Evaluating ecological risks and tracking potential factors influencing heavy metals in sediments in an urban river

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Abstract

Background: Heavy metal pollution of aquatic systems is a global issue that has received considerable attention. Canonical correlation analysis (CCA), principal component analysis (PCA), and potential ecological risk index (PERI) have been applied to heavy metal data to trace potential factors, identify regional differences, and evaluate ecological risks. Sediment cores of 200 cm in length were taken using a drilling platform at 10 sampling sites along the Xihe River, an urban river located in western Shenyang City, China, divided into 10 layers (20 cm each). The concentrations of the As, Cd, Cr, Cu, Hg, Ni, Pb and Zn were measured for each layer. Eight heavy metals, namely Pb, Zn, Ag, Cd, Cr, Cu, Ni, and Hg, were measured for each layer in this study.

Results: The average concentrations of the As, Cd, Cu, Hg, and Zn were significantly higher than their background values in soils in the region, and mainly gathered at 0–120 cm in depth in the upstream, 0–60 cm in the midstream, and 0–20 cm downstream. This indicated that these heavy metals were derived from the upstream areas where a large quantity of effluents from the wastewater treatment plants enter the river. Ni, Pb, and Cr were close or slightly higher than their background values. The decreasing order of the average concentration of the Cd was upstream > midstream > downstream, so were the Cr, Cu, Ni and Zn. The trend of the average concentration of the As was different to the Cd; being highest midstream, followed by upstream and then downstream. The potential factors of heavy metal pollution were Cd, Cu, Hg, Zn, and As concentrations, especially Cd and Hg, whose ecological risks were much higher than those of Ni, Cr,
and Pb. The ecological risk levels of all heavy metals were much higher upstream than midstream and downstream. **Conclusions:** Industrial discharge was the dominate source for eight heavy metals in the surveyed area, and rural domestic sewage has a stronger influence on the Hg pollution than industrial pollutants. These findings indicate that effective management strategies should be developed to protect the environmental quality of urban rivers.

**Keywords:** heavy metals; canonical correlation analysis; principal component analysis; potential ecological risk index

1. **Background**

With recent advances in industrialization and socio-economic development, heavy metal pollution of aquatic systems has become a global issue and has received considerable attention due to high biotoxicity, wide sources, non-biodegradability, and bio-enrichment in food webs [1–3]. River sediments have been identified as important carriers and sinks for the heavy metals discharged into the aquatic systems [4,5]. Heavy metals in river sediments mainly stem from rock weathering, soil erosion, runoff from agriculture, sewage treatment, and atmospheric precipitation [6,7]. The heavy metals loaded into the river environment can be transferred to and concentrated in sediments together with organic matter, Fe/Mn oxides, and sulfides by adsorption and accumulation on suspended fine-grained particles, however they cannot be permanently fixed in sediments [8–10]. Under ecological disturbances such as a decline in redox potential or pH and the degradation of organic matter, the heavy metals in sediments can be released back into the overlying water by various processes of remobilization, which may lead to secondary pollution [11–13].

Therefore, a variety of evaluation methods for heavy metals have been developed by international organizations and conventions, which have been applied to evaluate heavy metal pollution and the potential ecological risks in the sediments. These methods are
associated with the enrichment factor, pollution degree, pollution load index, pollution factor, and geo-accumulation index, which may be mainly used to evaluate the ecological risks according to the ratio of a single heavy metal concentration to the background value [14–19]. However, these methods neither provide information on the toxicity of heavy metals nor indicate the comprehensive toxic effects in the overall heavy metal assemblage. The potential ecological risk index, developed by the famous Swedish scholar Håkanson, is a well-known method for the evaluation of heavy metal pollution in sediments [20,21].

In this study, canonical correlation analysis (CCA), principal component analysis (PCA) and potential ecological risk index (PERI) were used to explore the spatial variations and trace potential factors, to identify regional differences, and to assess the potential ecological risks of heavy metals, namely As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn, in the sediments of the Xihe River, an urban river located in Shenyang City, China.

