with higher concentrations had lower stabilities. This was consistent with experimental results for OH-MWCNTs [20,44].

Effect of pH on As(III) and As(V) species distribution and toxicity

Figure 2 shows the distribution of As(III) and As(V) species in the natural water within the pH range of 5.0 to 9.0. For As(III), the neutral arsenite species H_3AsO_3 was always the dominant species at the pH values studied (Figure 2A), consistent with the findings of Sharma and Sohn [31]. As the pH increases from 5.0 to 9.0, the percentage of H_3AsO_3 decreases appreciably from 91.1% to 50.7%, whereas $H_2AsO_3^-$ increases from 0% to 49.3%. For As(V), there were 2 major species at the pH range of 5.0 to 9.0 (i.e., $HAsO_4^{2-}$ and $H_2AsO_4^-$; Figure 2B). The species $H_2AsO_4^-$ comprised 97.4% of the total species at pH 5.0, and its proportion decreased to 79.6% at pH 6.0. Meanwhile, the percentage of $HAsO_4^{2-}$ increased from 2.5% to 20.4%. At pH 6.6, there were almost equal concentrations of $H_2AsO_4^-$ and $HAsO_4^{2-}$ (Figure 2B). When the pH was further increased to 7.0, $HAsO_4^{2-}$, at a percentage of 71.9%, became more abundant than $H_2AsO_4^-$. Furthermore, at pH 8.0 and 9.0, $HAsO_4^{2-}$ was predominantly observed.

Figure 2 also shows the LC50 values of As(III) and As(V) for *D. magna* after 48-h exposures at pH 6.0, 7.0, and 8.0. The LC50 values of As for *D. magna* ranged from 3.87 mg/L to 5.49 mg/L and from 7.58 mg/L to 9.51 mg/L for As(III) and As(V), respectively. It is obvious that As(III) was more toxic than As(V). Many previous studies have reported that the 48-h LC50 values ranged from 1.51 mg/L to 9.10 mg/L of inorganic As for daphnids [28,45–47]. In comparison, the acute toxicity values obtained from the present study are within the range of such results for inorganic arsenicals in *D. magna*.

As can be seen, the LC50 of As(III) decreased from 5.15 mg/L to 4.02 mg/L when the water pH increased from 6.0 to 8.0 (Figure 2A). This means that the toxicity of As(III) was lower in an acidic environment than in an alkaline condition. This enhanced toxicity can be attributed to the increasing percentage of the $H_2AsO_3^-$ species (reaching 49.3%). Thus, the $H_2AsO_3^-$ species is more toxic to *D. magna* than H_3AsO_3. Fulladosa et al. [25] also found that $H_2AsO_3^-$ was the most toxic species for arsenite to luminescent bacteria (*Vibrio fischeri*). From Figure 2B, it can be seen that the LC50 of As(V) increased from 7.86 mg/L at pH 6.0 to 9.16 mg/L at 8.0 (Figure 2B). The dominance of $H_2AsO_4^-$ at pH 6.0 suggests that this species is responsible for the high toxicity. The toxicity reduction with the decreasing percentage of the $H_2AsO_4^-$ species also confirmed that among arsenate species, $H_2AsO_4^-$ is the most toxic to *D. magna*. Meanwhile, there is a positive correlation between the percentage of $H_2AsO_4^-$ species and

