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Article details

Xiao Y., Vijver M.G. & Peijnenburg W.J.G.M. (2018), Impact of water chemistry on the behavior and fate of copper nanoparticles, Environmental Pollution 234: 684-691.
Doi: 10.1016/j.envpol.2017.12.015

Environmental Pollution 234 (2018) 684–691

Contents lists available at ScienceDirect

Environmental Pollution

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

Impact of water chemistry on the behavior and fate of copper nanoparticles☆

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ARTICLE INFO

Article history:
Received 8 June 2017
Received in revised form
16 November 2017
Accepted 4 December 2017
Available online 21 December 2017

Keywords:
Copper nanoparticles
Water chemistry
Aggregation
Dissolution
Sedimentation

ABSTRACT

A full-factorial test design was applied to systematically investigate the contribution and significance of water chemistry parameters (pH, divalent cations and dissolved organic carbon (DOC) concentration) and their interactions on the behavior and fate of copper nanoparticles (CuNPs). The total amount of Cu remaining in the water column after 48 h of incubation was mostly influenced by divalent cation content, DOC concentration and the interaction of divalent cations and DOC. DOC concentration was the predominant factor influencing the dissolution of CuNPs, which was far more important than the effect of pH in the range from 6 to 9 on the dissolution of the CuNPs. The addition of DOC at concentrations ranging from 5 to 50 mg C/L resulted in a 3–5 fold reduction of dissolution of CuNPs after 48 h of incubation, as compared to the case without addition of DOC. Divalent cation content was found to be the most influential factor regarding aggregation behavior of the particles, followed by DOC concentration and the interaction of divalent cations and DOC. In addition, the aggregation behavior of CuNPs rather than particulate dissolution explained most of the variance in the sedimentation profiles of CuNPs. These results are meaningful for improved understanding and prediction of the behavior and fate of metallic NPs in aqueous environments.

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1. Introduction

Metallic nanoparticles (NPs) are increasingly applied in industrial and consumer products. The release of NPs into the aquatic environment can occur via different routes like industrial and municipal wastewater discharges (Boxall et al., 2007), runoff (Osmond and McCall, 2010) and leaching from consumer products (Hendren et al., 2011). Fueled by the inevitable release of NPs into the aqueous environment, concern about the adverse effects of these materials on aquatic biota has been growing. Among other particles, copper NPs (CuNPs) have been demonstrated to be highly toxic to a wide range of aquatic organisms (Hua et al., 2014; von Moos and Slaveykova, 2014; Song et al., 2015). It is therefore essential to comprehensively understand the behavior and fate of CuNPs in the aquatic environment across a range of water chemistry, in order to better interpret and predict their hazards to organisms.

After being emitted into aquatic environments, NPs are subject to undergo a series of environmental processes. These processes include dissolution and aggregation and subsequent sedimentation, which consequently affect their ultimate fate, bioavailability and hazards to organisms. Currently, it is widely known that the behavior and fate of NPs are highly dependent on the water chemistry. In particular, environmental parameters like pH, electrolytes (especially divalent cations) and natural organic matter (NOM) can strongly influence the ultimate fate of NPs in the environment. For example, pH can affect the aggregation and dissolution of metallic NPs by influencing the surface potential of the NPs (von der Kammer et al., 2010; Peng et al., 2017). The divalent cations Ca^{2+} and Mg^{2+} are able to efficiently compress the electrical double-layer of NPs and consequently enhance aggregation of NPs (Zhang et al., 2009; Van Hoecke et al., 2011). NOM has been widely reported to enhance the stability of metallic NPs via electrostatic and/or steric repulsion (Gao et al., 2012; Romanello and Fidalgo de Cortalezzi, 2013), and to alter their dissolution profiles through chelation and/or complexation of the metallic NPs (Majedi et al.,

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☆ This paper has been recommended for acceptance by Baoshan Xing.

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https://doi.org/10.1016/j.envpol.2017.12.015
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2014; Wang et al., 2015). Destabilizing effects of NOM on metallic NPs have also been reported, especially in the presence of divalent cations. This is due to the formation of bridges between NOM and cations (Stankus et al., 2011). Nevertheless, few studies have systematically investigated the issue of the extent in which environmental parameters present at environmental relevant concentrations affect the behavior and fate of NPs (Majedi et al., 2014; Son et al., 2015). This is amongst others due to a lack of full-factorial experiments to quantitatively investigate the individual and interactive effects of each environmental parameter on the behavior and fate of NPs in environment.

In this study, a factorial test design was applied to systematically investigate the behavior and fate of CuNPs in various aqueous matrices. The aim of this study is to evaluate the contribution and significance of each environmental parameter (i.e., pH, divalent cation content, and NOM concentration) and their interactions to the variations of behavior and fate of CuNPs across a range of water chemistry. This study is meaningful for improving our understanding and predictive potential of behavior and fate of NPs in freshwater environments.

2. Materials and methods

2.1. Preparation of CuNP suspensions

CuNPs (spherical surface area 30–50 m^2/g; purity 99.9%) were purchased from IoLiTec, with a nominal size of 25 nm. Stock suspensions of CuNPs (250 mg/L) were freshly prepared in MilliQ water after 30 min of bath-sonication to disperse the particles, prior to each experiment.