2. Materials and Methods

2.1. Description of study area

The Xihe River, a tributary of the Hunhe River, is located in the western part of Shenyang City, China, and extents 78.2 km through the Huanggu District, Tiexi District, Yuhong District, and Liaozhong County (Fig. 1). It experiences a temperate continental monsoon climate. The mean width of the Xihe River is 10.5 m, with a mean annual rainfall of 680.4 mm [22]. Its average runoff volume is about 924,500 m³ day⁻¹ and effluents from wastewater treatment plants are released directly into the river [23]. Therefore, large quantities of pollutants are discharged into the river, which can lead to water quality deterioration and ecosystem degradation. In particular, the continuous accumulation of heavy metals in the sediments may induce potential ecological risks.
Fig. 1. Watersheds of the Xihe River and location of the sampling sites.

2.2. Sampling and analysis

Field observations and sampling campaigns to collect sediments in the Xihe River were conducted during July 15–25, 2019. (Fig. 1). Ten sampling sites, three in the upstream and downstream regions, and four in the midstream regions, with a distance of 6,000 meters between each station were selected. Sampling sites #1–3 were located upstream in the industrial park region, where a large amount of effluents from the wastewater treatment plants (WWTP) was loaded into the river. Sampling sites #4–7 were situated in midstream in the town region, where a small amount of domestic wastewater and livestock breeding wastewater were discharged into the river. Sampling sites #8–10 were located downstream in farmland areas, where the partial return water from farmlands enters the river. Sediment cores 200 cm in length were collected at each site and divided into 10 horizons (0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm, 80–100 cm, 100–120 cm, 120–140 cm, 140–160 cm, 160–180 cm, and 180–200 cm). Each sediment horizon was thoroughly mixed and homogenized after carefully removing gravel, shells, and animal and plant residues. The samples were stored at 4 °C until they returned to the laboratory.
In a given sediment sample, 5 g of freeze-dried and sieved (Ø=100) sediment was digested in 5 mL solution of HNO₃ and HClO₄ at 140 °C for 3 h [24]. After the samples were cooled, the content of each tube was diluted to 25 mL with double distilled water. The digested samples were then filtered. The concentrations of the As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn were determined for each sample using ICP-MS, with sediment standard substance (Chinese geological reference materials GBW07309) used for quality control during the analysis process. Three parallel samples were set up and the relative standard deviations of the heavy metals for the multiple measurements were less than 5%.

2.3. Pollution assessment method

Recently, different indicators has been utilized to estimate the heavy metal pollution and risk in sediments. PERI was used in this study to represent the potential ecological risk index of heavy metals in the sediments of the Xihe River. It was calculated according to the following formula:

\[ C_f^i = \frac{C_f^i}{C_{n}^i} \]  

(1)

\[ E_r^i = T_r^i \cdot C_f^i \]  

(2)

\[ PERI = \sum_{i=1}^{n} E_r^i \]  

(3)

where \( E_r^i \) is the potential ecological risk associated with the heavy metal \( i \) in the sediments, which is the product of the toxic response factor \( T_r^i \) and the pollution factor \( C_f^i \). \( T_r^i \) is the toxic response factor of heavy metals \( i \) and has values of 10, 30, 2, 5, 40, 5, 5 and 1 for As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn, respectively [25]. \( C_f^i \) is the pollution factor of heavy metals \( i \), which is the ratio between the determined concentration in the sediment \( C_s^i \) and the background value \( C_{n}^i \) (Table 1). The background value for the soil in Liaoning Province were used as \( C_{n}^i \) [26]. According to Zhang et al [27], the individual ecological risk of heavy metals and integrated potential ecological risk can be classified into the categories shown in Table 2.
Table 1 Background values of the heavy metals in Liaoning Province of China.

| Heavy metals | As   | Cd   | Cr   | Cu   | Hg   | Ni   | Pb   | Zn   |
|--------------|------|------|------|------|------|------|------|------|
|              | mg kg$^{-1}$ |      |      |      |      |      |      |      |
| Background   | 8.80 | 0.16 | 57.66| 24.57| 0.05 | 25.60| 22.15| 59.84|

Table 2 Grade standards for potential ecological risks.

| Risk level | Low risk | Moderate risk | High risk | Very high risk | Disastrous risk |
|------------|----------|---------------|-----------|----------------|-----------------|
| Grade      | I        | II            | III       | IV             | V               |
| $E_r^i$    | <30      | 30-60         | 60-120    | 120-240        | >240            |
| PERI       | <100     | 100-200       | 200-400   | >400           |                 |

2.4. Statistical analysis

Variations in heavy metal values were presented using sediment depth and sampling site matrices. CCA and PCA were carried on the data matrices of the heavy metals to trace the potential factors and reveal the patterns of the sediment samples [28,29]. The score plots for heavy metals can characterize the potential factors of each principal components (PC), and the loading plots for the 10 sediment layers can be used to investigate the sedimentary characteristics of the heavy metals. All statistical analyses were performed using Canoco 5, SPSS 22, and Origin 8.0 software for windows.

