Introduction

Mercury (Hg) is a type of nervous poison element that exists in the atmosphere, water, and soil. It can threaten human health and the greater ecosystem either directly or indirectly [1-3]. For example, when consumed through food, high concentrations of mercury and methyl-mercury (MeHg) may affect humans’ and animals’ nervous and reproductive systems. In addition, Hg and MeHg can change birds’ sexual orientation by disrupting the release of gonadal hormones, and bring into play adverse effects such as endocrine disruption and neurodevelopmental impairment in carnivores.
in the city development area that restrict industrial and ecological functional reserve as permanent restrictions. In 2009 the Jiangsu Provincial Environmental Protection Bureau approved the establishment of the Wujiang Tongli Wetland, with an area of nearly 690 hm², of which water comprises 240 hm² along with land coverage of 450 hm². The wetland covers a diverse ecosystem with 170 species of plants, including ginkgo, metasequoia, and others; and a bird distribution consisting of 12 orders, 28 families, and 91 species (e.g., egrets, kingfishers, herons, etc.).

### Materials and Methods

#### Study Area

Tongli Wetland is located in the city of Wujiang in Jiangsu Province in eastern China. The wetland covers an area of nearly 690 hm², of which water comprises 240 hm² along with land coverage of 450 hm². In 2009 the Jiangsu Provincial Environmental Protection Office announced regional planning of the important ecological functional reserve as permanent restrictions in the city development area that restrict industrial and agricultural activities. And the pre-sampling survey also shows that the wetland had no industrial or agricultural activities. It is a good research area because it is less disturbed by artificial activities. The wetland has a diverse ecosystem with 170 species of plants, including ginkgo, metasequoia, and others; and a bird distribution consisting of 12 orders, 28 families, and 91 species (e.g., egrets, kingfishers, herons, etc.).

#### Sampling and Pretreatment

Soil composition: The soil type in this study area is waterlogged paddy, the main component is yellow pine soil, and the soil is viscous. It is formed by the accumulation of alluvial loamy parent material derived from the lake deposition. The soil is brownish yellow with a waterlogging layer typical development, and the edge block structure is obvious. At the same time, the structure surface has the continuous grey film and the section is covered with iron manganese rust spots. The soil in the forest zone is rich in dead branches and deciduous leaves, which makes it have an appreciable amount of organic matter and humus.

Soil samples were collected using a grid method (500 × 500 m) points, coarctation, or sparseness sampling depending on local topography and river development. For the purposes of this study, a soil sample refers to the mixture of a middle sampling point and four subsamples from four corners (south, north, east, and west) taken from the same sample site. Soil samples in Tongli Wetland: 15 soil samples that are affected by bird droppings (WS) (note: bird droppings covering the surface of the soil were removed in the process of collecting soil samples, so the soil does not contain bird droppings) and 15 soil samples that are not affected by bird droppings (OS) (Fig. 1). Bird droppings (BS) that covered the soil surface about 1-2 cm thick were gathered from four sites. A quadruplicate method was used to remove excess samples; 100-200 g of bird droppings were retained in total. In areas where bird activity is frequent, 15 fresh heron branch bird eggshells (ES) and 4 feathers (FS) were picked and placed in its own separate polyethylene plastic bags. All samples were collected during the summer.

After collection, soil and excrement samples were air-dried in the laboratory. All stones, plant root systems, organic residue, and visible invaders were removed. Then each sample was passed through a 0.85 mm mesh nylon sieve before being ground using an agate mortar and passed through a 0.15 mm mesh nylon sieve. In treating the eggshell samples, all residue was removed from the shell surface using a soft brush, after which samples were rinsed for 10 minutes with an ultrasonic cleaning device. Next, samples were washed 3-5 times with ultrapure water and then placed in a beaker and put into an oven to dry for 12 hours at 60°C.