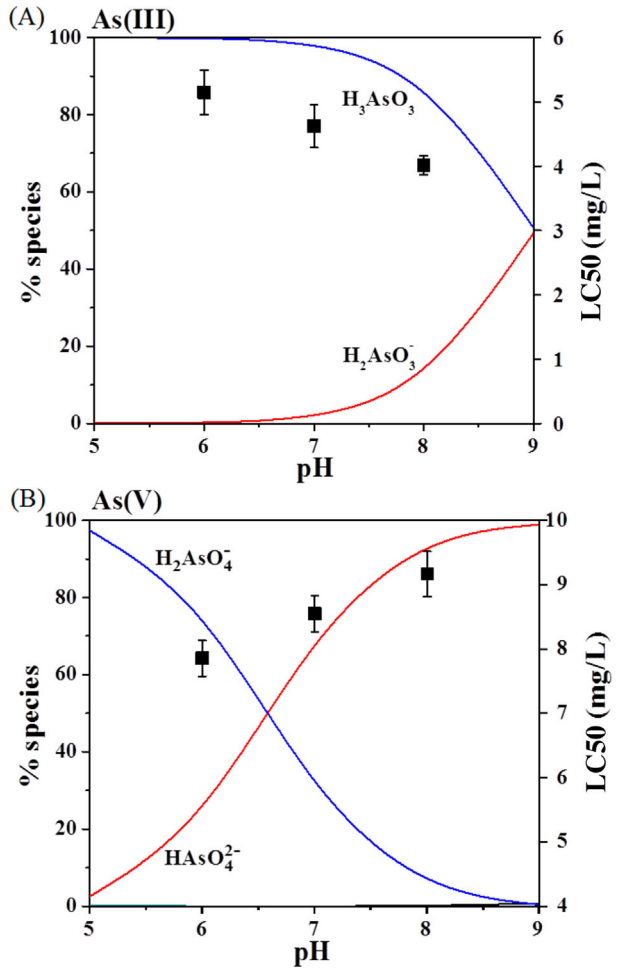

Figure 2. Effects of pH on As(III), (**A**) and As(V), (**B**) species distribution (lines) and toxicity (scatterplots) to *Daphnia magna* after 48-h exposures. Error bars represent 95% confidence interval. LC50 = median lethal concentration.

toxicity to *D. magna* [28]. Therefore, on the basis of LC50 values, it appears that pH values are of the greatest importance when the biological toxicity of As(III) and As(V) is compared.

Adsorption of As by OH-MWCNTs at different pH levels

Many studies have shown that different CNTs have different abilities to adsorb metals, and the oxygen-containing functional groups on the CNTs surface dominate the sorption capacity for metals from water [16,48,49]. It was also suggested that the adsorbed amount of metals onto CNTs increased quickly within the first 60 min and then slowly reached equilibrium in 10 h [49]. In the present study, the contact time of 24 h was selected to ensure that equilibrium conditions were reasonably well achieved. Figure 3 shows the amount of As(V) and As(III) adsorbed onto OH-MWCNTs at a CNT concentration of 5.0 mg/L at pH 7.0. The experimental data were analyzed using the linear Langmuir sorption isotherm model:

$$q_e = K_a q_m C_e/(1 + K_a C_e) \quad (1)$$

where q_e (mg/g) is the amount of sorbed As per unit weight of OH-MWCNTs at equilibrium, q_m (mg/g) is the maximum sorption capacity of OH-MWCNTs for As, C_e (mg/L) is the equilibrium As concentration, and K_a (L/mg) is the Langmuir affinity coefficient for adsorption. The adsorption data were

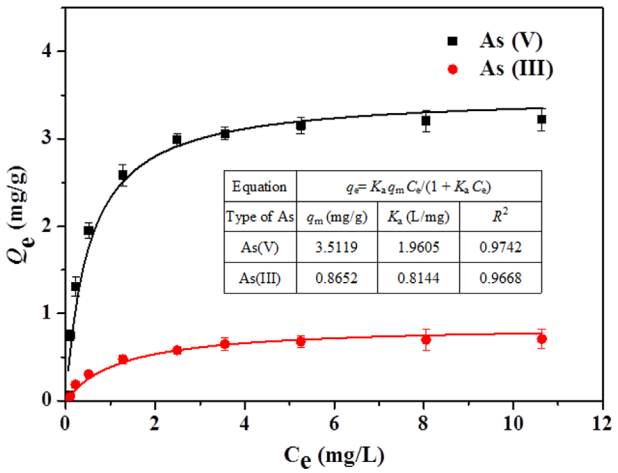

Figure 3. Adsorption isotherm for As(III) and As(V) at a hydroxylated multiwalled carbon nanotube (OH-MWCNT) concentration of 5.0 mg/L at pH 7.0. The adsorption isotherm was fitted by the Langmuir isotherm. The K_a, q_m, and R^2 values are shown in the inner table. All values are the means of 3 replicates, and error bars represent the standard deviations that are sometimes smaller than the dot sizes. Q_e is the amount of sorbed As per unit weight of OH-MWCNTs at equilibrium; C_e is the equilibrium As concentration.