3. Results and Discussion

3.1. Spatial distributions of heavy metals in sediments

Spatial distributions were obtained through the matrices of the heavy metals in the Xihe River (Fig. 2). The average concentrations of the heavy metals across all the sediments in decreasing order was as follows: Zn (363.04±571.13 mg kg$^{-1}$) > Cu (63.25±149.96 mg kg$^{-1}$) > Cr (35.55±58.96 mg kg$^{-1}$) > Ni (35.06±44.08 mg kg$^{-1}$) > Pb (21.88±13.68 mg kg$^{-1}$) > As (11.19±21.04 mg kg$^{-1}$) > Cd (4.12±10.00 mg kg$^{-1}$) > Hg (1.01±2.17 mg kg$^{-1}$). The average concentration of the As was the highest at the downstream sites, followed by the midstream and upstream (Fig. 2a). As concentrations were highest at the surface layers of all sites except for site #1 and site #2, which were much higher.
than the background values at sites #6, #9, and #10. Cd concentrations in all the layers of each site were far higher than their background values (Fig. 2b). The average concentration of Cd was the highest in the upstream, followed by the midstream and downstream. The concentrations in the 80–100 cm layer of site #1 and the 0–20 cm layer of site #6 were much higher than those in the other sediment layers. The average concentration trend of the Cr in the upstream, midstream, and downstream regions was similar to that of Cd. However, the concentrations in the whole layers at all sites were lower than their background value. The maximum concentration was observed in the 140–160 cm horizon of site #2 (Fig. 2c). For the midstream and downstream sites, the concentrations were highest in the 0–20 cm layers. At all sites, the Cu concentrations in all 10 layers were much higher than their background values (Fig. 2d). The trend of the mean Cu values in the upstream, midstream, and downstream regions was similar to that of Cd and Cr; being higher at the upstream sites. The maximum concentration of Cu occurred in the 60–80 cm layer of site #1.
The background values of Hg were higher than the concentrations found at any layer of any site (Fig. 2e). The decreasing order of the average Hg concentration was midstream > upstream > downstream. The maximum concentration occurred in the 0–20 cm layer of site #6. The maximum concentration of the Ni was found in the 0–20 cm layer at all sites except for site #1, where the maximum was in the 80–100 cm layer (Fig. 2f). The trend of Ni in the upstream, midstream, and downstream regions was the same as that of Cd, Cr, and Cu. Furthermore, the Ni concentrations in the layers 60–100 cm of site #1 and 0–20 cm of site #2 were higher than the background value, while the concentrations in the remaining layers were close to the background value. Interestingly, the concentrations of Pb were much lower at depths of the 40–100 cm (Fig. 2g). This indicated that the Pb content in the sediments was very low during a period of time. The Pb concentrations in all the layers were lower than their background values at all sites, especially at depths of 40–100 cm. Zn concentrations in all the layers were far higher than their background values at all the sites (Fig. 2h). The trend of the Zn concentrations was similar to that of Cd, Cr, Cu, and Ni, being highest at the upstream sites and getting lower as you go downstream. The maximum concentration occurred in the 80–100 cm layer of the site #1, while the minimum concentration in the 80–100 cm layer at the site #10.

3.2. Tracking potential factor and spatial classification

CCA was used could elaborate on an evident and graphic comprehension of the relationship between water pollution and the heavy metals [30,31]. In the CCA ordination biplot, the arrows for Cu, Cd, As, and Hg shoot in the positive direction of
the AX1, while the arrow for Pb goes in the negative direction (Fig. 3). This indicates that Cu, Cd, As, and Hg have negative correlations with Pb. The concentration values of Pb in the sediments were close to their background value or slightly higher. A positive direction for the AX1 might be concerned with the anthropogenic activities, while a negative direction might be related to natural processes. The arrow for Zn was positive for AX2, while the arrow for Ni and Cr was negative. The trends of Zn, Ni, and Cr were similar to those of Cu, Cd, Hg, As, and Pb. This indicated that the positive direction of the AX2 was associated with anthropogenic activities, while the negative direction was associated with natural conditions. Hence, the potential factors of heavy metal pollution were increased concentration of Cu, Cd, and Zn, instead of Pb, Ni, and Cr, whose concentrations in the sediments were close to their background values.

