Influence of heavy metal ions on the spectra and charge characteristics of DOM of municipal sewage secondary effluent

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ABSTRACT

The spectral and charged characteristics of dissolved organic matter (DOM) in municipal sewage secondary effluent influenced by heavy metal ions were studied by three-dimensional fluorescence spectroscopy, ultraviolet spectrometry, etc. As the Cu(II) concentration increased, the fluorescence intensity of the DOM’s fluorescence peak reduced. When the Cu(II) concentration was 100 μmol/L, the fluorescence intensity of the humic-like acid in the DOM reduced by 19.5%–27.6%. However, there was no obvious change in the fluorescence intensity when Cd(II) was added. According to the modified Stern–Volmer equation, the conditional stability constant lgK and the proportion of the fluorescent functional groups of the DOM binding with Cu(II) were 2.19–2.69 and 16%–48%, respectively. The UV253/UV203 value changed little with the additional dosage of metal ions below 10 μmol/L, which increased as the concentration of Cu(II) or Pb(II) increased from 10–100 μmol/L. As the metal concentrations increase, the absolute values of the Zeta potential and the particle sizes of the DOM basically increased. The results showed that Cu(II) had the strongest binding ability to the groups that emitted fluorescence in the secondary effluent DOM compared to Pb(II) and Cd(II).

Key words | charged characteristic, dissolved organic matter, heavy metal ions, secondary effluent, spectral characteristic

INTRODUCTION

Heavy metal pollution is a serious environmental problem. The degree of contamination depends mainly on the species, chemical forms and concentrations of heavy metals in the environment, food and organisms (Schaller et al. 2011). Heavy metals can enter organisms through a variety of ways, accumulate through the food chain, and further exacerbate the harm to the ecosystem. Some studies have found that people’s internal organs (such as the lungs or kidneys) can be subject to certain damage when exposed to an environment with high concentrations of heavy metals for a long time (Bocca et al. 2004). According to China’s 2014 Environmental Status Bulletin, the important fishery waters of rivers were mainly polluted by copper and cadmium, and the lakes and reservoirs were affected by copper. DOM is an active component that widely exists in water, soil and sediment. DOM can combine with heavy metals and affect the chemical behavior of heavy metals in the environment (Rangsivek & Jekel 2005; Zhu et al. 2010; Nebbioso & Piccolo 2013). The secondary effluent of municipal wastewater is an important water resource, most of which is discharged into receiving water bodies. The organic matter in the secondary effluent is based on dissolved organic carbon, which accounts for approximately 86% of the chemical oxygen demand (Shon et al. 2006; Zhu et al. 2010). Therefore, it is necessary to research the interaction of the heavy metal ions and DOM in the secondary effluent.

At present, the studies on the interactions between the DOM and heavy metals mainly include the following aspects. (i) The structure or molecular weight characterization of different components of DOM and its influence mechanism on the adsorption-desorption of heavy metals in particulate matter (Chen et al. 2002; Inaba & Takenaka 2005; He et al. 2008; Wang et al. 2010). Kaschl Römheld &
Chen (2002) divided DOM into hydrophilic and hydrophobic components with XAD-8 resin, and further subdivided them into six components via different eluents. Protein and amino sugars material had a strong binding capacity with Cd(II), while polysaccharides had a relatively weak combining ability. (ii) The migration rule of heavy metals affected by the DOM. DOM can form a coordination compound with heavy metals through complexing, thereby restraining the sediment of the heavy metals. In addition, the DOM can also interact with soil and minerals, enhancing its transfer (Huang et al. 2002; Lamelas et al. 2005; Sánchez-Marín et al. 2010). (iii) The biological availability of heavy metals affected by the DOM (Croteau & Luoma 2007; Nogueira et al. 2009; Wang et al. 2011; Shen 2012).

Many studies have suggested that DOM could form organic complexes with heavy metals, promote the desorption of heavy metals, and increase their biological availability; however, a few studies have also found that DOM could reduce the availability of heavy metals and reduce the biological toxicity in some cases (e.g. in high concentrations) (Trenfield et al. 2011).