For the study design, CuNP suspensions under a range of water chemistry conditions were prepared. The divalent cation composition of the exposure media was modified by adding $CaCl_2 \cdot 2H_2O$ and $MgSO_4 \cdot 7H_2O$ in a fixed molar ratio of 4:1. Aldrich humic acid (sodium salt) (HA) was used as a surrogate to natural dissolved organic carbon (DOC). A stock solution was prepared by dissolving HA in 0.002 N NaOH solution, followed by stirring for 24 h, filtration through a 0.2 μm cellulose acetate membrane, and storage at 4 °C prior to use. The total organic carbon (TOC) content of the prepared stock HA solutions was determined by a TOC analyzer (TOC-VCPH, Shimadzu Corporation). The pH values of the exposure media were adjusted by 0.1 M NaOH or 0.1 M HCl to reach values in between 6 and 9. It is worth to point out that the pH of the exposure media was adjusted to the desired value after mixing the DOC with the CuNP suspensions. CuNP suspensions were prepared freshly for all aqueous matrices tested. A factorial experiment design was adopted with 3 pH (6, 7.8, 9) × 3 divalent cation concentrations (0, 2.5, 10 mM) × 4 DOC concentrations (0, 5, 25, 50 mg C/L, Table 1). The range of each parameter employed in this study encompasses the range commonly observed in natural environments (Vijver et al., 2008; Ottofuelling et al., 2011; Hammes et al., 2013).

2.2. CuNP characterization

The morphology and primary size of the CuNPs in the exposure medium with 2.5 mM of divalent cations (composition of $CaCl_2 \cdot 2H_2O$ and $MgSO_4 \cdot 7H_2O$ in a fixed molar ratio of 4:1) and pH of 7.8 were characterized using transmission electron microscopy (TEM, JEOL 1010, JEOL Ltd., Japan). The hydrodynamic diameters of CuNP suspensions during 48 h of incubation were determined in triplicate by dynamic light scattering (DLS) on a Zetasizer Nano-ZS instrument (Malvern, Instruments Ltd., UK). At the same time points, the zeta-potential of each NP suspension was measured by ZetaPALS software based on the Smoluchowski equation. During the 48 h of incubation, all the prepared NP suspensions were stored in a climate chamber under a 16:8-h light-dark cycle (20 ± 1 °C). To maintain the desired pH value during the 48 h incubation, the pH of the exposure media was adjusted carefully with 0.1 M NaOH or 0.1 M HCl every 24 h. In addition, the actual divalent cation concentration (i.e., Ca^{2+} and Mg^{2+}) and actual DOC concentration in exposure suspensions were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) and listed (Table S1).

The variations of the concentration of total Cu and dissolved Cu-ions shedding from CuNPs in the water column, were monitored within 48 h of incubation. The actual concentration for each CuNP exposure suspension was around 800 μg/L, which is in the range (10–920 μg/L) of the predicted CuNP concentration in aquatic environments (Chio et al., 2012). The prepared CuNP suspensions across a range of water chemistry, as prepared according to Table 1, were stored in the climate chamber (16:8-h light-dark cycle; 20 ± 1 °C). At each sampling point (1, 12, 24, 36 and 48 h after preparation), two independent CuNP suspensions with the same water chemistry as duplicates were used to analyze the concentration of each Cu fraction. For each exposure suspension, a 5 mL sample collected carefully from the position around 2 cm below the surface of each NP suspension, was digested by addition of 1 drop of 65% nitric acid at room temperature for at least 1 d prior to ICP-OES analysis. In this way, the total amount of Cu remaining in the water column, including dissolved Cu and suspended particulate Cu was determined. Kinetics of the sedimentation of CuNPs within 48 h of incubation could be analyzed by determining the change of concentration of total Cu in the water column. At each sampling point,

Table 1
Overview of the systematic modification of the matrices used for testing the fate of CuNPs.

Trial no.	pH value	Cation conc. (mM)	DOC conc. (mg/L)
1	6	0	0
2	6	0	5
3	6	0	25
4	6	0	50
5	6	2.5	0
6	6	2.5	5
7	6	2.5	25
8	6	2.5	50
9	6	10	0
10	6	10	5
11	6	10	25
12	6	10	50
13	7.8	0	0
14	7.8	0	5
15	7.8	0	25
16	7.8	0	50
17	7.8	2.5	0
18	7.8	2.5	5
19	7.8	2.5	25
20	7.8	2.5	50
21	7.8	10	0
22	7.8	10	5
23	7.8	10	25
24	7.8	10	50
25	9	0	0
26	9	0	5
27	9	0	25
28	9	0	50
29	9	2.5	0
30	9	2.5	5
31	9	2.5	25
32	9	2.5	50
33	9	10	0
34	9	10	5
35	9	10	25
36	9	10	50

Conc. = concentration.

after drawing the 5 mL sample for total Cu concentration analysis, a 10 mL sample was pipetted from the water column of each CuNP exposure suspension and centrifuged at 30392 g for 30 min at 4 °C (Sorvall RC5Bplus centrifuge, Fiberlite F21-8 × 50y rotor). The supernatant was then filtered through a syringe filter with 0.02 μm pore diameter (Anotop 25, Whatman). The filtrate was digested by nitric acid and analyzed for the concentration of dissolved Cu shedding from CuNPs in the water column via ICP-OES.