well fitted using the Langmuir model, because the regression coefficients R^2 were 0.9742 and 0.9668 for As(V) and As(III), respectively (Figure 3). The maximum adsorption capacities of As(V) and As(III) were 3.51 mg/g and 0.87 mg/g, respectively. Thus, the OH-MWCNTs show low adsorption capacity toward As(III). This is consistent with previous studies showing that As(III) is less strongly adsorbed to a variety of sorbents (activated carbon and functionalized MWCNTs) than As(V) [50,51]. Many researchers have documented that there are obvious differences in the isosteric heat of adsorption between As(III) and As(V) [50,52]. These results suggest that physisorption occurs between As(III) and As(V) and OH-MWCNTs because of weak van der Waals forces.

The effect of solution pH on the adsorption of As(III) and As(V) onto OH-MWCNTs was determined over a pH range of 5.0 to 9.0 (Figure 4). The adsorption capacity of As(III) on OH-MWCNTs was constant at pH 5.0 to 7.0 (0.865 ± 0.026 mg As/g) and then decreased slightly from 0.865 mg As/g at pH 7.0 to 0.383 mg As/g at pH 9.0. By contrast, the capacity of As(V) adsorption by OH-MWCNTs was high at pH 5.0, and decreased

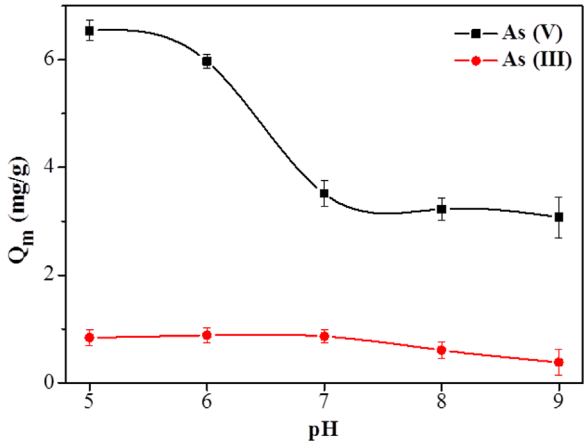

Figure 4. Effect of pH on the maximum sorption capacity (mg/g) of hydroxylated multiwalled carbon nanotubes for As(III) and As(V). Error bars represent 95% confidence intervals. Q_m = maximum sorption capacity.

from 6.54 mg As/g to 3.07 mg As/g with the initial pH increasing from 5.0 to 9.0. The point of 0 charge of OH-MWCNTs, which was measured to be approximately 6.1 [53,54], may help to further understand the influence of pH on As(III) and As(V) adsorption. Based on the As speciation, at a pH range between 5.0 and 9.0, As(III) is mainly present as neutral $H_3AsO_3^0$ and anionic $H_2AsO_3^-$ species whereas As(V) is present as the anionic species $HAsO_4^{2-}$and $H_2AsO_4^-$ (Figure 2). The surface charge of OH-MWCNTs might carry negative charges at pH > 6.1 because of the deprotonation of the surface hydroxyl groups. Thus, the adsorption capacity of the negatively charged As(III) and As(V) anions on OH-MWCNTs are reduced by the negatively charged surface of OH-MWCNTs because of electrostatic repulsion. With increasing pH, the concentration of OH^- increases, which can strongly compete with As(III) and As(V) anions to adsorb onto the surface of OH-MWCNTs. Therefore, it is conceivable that physisorption via electrostatic interaction plays a major role in the adsorption of As(III) and As(V) on OH-MWCNTs.

Together, Figures 2B and 4 show that as the pH increased, the capacity of As(V) adsorption decreased with the decreasing percentage of the $H_2AsO_4^-$ species. This possibly indicates that among the arsenate species, the $H_2AsO_4^-$ can easily adsorb onto the surface of OH-MWCNTs.