The current research focuses mainly on the interaction between the DOM in soil and lakes and heavy metals, while little is known about the DOM of the secondary effluent from sewage treatment plants. In addition, DOM from different sources has various degrees of humification, molecular sizes, functional group kinds and contents, all leading to the different binding characteristics of the DOM with heavy metals (Hulatt et al. 2009; Feng et al. 2013; Xie et al. 2015a; Yao et al. 2014). Therefore, in this paper, the binding characteristics of DOM in secondary effluent with additional Cu(II), Pb(II) and Cd(II) was studied using three-dimensional fluorescence spectrometry, ultraviolet-visible (UV-VIS) spectrophotometry, Zeta potentiometer and other analysis instruments. The stability constant for the heavy metal-DOM complex formation was explored, which provides the theoretical basis for the interaction law of the heavy metals with the DOM.

MATERIALS AND METHODS

Water sample collection and experiment content

Water samples were collected from the secondary effluent of three municipal sewage treatment plants in Xi’an. The orbal oxidation ditch process, anaerobic-anoxic-oxic process and anaerobic-anoxic-oxic process were used in municipal sewage treatment plants A, B and C, respectively. The water samples were additionally filtered with a 0.45-μm Millipore filter.

Cu(NO₃)₂, Pb(NO₃)₂ and Cd(NO₃)₂ solutions were prepared with AR copper nitrate, lead nitrate and cadmium nitrate with a concentration gradient of 0.01–10 mmol/L. The additional concentration of Cu(II), Pb(II) or Cd(II) in 50.0-ml samples to be tested was in the range of 0–100 μmol/L. The added solution volume was not more than 0.5 ml.

The three-dimensional fluorescence spectrum, UV spectra, Zeta potential and particle size were measured after the DOM reacted with the different heavy metal ions.

Analytical methods and experimental instruments

A Jasco FP-6500 spectrofluorometer with a Xenon lamp light source was used to determine the three-dimensional fluorescence contour line plots. The voltage of its photomultiplier tube was 700 mV, and the slit widths of the excitation and emission were respectively set to 5 nm and 1 nm at an excitation wavelength and emission wavelength of 220–480 nm and 230–550 nm, respectively. The scan interval was 5 nm at a scan speed of 5,000 nm/min. Ultrapure water was used as a blank correction. The fluorescence index (FI) is the ratio of the fluorescence intensities at emission wavelengths of 450 nm and 500 nm and an excitation wavelength of 370 nm (McKnight et al. 2001; Xiao et al. 2014; Gao et al. 2017). The FI represents the source of humus and its aromaticity sensitivity.

The absorption spectras of the DOM were scanned over 190–500 nm by a UV1650PC UV-Vis spectrometer (Shimadzu, Japan) with a path length of 1 cm and ultrapure water as a blank correction. UV₂₅₃/UV₂₀₅ is the absorbance ratio at the wavelengths of 253 nm and 205 nm (Huoivinen et al. 2003). E₃/E₄ is the absorbance ratio at the wavelengths of 300 nm and 400 nm, respectively, which is an index representing the humification degree and reflecting the aromaticity and molecular weight of organic matter (Xie et al. 2013b).

The Zeta potential and particle size were measured by a Nano-ZS ZEN3690 nanoparticle analyzer and Zeta potentiometer, which was set to scan three groups with 20 times per group. The Zeta potential and particle size were analyzed by an electrophoretic light scattering method and by a dynamic light scattering technique, respectively, which were used to identify the charge characteristics and aggregation properties of the colloids.
Coordination stability constant

It was assumed that the heavy metal ions were complexed by the fluorescent functional groups of the secondary effluent DOM at a ratio of 1:1. The quenching rate between the fluorescent molecules and the quenching agents followed the modified Stern–Volmer equation (Leher 1971), which was used to calculate the coordination stability constant below.

\[ \frac{F_0}{(F_0 - F)} = \frac{1}{f \cdot K \cdot [C]} + \frac{1}{f} \]  

where \( F_0 \) is the fluorescence intensity without adding metal ions, \( F_i \) presents the fluorescence intensity of the DOM at the addition of different concentrations of metal ions, \([C]\) is the added concentration of metal ions, \( f \) is the proportion of the fluorescent functional groups complexed by the metal ions, and \( K \) presents the modified Stern–Volmer quenching constant, which is the conditional stability constant.

\( F_0/(F_0 - F) \) and \( 1/[C] \) are employed to obtain the linear relationship. The slope and the intercept are \( 1/(fK) \) and \( 1/f \), respectively. \( K \) can be obtained by the ratio of the intercept to the slope, and \( f \) is the reciprocal of the intercept.