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Concentrations and Speciation of Mercury in Soils, Excrements, Eggshells, and Feathers

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dried samples were ground with an agate mortar and were passed through a sieve that is less than 0.15 mm in diameter and placed into new polyethylene plastic bags. The four feather samples were treated similarly to the eggshell samples, but in the final step dried feathers were cut using plastic scissors prior to being placed in plastic film bags.

Experimental Methods

Analysis of Physic-Chemical Properties

Total carbon (C_total), nitrogen (N_total), sulphur (S_total), and hydrogen (H) contents were measured in homogenized samples using an Elementar CHNS Elemental Analyzer at a combustion tube temperature of 1150 ºC and reduction tube temperature of 850 ºC. The method for determining total organic carbon (TOC) in soils and bird dropping samples was by dichromate oxidation-outer heating.

HgT Determination in Soil Sediment and Excrement

0.2000 g of soil and excrement samples were weighed in a 50 mL flask to which 20 mL mixed acid (8:2 HNO_3: HClO_4) was added using a small funnel. All samples were left overnight. Then, they were placed on an electric furnace and heated at 120ºC until the solution became colorless and clarified. The solution was then removed and cooled to room temperature, at which point the mixture was diluted to 50 mL using 0.5% HNO_3. Hg concentration in chemical species was determined using the same method as outlined in section 2.3.2.

Extraction and Determination of Mercury Speciation

The concentration of HgT in soil and excrement is the sum of Hg’s different chemical species, which can be differentiated by chemical and physical behavior [23]. For Hg speciation, a five-step selective sequential extraction procedure was applied; we followed the method developed by Zhang et al. [24]. Extractions were carried out using 1.000 g of samples. Five fractions were distinguished: (1) water-soluble (F1); (2) acid-soluble (F2); (3) alkali-soluble (F3); and (4) hydrogen peroxide-soluble (F4). In step 5, residue digestion with aqua regia was perspeciesed for mercuric sulfide (F5). As with HgT determination, Hg speciation was measured using AFS-820 double-channel atomic fluorescence spectrometry.

Quality Control

Glassware used during sample analysis was placed in 10% HNO_3 (v/v) immersion for over 24 hours and was sanitized according to procedures suggested by Fang [22]. GBW07403 (GSS-3) certified reference materials were added in the digestion process to ensure quality and accurate measurements. These results are listed in Table 1. Quality control also included the use of blanks (6 blank samples for 30 soil samples) and 3 duplicate samples analyzed in each digestion batch (25 samples per batch). The instrument’s Hg detection limit was 0.001 ng/mL; these results confirmed that the Hg analysis was sufficiently credible for our study.

Data Analysis

All data were analyzed in Excel 2010 and SPSS 20.0. Non-parametric tests and Kruskal-Wallis tests were used to evaluate the effects of bird droppings on soil fluorescence spectrometry with a hollow cathode lamp of mercury and high purity argon gas, Hg concentration in the resultant solution was determined to be 5% HCl with 2% K_2Cr_2O_7 (w/v) + 0.5% KOH (w/v) as the reducing agent.

HgT Determination in Eggshells and Feathers

0.4000 g of eggshell and feather samples were weighed in a 50 mL flask to which 20 mL mixed acid (8:2 HNO_3: HClO_4) was added using a small funnel. All samples were left overnight. Then, they were placed on an electric furnace and heated at 120ºC until the solution became colorless and clarified. The solution was then removed and cooled to room temperature, at which point the mixture was diluted to 50 mL using 0.5% HNO_3. Hg concentration in chemical species was determined using the same method as outlined in section 2.3.2.
samples’ HgT concentrations and chemical species at a 0.05 significance level for all analyses.