Effect of OH-MWCNTs on As toxicity under different pH levels

The toxicity of As(III) and As(V) to *D. magna* was measured at 5 OH-MWCNT concentrations of 0.1 mg/L, 0.5 mg/L, 1.0 mg/L, 2.5 mg/L, and 5.0 mg/L (Figure 5). In this OH-MWCNT concentration range, the OH-MWCNTs themselves have no significant toxicity to *D. magna*, and 5 mg/L is below the lowest observed effect concentration (data not shown). As shown in Figure 5, compared with the only As group, the 48-h LC50 values of As(III) and As(V) decreased with the addition of OH-MWCNTs at each pH level. After 48 h of exposure, maximum decreases (of 14.1% and 14.9% for As(III) and As(V), respectively) were observed in the treatment containing 1.0 mg/L of OH-MWCNTs at pH 6.0. As can be seen in Figure 5A, the 48-h LC50 values of As(III) were significantly ($p < 0.05$) decreased in the presence of 1.0 mg/L, 2.5 mg/L, and 5.0 mg/L OH-MWCNTs at each pH level. The 48-h LC50 values of As(V) were also significantly ($p < 0.05$ for pH 7.0 and 8.0; $p < 0.01$ for pH 6.0) decreased in the presence of 1.0 mg/L, 2.5 mg/L, and 5.0 mg/L OH-MWCNTs (Figure 5B). It is suspected that not only the aqueous As(III) and As(V) but also the adsorbed As contributed to the overall toxicity. Therefore, the presence of OH-MWCNTs could enhance the toxicity of arsenic. The results were consistent with those reported by other researchers [18–20].

It is noteworthy that the As(V) LC50 values were highly significantly ($p < 0.01$) decreased in the treatment groups with the addition of 1.0 mg/L, 2.5 mg/L, and 5.0 mg/L OH-MWCNTs at pH 6.0 (Figure 5B). The reason is that, at pH 6.0, OH-MWCNTs can carry much more toxic species $H_2AsO_4^-$ into the gut of *D. magna*, leading to the significantly enhanced toxicity ($p < 0.01$). According to previous research [55], the enhanced As accumulation in *D. magna* may contribute to the increased toxicity. Thus, the total amount of As accumulated in *D. magna* with or without OH-MWCNTs was analyzed.

Accumulation of As in D. magna *at pH 6.0*

To explain the enhanced toxicity, accumulated As in *D. magna* was measured after 48-h exposure to 3 different