2.3. Data analysis

A univariate general linear model with a Tukey's post Hoc test was developed using SPSS 16.0 to analyze the significance and the contribution (%) of each treatment and their interaction effects on aggregation, zeta-potential and dissolution of CuNPs and on the total amount of Cu remaining in the water column after 48 h of incubation. Data were checked for normality and the homogeneity before analysis of variance (ANOVA). The significant level in all calculations was set at $p < 0.05$.

3. Results and discussion

3.1. Total Cu suspending in the water column upon modification of water chemistry

The total amount of Cu suspended in the water column represents the level of Cu to which pelagic organisms are likely exposed (Adeleye et al., 2014). It includes dissolved Cu shedding from CuNPs which freely suspended in the water column and suspended particulate Cu and may also include the dissolved Cu complexed by DOC. The dynamics of the total amount of Cu remaining in the water column within 48 h of incubation upon various environmental conditions are presented in Fig. 1. Due to sedimentation, the total amount of Cu in the water column dropped after suspending the particles in the various media tested in this study. Especially in the DOC containing-suspensions at 10 mM of divalent cations, the majority of the total added Cu was removed from the water column by sedimentation within the first 12 h of incubation (Fig. 1). The average Cu concentration remaining in the water column after 48 h of incubation corresponding to the magnitude of each factor, is given in Fig. 2. The average value for each factor enables comparison among the levels of that factor (Majedi et al., 2014). It is clear that the average total Cu concentration decreased with the addition of DOC and with increasing divalent cation content, especially at 10 mM of divalent cations. The lowest amount of Cu determined in the water column was observed for the cases of 10 mM of divalent cations and 50 mg C/L at pH 7.8 and pH 9 (Fig. 1), at which only 14% of the initially added CuNPs remained in the water column after 48 h of incubation.

Our results demonstrated that the divalent cation content was the most influential factor regarding the total concentration of Cu suspending in the water column, which explained around 60% of the variation of total concentration of Cu remaining in the water column after 48 h of incubation (Table 2). The next most important factor was DOC concentration, as this parameter explained approximately 15% of the change of concentration of Cu suspended in the water column after 48 h of incubation. Our results are similar with the findings of Peeters et al. (2016), who found that in landfill leachate the settling effect of ionic strength prevailed the dispersion effect of NOM on Fe-based NPs. The pH was shown to insignificantly affect the concentration of Cu remaining in the water column after 48 h ($p > 0.05$, Table 3). pH has been reported to significantly affect the sedimentation of NPs only when it approaches the point of zero charge (PZC) of NPs, at which NPs are expected to aggregate most and aggregation-accompanied sedimentation occurs (Hsiung et al., 2016; Peng et al., 2017). In this study, pH in the range of 6–9 did not significantly affect the zeta-potential of CuNPs and the PZC of CuNPs was not included in the pH range investigated (Table 3). This is probably why pH had an insignificant effect on the total Cu concentration in the water column. The interaction between divalent cations and DOC could explain around 13% of the variation of the total Cu concentration in the water column after 48 h of incubation (Table 2). The interactive effect of divalent cations and DOC may arise from their effect on aggregation, as discussed in more detail below.

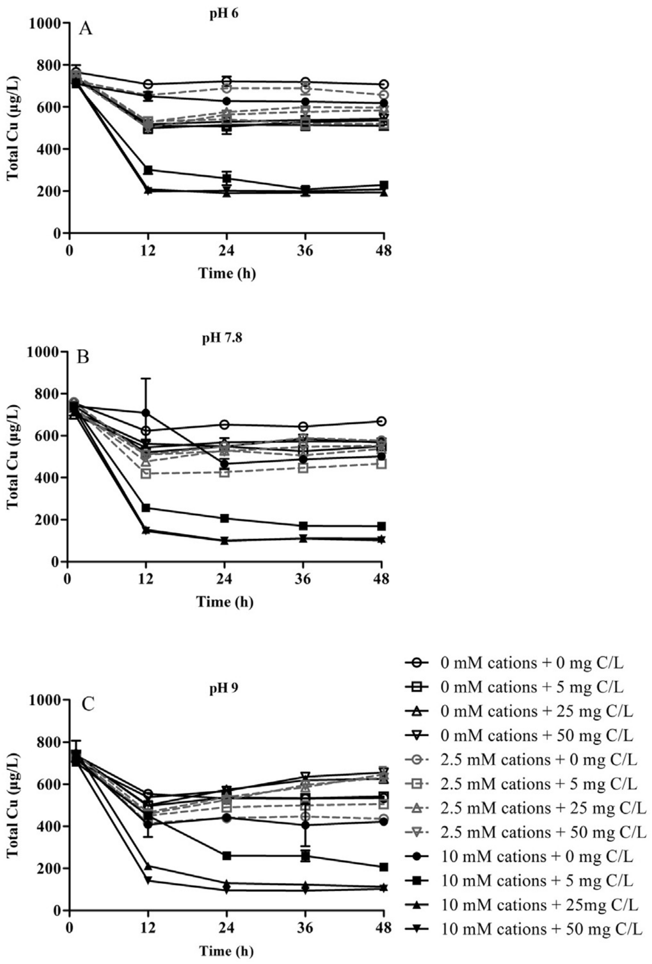

Fig. 1. The total amount of Cu remaining in the water column during 48 h of incubation of CuNPs across a range of pH, divalent cations and DOC concentrations. All data are presented as the mean ± standard deviation ($n = 2$).