Results and Discussion

Physical and Chemical Characteristics of Soil and Excrement

The average pH value of soil samples taken from Tongli Wetland was 4.9 (i.e., acidic). The pH value of OS was significantly higher than that of WS (P ≈ 0.005<0.05) by 13.04% (see Table 2). The average content of C$_{tot}$, N$_{tot}$, S$_{tot}$, and H in wetland soil was higher than that of soil in typical subtropical regions, reaching 2.72, 24.37, 9.93, and 0.69 g•kg$^{-1}$, respectively. The content of TOC is 0.04±0.01 g•kg$^{-1}$. The average content of S$_{tot}$ in WS was 104.86% higher than in OS. Similarly, the average content of N$_{tot}$ in WS was significantly higher than in OS (P = 0.037<0.05) by 22.95%. The same appeared to be true of C$_{tot}$ and H content: those in WS were higher than in OS, although we found no noteworthy statistical differences. The same appeared to be true of TOC in WS, which were higher than in OS, although we found no noteworthy statistical differences. This indicates that the nutrients in the soil are different in the WS and OS.

The pH value of BS (4.6) also indicated acidity, and its concentrations of C$_{tot}$, N$_{tot}$, and H were higher than those found in OS and WS. We found similar results for C/N and C/H. The concentration of S$_{tot}$ in BS was higher than in OS but slightly lower than in WS.

Distribution Characteristics of HgT Concentrations in Different Media

As presented in Fig. 2, there were clear variations in Hg concentrations across different media. For OS, the results suggested that the average HgT concentration was 0.104±0.032 mg/kg. In comparison, Hg concentrations in WS and BS were higher than in OS at 0.194±0.155 and 0.182±0.020 mg/kg, respectively. In line with the environmental quality standard for soils in China (GB 15618-1995), Hg concentration in one soil sample (6.67%) exceeded Grade 1 of GB 15618-1995 (0.15 mg/kg) (Grade 1 is the standard that is a limiting value in order to maintain the soil environment quality of the natural background in China). There were 4 WS samples with HgT concentrations above the standard value (i.e., with standard ratios in excess of 22.67%). The HgT concentration of all BS samples also exceeded the standard value (i.e., with standard ratios exceeding 100%). The concentration of HgT of the soil in this study area was compared with soil background value of mercury in the local (0.163 μg/g) [25], the phenomenon of enrichment is present in the WS, but is not found in the OS.

In ES, the average Hg concentration was 0.021±0.013 mg/kg. The average Hg concentration in feathers was higher at 0.071±0.023 mg/kg; thus, HgT enrichment from feathers was greater than from eggshells. Research has shown that Hg concentration

### Table 1. Parameters and determination about standard sample (± standard deviation) (mg/kg).

<table>
<thead>
<tr>
<th></th>
<th>Certified values</th>
<th>Actual measured value</th>
<th>Measurement times</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSS-3</td>
<td>0.060±0.004</td>
<td>0.069±0.003</td>
<td>12</td>
</tr>
</tbody>
</table>

### Table 2. Physical and chemical characters in soil and excrements from Tongli in summer.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>N$_{tot}$ (g/kg)</th>
<th>C$_{tot}$ (g/kg)</th>
<th>H (g/kg)</th>
<th>S$_{tot}$ (g/kg)</th>
<th>TOC (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS (n = 15)</td>
<td>Average Content</td>
<td>5.2</td>
<td>2.44</td>
<td>22.67</td>
<td>9.65</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Standard Deviation</td>
<td>0.8</td>
<td>0.82</td>
<td>8.87</td>
<td>2.13</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Coefficient of Variation (%)</td>
<td>15.38</td>
<td>33.61</td>
<td>39.13</td>
<td>22.07</td>
<td>31.11</td>
</tr>
<tr>
<td>WS (n = 15)</td>
<td>Average Content</td>
<td>4.6</td>
<td>3.00</td>
<td>26.06</td>
<td>10.21</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Standard Deviation</td>
<td>0.8</td>
<td>0.77</td>
<td>8.90</td>
<td>1.53</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>Coefficient of Variation (%)</td>
<td>17.39</td>
<td>25.67</td>
<td>34.15</td>
<td>14.99</td>
<td>30.11</td>
</tr>
<tr>
<td>BS (n = 4)</td>
<td>Average Content</td>
<td>4.6</td>
<td>6.24</td>
<td>81.50</td>
<td>15.34</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>Standard Deviation</td>
<td>0.2</td>
<td>1.48</td>
<td>26.06</td>
<td>3.55</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Coefficient of Variation (%)</td>
<td>4.35</td>
<td>23.77</td>
<td>31.98</td>
<td>23.12</td>
<td>30.00</td>
</tr>
</tbody>
</table>
Concentrations and Speciation...