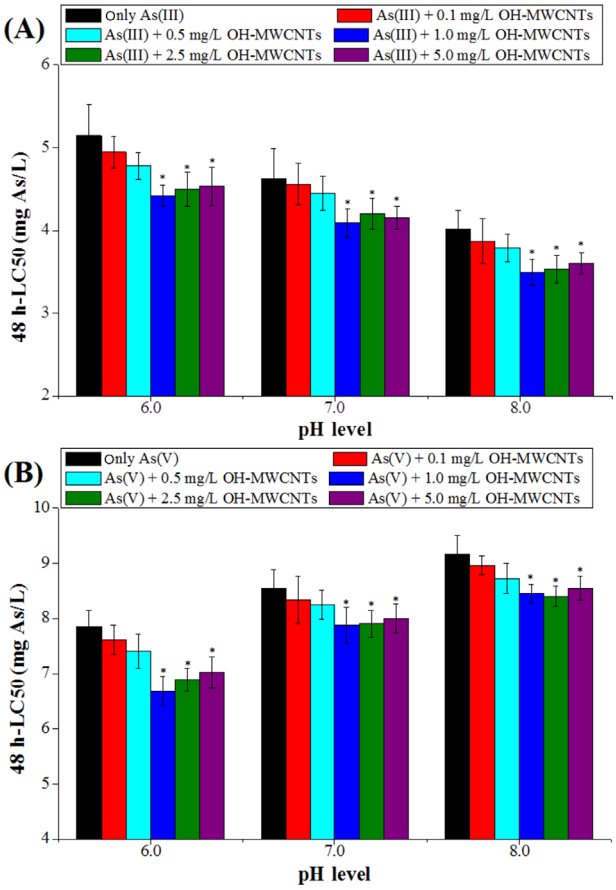

Figure 5. The 48-h median lethal concentration (LC50) values of As(III), (**A**) and As(V), (**B**) in the presence of different concentrations of hydroxylated multiwalled carbon nanotubes (OH-MWCNTs) at pH 6.0, 7.0 and 8.0. Data are means ± standard deviation; $n = 4$ for each data point. *Different from control (only As; $p < 0.05$); **different from control (only As; $p < 0.01$).

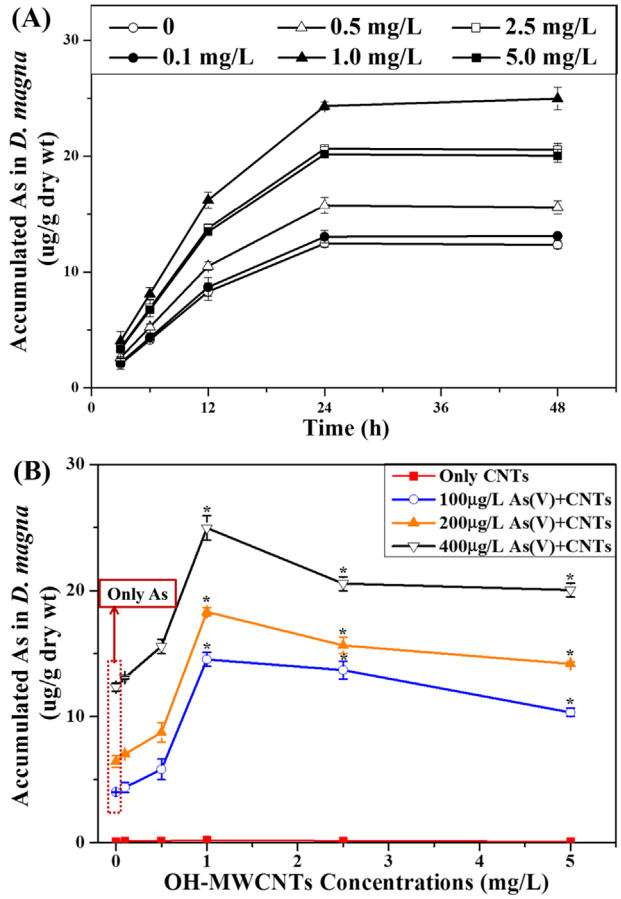

Figure 6. Accumulated As(V) (μg/g dry wt) in *Daphnia magna* during 48-h exposure to As (400 μg/L), (**A**) and after 48-h exposure to different As concentrations (0 μg/L, 100 μg/L, 200 μg/L, and 400 μg/L), (**B**) in the presence of different hydroxylated multiwalled carbon nanotube (OH-MWCNT) concentrations (0 mg/L, 0.1 mg/L, 0.5 mg/L, 1.0 mg/L, 2.5 mg/L, and 5.0 mg/L) at pH 6.0. All values are the means of 3 replicates, and error bars represent the standard deviations. *Different from control (only As; $p < 0.05$). CNT = carbon nanotube.