3.2. Dissolution of CuNPs upon modification of water chemistry

Dissolution of NPs is a key process that determines to a considerable extent the bioavailability and toxicity of NPs to organisms. The results of the quantification of ions released from CuNPs after 48 h of incubation in media across a range of water chemistry are given in Fig. 3. In the absence of DOC, around 75%, 65% and 55% of the total added Cu was dissolved after 48 h of incubation at pH 6, 7.8 and 9, respectively (Fig. 3). The CuNPs used in this study displayed a relatively high solubility. In our previous study, less than 25% of ions released from CuNPs (with a pristine size of 50 nm) after 48 h of incubation in the suspension with 2.5 mM of divalent cations and pH of 7.8, were determined (Xiao et al., 2016). This difference in terms of solubility may arise from

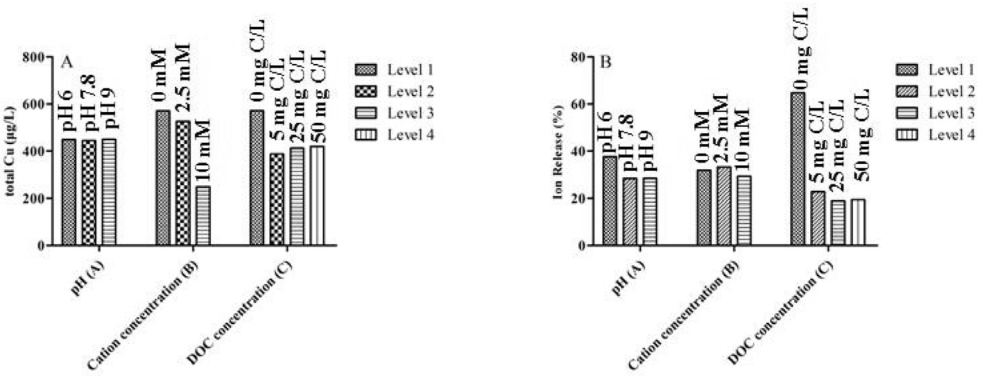

Fig. 2. Effect of each level of pH, divalent cations and dissolved organic carbon on the average of total amount of Cu remaining in the water column and on the percentages of ions released from CuNPs after 48 h of incubation.

Table 2
ANOVA techniques showing the treatments and their interaction effects on the total amount of Cu[a] remaining in the water column and dissolution[b] of CuNPs and hydrodynamic diameter[c] and zeta-potential[d] of CuNP suspensions after 48 h of incubation.

Source	Sum of square	Mean square	*F*-value	*p*-value	PC(%)
Total amount of Cu					
pH (A)	357	178	1.05	0.361	0.01
Divalent cation conc. (B)	1469155	734577	4311.00	<0.001	59.93
DOC conc. (C)	377406	125802	738.32	<0.001	15.39
A × B	77219	19305	113.30	<0.001	3.15
A × C	177427	29571	173.55	<0.001	7.24
B × C	306866	51144	300.16	<0.001	12.52
A × B × C	36946	3079	18.07	<0.001	1.51
Error	6134	170	–	–	0.25
Total	2451511	–	–	–	–
Dissolution					
pH (A)	1351	676	220	<0.001	4.56
Divalent cation conc. (B)	195	94	32	<0.001	0.66
DOC conc. (C)	26688	8896	2902	<0.001	90.05
A × B	415	104	34	<0.001	1.40
A × C	774	129	42	<0.001	2.61
B × C	55	9	3	<0.001	0.19
A × B × C	48	4	1	0.262	0.16
Error	110	3	–	–	0.37
Total	29636	–	–	–	–
HDD					
pH (A)	3483836	1741918	49.3	<0.001	7.47
Divalent cation conc. (B)	21420000	10710000	302.98	<0.001	45.95
DOC conc. (C)	10860000	3620919	102.42	<0.001	23.30
A × B	1046549	261637	7.40	<0.001	2.25
A × C	1267398	211233	5.98	<0.001	2.72
B × C	4908854	818142	23.14	<0.001	10.53
A × B × C	1080975	90081	2.55	<0.001	2.32
Error	2545557	35355	–	–	5.46
Total	46620000	–	–	–	–
Zeta-potential					
pH (A)	15	8	1.58	0.213	0.09
Divalent cation conc. (B)	14031	7015	1474.00	<0.001	86.20
DOC conc. (C)	1042	347	72.96	<0.001	6.40
A × B	173	43	9.08	<0.001	1.06
A × C	56	9	1.96	0.08	0.34
B × C	505	84	17.69	<0.001	3.10
A × B × C	113	9	1.99	0.038	0.69
Error	343	5	–	–	2.10
Total	16277	–	–	–	–

PC = Percentage contribution. Conc. = concentration. HDD = Hydrodynamic diameter.

[a] The coefficient of determination (R^2) of the model for total amount of Cu was 0.997, suggestive of good model fit with sufficient predictive power.

[b] The coefficient of determination (R^2) of the model for dissolution was 0.996, suggestive of good model fit with sufficient predictive power.

[c] The coefficient of determination (R^2) of the model for aggregation was 0.945, suggestive of good model fit with sufficient predictive power.