in feathers is responsible for 93% of Hg in a bird’s body [12]. It is important to note that different migration stopovers may convolute the interpretation and utility of HgT concentration in bird feathers as an indicator of Hg exposure in the sampling area [11, 26-27]. Because there were higher Hg concentrations in feathers than in eggshells in this study, we can derive information about Hg in the local environment; namely, Hg in feathers represents circulating concentrations in the blood during a brief period of feather speciation, which in turn indicates local exposure and mobilization from internal tissues [28]. Once feathers mature, their vascular connection atrophies, leaving the feather as a record of blood levels at the point of speciation. The concentration of metals in feathers, however, remains constant [29]. This explains why feathers could absorb mercury in a bird’s body. However, due to a lack of albumen, vitelline, and embryo, eggshells can only retain part of a bird’s Hg speciation, rendering feathers a more accurate representation of Hg levels.

The results showed that WS and BS are highlighted differences from OS by Non-parametric and Kruskal-Wallis tests. In particular, significant differences were found between OS and WS (P = 0.044<0.05) and OS and BS (P = 0.011<0.05); the difference between WS and BS was not statistically significant. Hg concentration in BS was significantly higher than in WS, echoing previous research findings [20-21]. Because Hg is a typical heavy metal element with biological enrichment characteristics [30], it can be absorbed in a bird’s body and thus appear in excrement [21, 31]. Excrement represents unabsorbed remnants of multiple food items, often at higher concentrations than multiple food items, to provide a non-destructive, quantifiable means of monitoring Hg contamination in the food chain [31].

The above results provide interesting insight. In its natural state, excrement from Ardeid birds as found in soil increases the HgT concentration of surface soil. More than that, though, increased Hg concentrations were also found in the three chemical species (i.e., water-soluble, acid-soluble, and residual mercury) – possibly due to chemical species trans-speciation from residual mercury to available mercury in bird droppings under acidic soil and microbial activity conditions.

Chemical Speciation Analysis of Hg in Soil and Excrement

In OS, Hg concentrations in different speciation (i.e., F1, F2, F3, F4, and F5) were 0.015±0.005, 0.022±0.016, 0.030±0.006, 0.034±0.008, and 0.024±0.003 mg/kg, respectively. In WS, Hg concentrations in different speciation were 0.018±0.004, 0.029±0.005, 0.052±0.015, 0.043±0.006, and 0.035±0.008 mg/kg. In BS, concentrations were 0.019±0.002, 0.037±0.008, 0.024±0.002, 0.021±0.001, and 0.078±0.020 mg/kg (see Fig. 3).

Distinct characteristics appeared in different chemical species of Hg across OS, WS, and BS. The concentration of F1 was basically equal in OS and WS. However, the concentrations of F2, F3, F4, and F5 in WS were higher than in OS by ratios of 31.8%, 73.3%, 26.5%, and 45.8%, respectively. When comparing BS with WS, we found a slight difference in the concentration of Hg in F1. In F3 and F4, by comparing BS with WS, Hg concentrations in WS were higher than in BS at ratios of 116.7% and 104.8%, respectively. However, Hg concentrations of F2 and F5 in BS were higher than in WS at ratios of 27.6% and 122.9%.

The above results provide interesting insight. In its natural state, excrement from Ardeid birds as found in soil increases the HgT concentration of surface soil. More than that, though, increased Hg concentrations were also found in the three chemical species (i.e., water-soluble, acid-soluble, and residual mercury) – possibly due to chemical species trans-speciation from residual mercury to available mercury in bird droppings under acidic soil and microbial activity conditions.
And studies have shown that it is easy for water- and acid-soluble mercury to transport and transform, with the pH and Eh changes in soil, their concentration in the soil would change [24].