RESULTS AND DISCUSSION

Fluorescence characteristics

Figures 1, 2 and 3 shows the fluorescence contour line spectra of the secondary effluent DOM reacting with Cu(II), Pb(II) and Cd(II), respectively.
Pb(II) or Cd(II) when the additional concentrations of the metal ions were 1 μmol/L, 10 μmol/L and 50 μmol/L in sewage treatment plant A. The fluorescence intensity of the fluorescence peak and the FI are shown in Figures 4, 5 and 6. There was an obvious fluorescence peak in the fluorescence contour line spectra of the sewage effluent DOM from the three municipal sewage treatment plants, with an excitation wavelength and emission wavelength at 350–355 nm and 431–435 nm respectively. These results indicate that the secondary effluent DOM contained humic-like acids (Zsolnay et al. 1999). The fluorescence intensity decreased with the increase of the additional Cu(II) concentration, as seen in Figure 4(a). The fluorescence intensity of the DOM in sewage treatment plants A, B and C decreased 0.7%, 0.87% and 0.7%, respectively, with 0.1 μmol/L of the added Cu(II) concentration, and reduced 19.5%, 27.6% and 26.5% after adding 100 μmol/L of the Cu(II) solution. The above phenomenon indicates that the Cu(II) could complex with humic-like acids emitting fluorescence, which leads to a fluorescence quenching (Sun et al. 2017). The fluorescence intensity of the secondary effluent DOM in sewage plants A and B showed a slight decrease with an increase in the concentration of Pb(II), as seen in Figure 5(a). There was no obvious change in the fluorescence intensity when the secondary effluent DOM in sewage plant C was augmented with Pb(II) (Figure 5(a)) and in the three sewage plants when Cd(II) was added (Figure 6(a)).

As the Cu(II) concentration increased, the FI value slightly increased, as shown in Figure 4(b). Furthermore, the FI value changed little when the Cu(II) concentration

Figure 4 | Change of fluorescence intensity and the FI of the DOM reacting with Cu(II). (a) Fluorescence intensity and (b) FI.

Figure 5 | Change of fluorescence intensity and the FI of the DOM reacting with Pb(II). (a) Fluorescence intensity and (b) FI.

Figure 6 | Change of fluorescence intensity and the FI of the DOM reacting with Cd(II). (a) Fluorescence intensity and (b) FI.
ranged from 30 μmol/L to 80 μmol/L. The FI values at a dosage of 0.1 μmol/L Cu(II) in treatment plants A, B and C were 2.5, 2.04 and 2.12, and at a dosage of 100 μmol/L, Cu(II) values were 2.7, 2.13 and 2.22, respectively. Thus, the FI values under which the DOM bound with the Cu²⁺ ranged from 2.04–2.7.

When the addition of Pb(II) or Cd(II) was between 0.1 μmol/L and 100 μmol/L, the FI values under which the DOM reacted with Pb(II) in treatment plants A, B and C were 2.42–2.47, 2.05–2.11 and 2.13–2.20, respectively, as shown in Figure 5(b), and were 2.47–2.50, 2.03–2.12 and 2.10–2.16, respectively, when Cd(II) was added, as shown in Figure 6(b). The change of the FI value was irregular and small with an increase in Pb(II) or Cd(II) concentration.

Based on the FI value, one can conclude that the secondary effluent DOM was mainly from a biological source, and the aromatic degree of the humus contained in the DOM was relatively low (Cory & McKnight 2005).

The FI is negatively correlated with humus aromaticity (McKnight et al. 2003). The FI values of the secondary effluent DOM in sewage treatment plants A, C and B reduced in turn, indicating that their humification degree and aromatic sensitivity had increased.

### Coordination function constants

The modified Stern–Volmer equations and the linear relationships between the $F_0/(F_0-F)$ and $1/C$ are shown in Table 1.

<table>
<thead>
<tr>
<th>Fluorescence peak</th>
<th>lgK/(L/mmol)</th>
<th>f/[%]</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sewage plant A</td>
<td>2.69</td>
<td>16</td>
<td>0.9981</td>
</tr>
<tr>
<td>Sewage plant B</td>
<td>2.67</td>
<td>19</td>
<td>0.9984</td>
</tr>
<tr>
<td>Sewage plant C</td>
<td>2.19</td>
<td>48</td>
<td>0.9917</td>
</tr>
</tbody>
</table>

Figure 7. The coordination function constants of the DOM reacting with Cu(II) are shown in Table 1.