[d] The coefficient of determination (R^2) of the model for zeta-potential was 0.979, suggestive of good model fit with sufficient predictive power.

the different pristine size. On the other hand, the percentage of ions released from CuNPs in the water column was decreased 3–5 fold by the addition of DOC ranging from 5 to 50 mg C/L after 48 h of incubation, compared to the case without addition of DOC (Fig. 3). At pH 6, for example, the percentage of dissolution of CuNPs decreased from 75% without addition of DOC to 27% upon addition

Table 3
Hydrodynamic diameter and zeta-potential of CuNPs during 48 h of incubation in waters across a range of pH, divalent cations and DOC concentrations.

Trial no.	Hydrodynamic diameter (nm)[a]			Zeta-potential (mV)[a]		
	1 h	24 h	48 h	1 h	24 h	48 h
1	413 ± 29	556 ± 49	600 ± 72	−31 ± 1	−26 ± 5	−29 ± 4
2	134 ± 47	122 ± 37	127 ± 18	−39 ± 2	−35 ± 1	−35 ± 1
3	82 ± 16	93 ± 9	87 ± 8	−41 ± 5	−39 ± 2	−40 ± 3
4	80 ± 12	72 ± 21	77 ± 8	−45 ± 5	−41 ± 1	−42 ± 4
5	754 ± 217	903 ± 194	1383 ± 360	−13 ± 2	−14 ± 2	−9 ± 1
6	141 ± 34	127 ± 16	159 ± 25	−14 ± 1	−11 ± 1	−12 ± 1
7	110 ± 24	115 ± 18	149 ± 16	−12 ± 1	−12 ± 2	−12 ± 1
8	89 ± 19	105 ± 17	117 ± 13	−12 ± 1	−13 ± 2	−12 ± 2
9	780 ± 106	947 ± 61	854 ± 47	−7 ± 1	−8 ± 1	−9 ± 1
10	815 ± 80	988 ± 58	1047 ± 56	−11 ± 1	−9 ± 1	−10 ± 2
11	1099 ± 145	1047 ± 88	1135 ± 199	−11 ± 1	−10 ± 2	−11 ± 1
12	1156 ± 183	1138 ± 64	993 ± 162	−13 ± 1	−11 ± 1	−11 ± 2
13	465 ± 84	641 ± 173	518 ± 80	−34 ± 4	−27 ± 1	−26 ± 1
14	323 ± 65	191 ± 5	156 ± 14	−36 ± 12	−26 ± 2	−27 ± 4
15	191 ± 30	159 ± 14	236 ± 27	−42 ± 1	−42 ± 2	−39 ± 4
16	157 ± 14	168 ± 10	148 ± 8	−40 ± 4	−41 ± 3	−43 ± 6
17	637 ± 105	1008 ± 116	1650 ± 335	−17 ± 1	−12 ± 3	−10 ± 1
18	369 ± 41	486 ± 21	512 ± 23	−18 ± 1	−16 ± 1	−16 ± 2
19	373 ± 60	457 ± 23	468 ± 16	−19 ± 1	−18 ± 1	−17 ± 1
20	359 ± 19	445 ± 27	495 ± 17	−19 ± 1	−16 ± 3	−15 ± 1
21	795 ± 138	1301 ± 356	1773 ± 221	−10 ± 1	−8 ± 1	−7 ± 1
22	1004 ± 198	1273 ± 83	1287 ± 442	−15 ± 1	−14 ± 1	−13 ± 0
23	1217 ± 189	1441 ± 300	1398 ± 191	−16 ± 1	−15 ± 0	−14 ± 1
24	1540 ± 107	1208 ± 214	1605 ± 446	−16 ± 1	−14 ± 1	−14 ± 1
25	633 ± 58	824 ± 78	669 ± 119	−29 ± 1	−28 ± 1	−32 ± 1
26	385 ± 27	323 ± 38	324 ± 53	−32 ± 3	−31 ± 1	−31 ± 2
27	274 ± 43	236 ± 39	223 ± 4	−40 ± 3	−39 ± 4	−42 ± 3
28	263 ± 18	203 ± 13	212 ± 21	−42 ± 4	−42 ± 3	−42 ± 2
29	745 ± 93	1307 ± 172	2436 ± 490	−9 ± 2	−6 ± 3	−5 ± 4
30	456 ± 38	567 ± 45	553 ± 47	−14 ± 1	−14 ± 0	−13 ± 1
31	411 ± 13	559 ± 48	579 ± 22	−14 ± 0	−10 ± 6	−13 ± 0
32	403 ± 14	534 ± 50	559 ± 40	−14 ± 0	−14 ± 0	−13 ± 0
33	646 ± 141	1319 ± 163	2278 ± 407	−11 ± 1	−8 ± 1	−7 ± 1
34	1200 ± 113	1396 ± 74	1377 ± 35	−12 ± 1	−12 ± 1	−11 ± 0
35	1369 ± 45	1313 ± 101	1293 ± 158	−12 ± 0	−12 ± 0	−11 ± 0
36	1528 ± 52	1173 ± 106	1393 ± 99	−12 ± 0	−12 ± 0	−11 ± 0

[a] Hydrodynamic diameters and zeta-potentials are expressed as means ± SD ($n = 3$).

of 5 mg DOC/L after 48 h of incubation (Fig. 3). The reduction of dissolution of metallic NPs by the addition of DOC has also been reported by some other studies (Liu and Hurt, 2010; Conway et al., 2015). The NOM-dependent reduction of the concentration of dissolved Cu shedding from CuNPs in the water column was possibly due to the combination of complexation of dissolved Cu by DOC and of coating by DOC of the surface of Cu particles (Conway et al., 2015).