Because soil in Tongli Wetland is typically acidic and covered in bird droppings, Hg in droppings leaches readily into the soil surface as it rains. Studies have shown that Hg’s migration efficiency is enhanced when there is acidity and high concentrations of organic matter [32].

**Correlation between Hg and Physic-Chemical Properties**

A Spearman rank correlation was used to analyze the correlation between Hg and physico-chemical properties (see Table 3). There was an extremely significant correlation between concentrations of HgT and N$_{tot}$ and C$_{tot}$ in soil and excrement ($\rho = 0.677$, 0.530, $p<0.01$). We also found a significant correlation between concentrations of HgT and H in soil and excrement ($\rho = 0.432$, $p<0.05$).

Similar studies are consistent with the results of this study. Some studies have shown that there is a significant correlation between TOC, total Carbon, and total Hg in forest soils and sediments [33]. The results showed that TOC, C$_{tot}$, N$_{tot}$, and H in soil and bird droppings were positively correlated with HgT in soil and bird droppings, and higher concentrations of TOC, C$_{tot}$, N$_{tot}$, and H could enhance the concentration of HgT in soil. Studies have shown that organic matter has a great effect on soil adsorption and fixation of Hg, the soil organic matter increases by 1%, and the fixed rate of Hg can reach 30% or so. Organic matter and humus in soil have a strong binding effect on Hg by adsorption, desorption, complexation, chelation, etc., thus affecting the absorption and transfer of Hg in soil [34]. Therefore, in the soil background with high organic matter content, the content of Hg is also high, and there are good correlations between Hg and TOC, and N$_{tot}$ and C$_{tot}$ in soil sediments. The entry of bird droppings will increase the content of N$_{tot}$, C$_{tot}$ and TOC in the soil, over time, and with the microbial activity its nutrient elements will be converted to organic matter and humus, which would increase the adsorption of Hg in sediment and soil and increase the concentration of Hg.

HgT concentrations in soil and excrement were further positively correlated with N$_{tot}$, H, and C. C/N ratios indicate the degree of decomposition: high C/N ratios represent fresh, undecomposed organic C, while low C/N ratios are indicative of older, decomposed fractions [33]. Hg/C ratios increase exponentially with decreasing C/N ratios (see Fig. 4). Possible reasons for increasing Hg and Hg/C levels in decomposing excrements and C pools include selective Hg sorption to different organic matter fractions [33, 35]. Fresh bird droppings could promote mercury enrichment in soil. As time goes by, bird droppings and humus that are highly decomposed will likely increase HgT concentration in soil when they remain there for an extended period, which explains why Hg concentrations were higher in WS than in OS. In terms of the relationship between Hg and H content in soil, if H concentration increases, it can promote the growth of plants and rhizosphere-hydrogenated bacteria [36]. The same can be said for organic matter loss slowing and increasing in alkali-

![Fig. 4. Hg to C/N relationships observed in soils and excrements.](image-url)
Concentrations and Speciation of Mercury in Tongli Wetland Soil

Due to high H concentrations in excrement in the sampled soil, nutrient elements could increase and thus facilitate growth of plant root systems and promote absorption of available mercury from soil and excrement. Therefore, Hg concentration in WS compared to OS was small in F1, but higher in F2, F3, F4, and F5.

Correlation analysis between physical and chemical properties and Hg speciation in the soil (Table 4) shows a significant positive correlation between TOC and F4, while pH exhibits significant negative correlation with F3, F4, and F5; pH was positively correlated with F2, S and F3, F4 and F5 showed significant positive correlations. It is indicated that the contents of TOC, C_{tot}, N_{tot}, H, and S_{tot} in soil and bird droppings affect the change of Hg speciation – especially the effect on the available state (the sum of the concentration of F1, F2, F3, and F4) being greater, but the influence of the residue state Hg (the concentration of F5) is not statistically significant.