As(V) concentrations (100 μg/L, 200 μg/L, and 400 μg/L) in the presence of OH-MWCNTs (0 mg/L, 0.1 mg/L, 0.5 mg/L, 1.0 mg/L, 2.5 mg/L, and 5.0 mg/L) at pH 6.0 (Figure 6). The accumulation results of As in *D. magna* exposed to 400 μg/L As(V) both with and without OH-MWCNTs are shown in Figure 6A. Arsenic concentrations in *D. magna* in each group increased with increased exposure time, and then remained unchanged after 24 h of exposure. In the control group, As concentrations in *D. magna* increased gradually and after 48 h of exposure reached 12.3 μg/g. When exposed to As-contaminated water in the presence of OH-MWCNTs, the daphnia accumulated considerably more As. As can be seen from Figure 6A, As concentrations in the daphnia in the presence of 1 mg/L OH-MWCNTs reached a maximum value (24.9 μg/g) after 48 h of exposure, which increased by 102% more than without CNTs, suggesting that the presence of 1 mg/L OH-MWCNTs greatly enhanced the accumulation of As in daphnia. Figure 6B shows that the accumulation of As in *D. magna* increased slowly at low OH-MWCNTs concentrations (0.1 mg/L and 0.5 mg/L) and increased rapidly to the maximum at 1 mg/L of OH-MWCNTs, with specific values of 14.6 μg As/g, 18.3 μg As/g, and 25.0 μg As/g for 100 μg/L, 200 μg/L, and 400 μg/L As(V) exposure, respectively. A significantly increased As accumulation ($p < 0.05$) was observed when the concentration of OH-MWCNTs reached 1.0 mg/L or higher. However, the accumulated As in *D. magna* was slightly decreased (range of 19.7–28.8%) at higher OH-MWCNT concentrations of 2.5 mg/L and 5 mg/L, compared with the maximum As accumulation. This trend is consistent with the toxicity variation shown in Figure 5B. This demonstrates that high OH-MWCNT concentration (1 mg/L, 2.5 mg/L and 5 mg/L) can effectively increase As(V) concentration in the *D. magna* due to the adsorption capacity of the CNTs. Generally, OH-MWCNTs increased the As accumulation in *D. magna* in a concentration-dependent manner, which was similar to the result of the toxicity experiment.

Similar results were also reported by other researchers. Wang et al. [55] investigated the combined effect of TiO_2 nanoparticles and As(V) and found that the significantly enhanced toxicity and accumulation of As(V) to *Ceriodaphnia dubia* was dependent on the nano-TiO_2 concentrations. In their findings, As(V) adsorption on the TiO_2 surface was high at lower concentrations of TiO_2, resulting in increased toxicity. When the concentration of TiO_2 reached a certain value, both the dissolved and adsorbed As(V) concentration were significantly reduced. In the present study, different pH levels were introduced, to study the effects of OH-MWCNTs on the toxicity and accumulation of As(V) in *D. magna*. Compared with pH 7.0 and 8.0, As(V) was more toxic to *D. magna* at pH 6.0, because of the dominance of the arsenic species $H_2AsO_4^-$. Moreover, OH-MWCNTs had a highly significant effect on the toxicity of As(V) at pH 6.0. Therefore, it is concluded that the enhanced

accumulation of $H_2AsO_4^-$ in *D. magna* plays an important role in the combined toxicity. The accumulation of As in *D. magna* could be related to the adsorption of $H_2AsO_4^-$ on OH-MWCNTs. To gain a comprehensive understanding of the adsorption mechanism of the different arsenic species on OH-MWCNTs, further research is required.

CONCLUSIONS

The present study shows that the presence of OH-MWCNTs could significantly enhance the toxicity of As. The toxicity-enhancing effect of OH-MWCNTs could be because the adsorption of As onto the OH-MWCNTs and the uptake of OH-MWCNTs as a fake food increased the exposure of *D. magna* to As. The species distribution and toxicity of As(III) and As(V) was determined at pH 6.0, 7.0, and 8.0, and the results revealed that the $H_2AsO_3^-$ species is more toxic at pH 8.0 for As(III) and that $H_2AsO_4^-$ is the dominant species responsible for the high toxicity at pH 6.0 for As(V). These findings suggest that the adsorption of certain arsenic species onto OH-MWCNTs was the key factor contributing to the enhanced toxicity to an aquatic organism. The present study contributes to our current understanding of the potential environmental risks of CNTs.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3340.

Acknowledgment—The present study was supported by the National Natural Science Foundation of China (grants 41071319 and 21377051) and the Major Science and Technology Program for Water Pollution Control and Treatment of China (grant 2012ZX07506-001). Also, the authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of the present study through Research Group Project RGP- VPP-240.

Data availability—All data, associated metadata, and calculation tools are available on request from the corresponding author (wangzun315cn@163.com).

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