The average percentage of ions released from CuNPs ranked according to the importance of the water properties considered, is given in Fig. 2. The highest percentage of dissolved Cu shedding from CuNPs was occurred when no DOC was added. The addition of DOC at a concentration of 5 mg C/L significantly reduced the percentage of dissolved Cu from 65% without addition of DOC to around 29%. The highest solubility of CuNPs occurred at the situation with 2.5 mM of divalent cations, pH of 6 and without addition of DOC (Fig. 3). Furthermore, our results showed that more than 90% of the variation of the dissolution of CuNPs could be explained by the DOC concentration (Table 2). It is worth to note that pH, which is often considered as the most influential factor on NP dissolution (Majedi et al., 2014; Odzak et al., 2014), only accounted for 5% of the variation of the dissolution of CuNPs in this study (Table 2). According to the dissolution mechanisms of metallic NPs (Bian et al., 2011; Wang et al., 2016), reaction of metal-NPs with protons is expected only under acidic conditions on the basis of the reactivity of nanomaterials. The CuNPs used in this study displayed a relatively high reactivity due to their small pristine size (as mentioned above), which hence may overshadow the influence of pH on the dissolution process of CuNPs. Bian et al. (2011) found that pH was more likely to affect dissolution of metallic NPs under highly acidic and alkaline conditions (lower than pH 6 and higher than pH 9) than in the pH range from 6 to 9. Hence, consideration of the role of pH in the dissolution of metallic NPs needs to be interpreted with care. In our study, divalent cations were found to play a negligible role in the dissolution of CuNPs (Table 2). According to Debye–Hückel law, one would expect a rise in the solubility with increasing ionic strength to reduce chemical potential of the solution (Debye and Hückel, 1923). However, it was found that aggregation of CuNPs was enhanced with increasing divalent cation content (as discussed below). The enhanced aggregation may result in a reduction of dissolution of NPs, as the enhanced aggregation reduced the surface area of NPs. Hence, the effect of enhanced aggregation on dissolution of CuNPs with increasing divalent cation content would likely counteract the effect of reduced chemical potential on dissolution.

3.3. *Aggregation of CuNPs upon modification of water chemistry*

The image captured by the transmission electron microscopy demonstrated that the pristine shape of the CuNPs was spherical and CuNPs aggregated rapidly after submersion into the exposure medium (Fig. S1). Since no individual well-defined NPs could be determined by TEM, size analysis was not conducted. In this study, the aggregation behavior of the CuNPs was strongly modified by

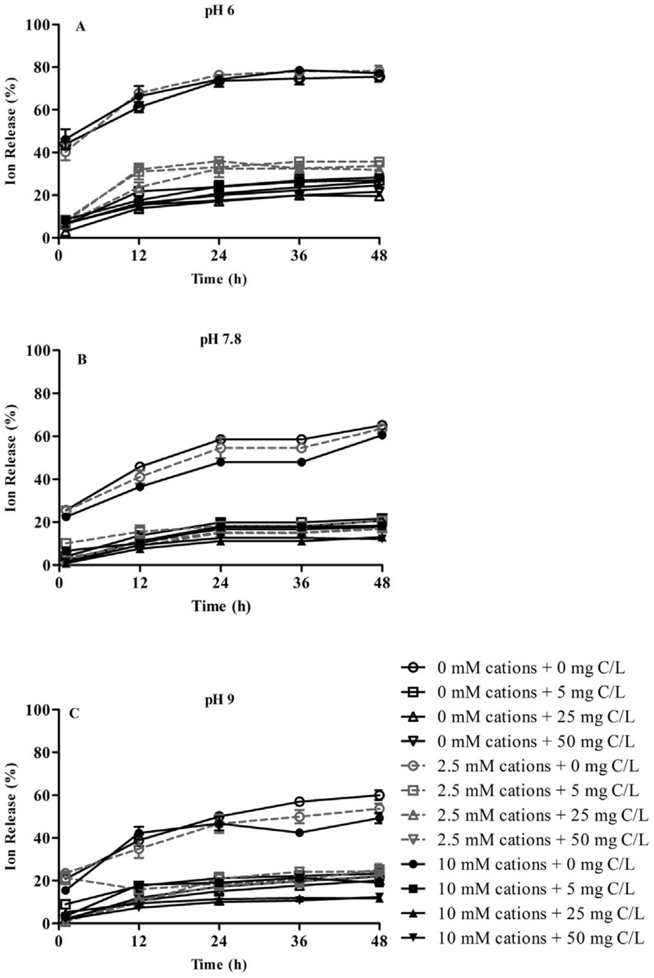

Fig. 3. Dissolution profiles of CuNPs across a range of pH, divalent cations and DOC concentrations. All data are presented as the mean ± standard deviation ($n = 2$).

the composition of the media (Table 3). The average hydrodynamic diameter and zeta-potential of the CuNP suspensions as a function of the environmental parameters tested in this study, are presented in Fig. 4. It is evident that the hydrodynamic diameters of particles increased with a rise in pH and with increasing divalent cation content, while the hydrodynamic diameter of particles dropped with the addition of DOC. The smallest hydrodynamic diameter was around 80 nm after 48 h of incubation in the suspension with 50 mg DOC/L and pH of 6 when no divalent cations were added (trial 4 in Table 3). The addition of divalent cations largely reduced the absolute value of the zeta-potential of CuNP suspensions (Fig. 4).