The correlation analysis of Hg speciation in soil showed that there was a significant positive correlation between F1 and F3, F3 and F4 and F5 showed significant positive correlations, similarly; there was also a significant positive correlation between F4 and F5. The results showed that there may be a transformation between the available states of mercury. Meanwhile, there is also a possibility for mutual transformation between the available states and the residual state of mercury.

### Table 4. Correlation between speciation of mercury and physico-chemical properties.

<table>
<thead>
<tr>
<th></th>
<th>TOC</th>
<th>pH</th>
<th>N_{tot}</th>
<th>C_{tot}</th>
<th>H</th>
<th>S_{tot}</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.081</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N_{tot}</td>
<td>0.584&quot;</td>
<td>-0.251</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_{tot}</td>
<td>0.460&quot;</td>
<td>-0.314</td>
<td>0.918&quot;</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>0.395&quot;</td>
<td>-0.332</td>
<td>0.840&quot;</td>
<td>0.872&quot;</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>S_{tot}</td>
<td>0.471&quot;</td>
<td>-0.630&quot;</td>
<td>0.411&quot;</td>
<td>0.419&quot;</td>
<td>0.281</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>F1</td>
<td>0.137</td>
<td>-0.325</td>
<td>-0.056</td>
<td>0.060</td>
<td>0.095</td>
<td>0.331</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>F2</td>
<td>0.326</td>
<td>0.376&quot;</td>
<td>0.347</td>
<td>0.053</td>
<td>0.119</td>
<td>-0.187</td>
<td>-0.213</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>0.352</td>
<td>-0.588&quot;</td>
<td>0.185</td>
<td>0.133</td>
<td>0.169</td>
<td>0.677&quot;</td>
<td>0.369&quot;</td>
<td>-0.120</td>
<td>1</td>
<td></td>
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<tr>
<td>F4</td>
<td>0.411&quot;</td>
<td>-0.607&quot;</td>
<td>0.265</td>
<td>0.244</td>
<td>0.331</td>
<td>0.679&quot;</td>
<td>0.198</td>
<td>-0.175</td>
<td>0.673&quot;</td>
<td>1</td>
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<tr>
<td>F5</td>
<td>0.379&quot;</td>
<td>-0.522&quot;</td>
<td>0.192</td>
<td>0.129</td>
<td>0.039</td>
<td>0.838&quot;</td>
<td>0.288</td>
<td>-0.150</td>
<td>0.786&quot;</td>
<td>0.634&quot;</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: **The correlation is extremely significant when the confidence level is 0.01. *The correlation is significant when the confidence level is 0.05. F1: Water-soluble mercury, F2: Acid-soluble mercury, F3: Alkali-soluble mercury, F4: Hydrogen peroxide-soluble mercury, F5: Residual mercury.

### Conclusions

Bird droppings influence the physico-chemical properties and accumulation of Hg in Tongli Wetland soil. Hg enrichment from heron branch bird feathers is stronger than HgT concentrations in eggshells. Accompanied by bird activity, HgT concentrations in soil will increase, thereby rendering HgT concentrations in soil with bird droppings higher than in soil without. Numerical analysis revealed that concentrations of water-soluble, acid-soluble, alkali-soluble, hydrogen peroxide-soluble, and residual mercury in soil that is affected by bird droppings were higher in soil that is not affected by bird droppings. Thus, we can conclude that a positive correlation exists between Hg concentrations in soil or excrement and C_{tot}, N_{tot}, and H concentrations. Furthermore, there is an exponential proportional relationship between C/N and Hg/C. Fresh bird droppings may promote Hg enrichment in soil. Over time, highly decomposed bird droppings and humus are likely to be more conducive to Hg accumulation than when bird droppings first enter the soil.

### Acknowledgements

The revision of this article was assisted by Associate Professor Wu Li from the College of Geography and Tourism at Anhui Normal University. The authors gratefully acknowledge the financial support of the National Natural Science Foundation of China (No. 41371480).
Conflict of Interest

The authors declare no conflict of interest.

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