Figure 7(a) shows that the $F_0/(F_0-F)$ and $1/C$ had strong linear relationships when the DOM bound with Cu(II), and the $R^2$ values were 0.9981, 0.9984 and 0.9917 for treatment plants A, B and C, respectively. The conditional stability constants $lgK$ were 2.69, 2.67 and 2.19, as seen in Table 1, and the proportion of the fluorescent functional groups complexing with Cu(II) were 16%, 19% and 48%, respectively. However, the linear relationships of the $F_0/(F_0-F)$ and $1/C$ with the addition of the Pb(II) or Cd(II) were very weak, as shown in Figure 10(b) and 10(c). This is consistent with the variation trend of the fluorescence intensity.

### UV$_{253}$/UV$_{203}$ and E$_3$/E$_4$

The UV$_{253}$/UV$_{203}$ value changed little when the additional dosage of three kinds of metal ions was less than 10 μmol/L (Figures 8(a), 9(a) and 10(a)). The UV$_{253}$/UV$_{203}$ increased with the increased concentration of Cu(II) or
Pb(II) at the additional dosage of 10–100 μmol/L. One can conclude that the substitution degree of the benzene ring from the DOM increased gradually (Korshin et al. 1997). The UV$_{253}$/UV$_{203}$ value changed little with the addition of Cd(II), which indicates that the substitution degree of the benzene ring contained in the DOM was essentially unchanged.

Figures 8(b), 9(b) and 10(b) illustrate that the E3/E4 values of the DOM in municipal treatment plants B and C changed slightly after adding heavy metals, indicating that the humification degree of the humic acid was basically stable, while the E3/E4 value of the DOM in treatment plant A decreased at the additional dosage of 50–100 μmol/L.

The E3/E4 values of the secondary effluent DOM in sewage treatment plant A were greater than those in plants B and C, indicating that its humification degree and aromatic sensitivity were lower. This is mainly because the source, composition and structure of the secondary effluent DOM are diverse and complex, leading to the different results.

**Charged characteristic and aggregation property**

Figures 11, 12 and 13 show the charged characteristics and aggregation property of the secondary effluent DOM in three urban sewage treatment plants mixed with different metal ions. The Zeta potential gradually decreased with an
increase in the metal ion concentrations, as seen in Figures 11(a), 12(a) and 13(a). When the added concentrations of the Cu(II), Pb(II) or Cd(II) were 0.1 μmol/L and 100 μmol/L, the Zeta potential values of the secondary effluent DOM in the three sewage treatment plants were within −4.65 to −7.94 mV and −11.7 to −21.6 mV, respectively. Because the DOM complexed with heavy metal ions (Chappaz & Curtis 2013; Yuan et al. 2015; Lu et al. 2017), the acidic functional groups of the DOM molecule dissociated gradually, and the amount of protons released increased as the metal ion concentrations increased.

The particle sizes of the DOM increased substantially as the metal ion concentration increased. The particle sizes were 65–351 nm and 332–1,249 nm when the added Cu(II), Pb(II) or Cd(II) concentrations were 0.1 μmol/L and 100 μmol/L respectively. The main reason for this observation is that the metal ions were regarded as the centers of the new colloidal particles, which made multiple DOM molecules link together, leading to an increase in the particle sizes (Guggenberger et al. 1994; Lu & Allen 2002). Additionally, the DOM molecules stretched, or the particles collided with each other because of an increase in repulsion, causing the particle size to increase.

**CONCLUSIONS**

The secondary effluent DOM could complex with Cu(II), which led to a clear fluorescence quenching phenomenon. The fluorescence quenching rate had a strong linear
relationship with the reciprocal of the additional Cu(II) concentration. There was no obvious change in the fluorescence intensity of the secondary effluent DOM augmented with Cd(II). The conditional stability constants $\log K$ and the proportion of the fluorescent functional groups complexing with Cu(II) were 2.19–2.69 and 16–48%, respectively.

The $UV_{253}/UV_{203}$ value changed little at a lower additional dosage of the metal ions, increasing at a higher additional concentration of Cu(II) or Pb(II). The $UV_{253}/UV_{203}$ value changed little with the addition of Cd(II). The E3/E4 values changed slightly after adding the heavy metals. Perhaps the E3/E4 value decreased at a higher additional dosage of metal ions. The absolute value of the Zeta potential and the particle size increased with the increased concentration of Cu(II), Pb(II) and Cd(II).

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