The aggregation behavior of the CuNPs was significantly modified by the divalent cation content, the DOC concentration and pH as well as by their interactions ($p < 0.05$, Table 2). Among these three parameters, the divalent cation content was the most influential factor for the variation of the aggregate size of suspensions of CuNPs. The factor of divalent cation content explained around 46% of the change of hydrodynamic diameter of CuNPs in exposure media across a range of water chemistry (Table 2). Meanwhile, the divalent cation content was also the predominant factor in influencing the zeta-potential of CuNP suspensions ($p < 0.05$, Table 2), which was far more important than DOC in determining the zeta-potential of the suspensions. This is probably due to a rapid adsorption of divalent cations on the surface of DOC and NPs, neutralizing the negative charges and reducing the absolute value of the zeta-potential of the suspensions. According to the Derjaguin- Landau-Verwey-Overbeek theory, the stability of colloidal particles in aqueous environments is determined by the sum of Van der Waals forces and electrical double layer repulsions. In this study, the addition of divalent cations significantly decreased the absolute values of the zeta-potential of CuNP suspensions ($p < 0.05$, Table 2), leading to the reduction of electrical double layer repulsion between particles. Enhanced aggregation of CuNPs with the addition of divalent cations was hence likely due to net attractive force between particles as a result of prevailing Van der Waals attraction.

The second most important factor was the DOC concentration, which explained around 23% of the variation in aggregate size of CuNPs (Table 2). The interaction between the divalent cations and DOC was also important in influencing the aggregation of CuNPs, accounting for 11% of the variation of aggregate sizes (Table 2). The interaction between divalent cations and DOC can influence the aggregation of NPs via affecting the surface charge of NPs and via their bridging effect (Delay et al., 2011). In this study, the stabilizing effect of DOC on CuNPs decreased with a rise in divalent cation content (Table 3 and Fig. 4). At 2.5 mM of the cations, the average hydrodynamic diameter of the CuNP aggregates was approximately 500 nm upon addition of DOC with a concentration in between 5 and 50 mg C/L (trials 18, 19 and 20 in Table 3), which was larger than the case in which no cations were added (around 200 nm, trials 14, 15 and 16 in Table 3) and much smaller than the case in which no DOC was added (around 1650 nm, trail 17 in Table 3).

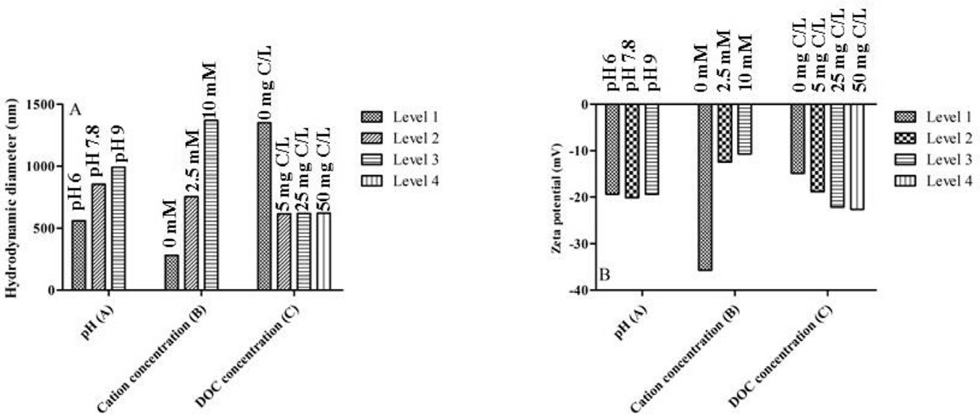

Fig. 4. Effect of each level of pH, divalent cations and dissolved organic carbon on the average of hydrodynamic diameter and zeta-potential of CuNP suspensions.

These results suggest that DOC could still stabilize the NPs at 2.5 mM of divalent cations. In fact, when the cations were added the zeta-potential of CuNP suspensions was higher than −20 mV even in the presence of DOC for up to 50 mg C/L (Table 3). Colloidal suspensions are generally inherently unstable and tend to aggregate if the absolute value of the zeta-potential is smaller than 30 mV (Schramm, 2005). This suggests that the stabilization effect of DOC on CuNPs in the divalent cations-containing suspensions is likely to result from steric hindrance imparted by DOC. Similarly, Delay et al. (2011) also found that even though addition of 1 mM of Ca^{2+} into aqueous Ag NP systems containing NOM resulted in electrostatic destabilization of the particles, the steric repulsion imparted by NOM still stabilized the particle aggregates and allowed them to disperse uniformly in suspension. However, in our study, the addition of DOC into the CuNP suspensions containing 10 mM of divalent cations led to the formation of HA-flocs, which then settled quickly. The formation of flocs is attributed to the bridging effects between divalent cations and DOC, which can result in an enhanced aggregation and sedimentation of NPs (Stankus et al., 2011; Romanello and Fidalgo de Cortalezzi, 2013). The massive sedimentation of CuNPs observed in DOC-containing suspensions at 10 mM of divalent cations (Fig. 1), as mentioned above, hence could arise from the interparticle bridging by the formed HA-flocs. In order to quantify the interaction between DOC and divalent cations, we first determined the concentrations of DOC in the water column across a range of divalent cation contents within 48 h. The results showed that the DOC concentrations in the water column at 0 and 2.5 mM of divalent cations maintained similar within 48 h of incubation, while at 10 mM of cations, the concentration of DOC within the range between 5 and 50 mg C/L dropped significantly over time (Fig. S2). Furthermore, the concentration of the divalent cations in the presence of 25 mg DOC/L was also monitored within 48 h of incubation. It was found that in the CuNP suspension containing 25 mg/L of DOC and 10 mM of divalent cations, both the concentrations of Ca^{2+} and Mg^{2+} in the water column decreased by around 30% after 48 h of incubation (Fig. S3). Our results support the findings reported by Stankus et al. (2011) that both Ca^{2+} and Mg^{2+} could bridge with DOC, resulting in an enhanced aggregation of Au NPs. In addition, it is worth to point out that the average aggregate sizes measured at 1 h of the DOC-containing suspensions of CuNPs at 10 mM of divalent cations may mainly arise from the HA-flocs rather than from CuNPs, as the hydrodynamic diameters of suspensions without CuNPs (combination of DOC and 10 mM of divalent cations) were similar to the suspensions with CuNPs (Table 3 and Fig. S4). However, the HA-flocs sediment rapidly and intensively, and after 12 h of sedimentation there was no significant change of DOC concentration in the water column (Fig. S5). The average hydrodynamic diameters of DOC-containing suspensions at 10 mM of divalent cations measured at 24 h and 48 h were similar (around 600 nm, Fig. S4), and smaller than the average hydrodynamic sizes of suspensions with CuNPs (Table 3). Hence, employing the average hydrodynamic diameters measured at 48 h to analyze the impact of environmental parameters on the aggregation of CuNPs, could reduce the influence of HA-flocs on the average sizes of CuNP aggregates.

Aggregation of particles tends to be most when pH approaches the point of zero charge (PZC) (Adam et al., 2014). In this study, it was found that the PZC of CuNPs was not within the pH range from 6 to 9. To date, different PZC values of Cu-based particles have been reported. Li and Chang (2004) suggested that the PZC of Cu-based NPs was located at a pH of 9.9 (needle-shaped particles with one dimension < 50 nm). Similarly, Sousa and Teixeira (2013) found that the PZC of Cu-based NPs (round particle < 50 nm) was near pH 10. On the other hand, Guedes et al. (2009) found that the PZC of Cu-based particles (with an average size of 1.9 μm) was at pH 7.9. Although pH in the range from 6 to 9 was found to have an insignificant effect on the zeta-potential of the CuNP suspensions ($p > 0.05$, Table 2), it still significantly influenced the hydrodynamic diameter of CuNPs, accounting for 7% of the variation of the average aggregate size of the CuNPs ($p < 0.05$, Table 2). This may result from the effect of pH on the dissolution of CuNPs, which could further affect the hydrodynamic diameter of particles (Domingos et al., 2009; Song et al., 2014). It is interesting to point out that the interactions between pH and other factors could also significantly affect the aggregation of CuNPs ($p < 0.05$, Table 2). For instance, DOC was more pronouncedly inhibiting aggregation at pH 6 than at pH 7.8 and 9, as the average aggregate sizes at pH 6 were much smaller than at pH 7.8 and 9 after 48 h of incubation (Table 3). This may arise from the effect of pH on the deprotonation/protonation and adsorption capacity of DOC (Yang et al., 2009; Van Hoecke et al., 2011), leading to a change of stabilizing effect of DOC on CuNPs. It is important to point out that the order in terms of the significance of each factor in influencing the aggregation behavior of CuNPs was similar to the order of significance of each factor in influencing the total amount of Cu in the water column (Table 2). These results indicate that the aggregation behavior of CuNPs rather than their dissolution behavior determined the variation of sedimentation profiles of CuNPs.

4. Conclusions

This study systematically investigated the main and interactive effects of pH and concentrations of divalent cations and DOC in the exposure medium on the behavior and fate of CuNPs. The results demonstrated that the total amount of Cu remaining in the water column after 48 h of incubation was mostly influenced by divalent cation content, DOC concentration and the interaction of divalent cations and DOC. DOC concentration was the predominant factor in explaining the variation of dissolution of CuNPs, which was far more important than the effect of pH in the range from pH 6 to pH 9 on the dissolution of CuNPs. The divalent cation content was the most influential factor regarding the aggregation behavior, followed by the DOC concentration. The interaction between divalent cations and DOC also played an important role in influencing the aggregation of CuNPs. In addition, the sedimentation profile of Cu was more influenced by the aggregation behavior of CuNPs than by their dissolution.

The results provided in this study are helpful for improving our understanding and the prediction potential on how and to what extent environmental factors affect the behavior and fate of metallic NPs upon a range of environmental conditions. In addition, our results can help to optimize the testing guidelines for assessing the toxicity of NPs for the aim of improving the reproducibility of NP toxicity tests and making sure that the test organisms are exposed mainly to NPs rather than to larger aggregates and to the ions released from the NPs.

Acknowledgements

Yinlong Xiao is financially supported by the Chinese Scholarship Council (CSC). M.G. Vijver was funded by VIDI 864.13.010. We would like to thank Marja Wouters for the preparations of the DOC suspensions and for measuring DOC concentrations. The research described in this work was performed within the framework of the “NANOFASE” project supported by the European Union's Horizon 2020 research and innovation programme under grant agreement number 642007.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.12.015.

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