Fig. 3. Ordination biplot based on the CCA of the relationships between the heavy metals and the sediment layers of the sampling sites.

In the ordination biplot, the sediment layers of the sampling sites were roughly divided into two clusters by the straight line y=x (Fig. 3). Cluster A was located under the
straight line y=x and contained all sediment layers of sites #1 and #2, and the minor
partial layers of sites #4–10. Cluster B was located on the straight-line y=x and
contained most layers of sites #3–10. This indirectly indicates the pollution of heavy
metals was more serious upstream than pollution in the midstream and downstream.
Moreover, in a given sampling site, the dispersion of the ten layers was relatively larger,
which was situated in more than two quadrants. Therefore, the ten layers in each site
should be analyzed and discussed independently.

3.3. Identifying regional differences
PCA was carried out on the heavy metals of the ten layers of each sediment profile,
which yielded two PCs. The loadings of the PCs could indicate the abundance of the
heavy metals, where values over 0.7 should be the potential factors. The scores of the
PCs could discriminate the similarity and dissimilarity of the sediment layers.

For site #1 (Fig. 4a), PC1, with 75.66% of the total variance, had strong positive
loadings on Zn, Cd, Ni, and As, and negative loadings on Pb. PC2 with 24.14% of the
total variance had positive loadings on Cu. The No. 3 to 5 layers were in the first and
fourth quadrants, while the other layers were in the third quadrant and their dispersion
was much lower. These behaviors illustrated that Zn, Cd, As, Ni, and Cu were potential
factors of heavy metal pollution, whose variations were much higher in the No. 3 to 5
layers than those in the other layers (Fig. 4a). For site #2 (Fig. 4b), PC1 (30.87%)
included Zn, Cr, and Cd, which indicated that these heavy metals were potential factors.
The No. 2 to 5 layers were in the third quadrant, while the other layers were in the other
three quadrants. This indirectly demonstrates that the variations of the heavy metal in
the former were lower than those in the latter. For site #3 (Fig. 4c), PC1 (26.60%)
contained Ni and Pb, and PC2 (20.37%) contained Zn. Interestingly, the PC1 and PC2
scores of the first layer were much larger than those of the other layers, which indirectly
proved that the concentrations of Pb, Ni, and Zn in the first layer were higher than those
in the other layers. Regardless of background, the potential factors of heavy metal
pollution mainly included As, Cd, Cu, and Zn in the upstream region, whose concentrations were much higher in the 0–120 cm region than those in the 120–200 cm depth, except for Ni in site #2.
Fig. 4. Principal component analysis (PCA) based on the heavy metals of sediments, (a) site #1, (b) site #2, (c) site #3, (d) site #4, (e) site #5, (f) site #6, (g) site #7, (h) site #8, (i) site #9, (j) site #10. Abbreviations: No. 1 = 0–20 cm; No. 2 = 20–40 cm; No. 3 = 40–60 cm; No. 4 = 60–80 cm; No. 5 = 80–100 cm; No. 6 = 100–120 cm; No. 7 = 120–140 cm; No. 8 = 140–160 cm; No. 9 = 160–180 cm; No. 10 = 180–200 cm. (Variables with weaker impact located at lower left corner.)

For site #4 (Fig. 4d), PC1 (72.39%) contained Cu, Cd, Zn, Ni, Cr, and Hg, increased concentrations of which were the potential factors of heavy metal pollution. The first layer was located on the right side of the y-axis, while the remaining layers were on the left side of the y-axis. Furthermore, the No. 7–10 layers were clustered intensively. These results indicate that the heavy metals were enriched in the surface layer (except for the Pb), and continuously decreased with the depth. For site #5 (Fig. 4e), PC1 (73.49%) contained Zn, Cu, Cr, Cd, Hg, and As, which were potential factors of heavy metal pollution. The distributions of the ten sediment layers were similar to site #4, which also contributed to the variations of the heavy metals being similar to site #4. For site #6 (Fig. 4f), PC1 (81.64%) again contained Zn, Cu, Cd, Hg, and As. The distribution of the ten layers was roughly similar to sites #4 and #5, which indicated that the variations of the heavy metals resembled the sites #4 and #5. PC1 (69.45%) for site #7 (Fig. 4g) also contained Hg, Cu, As, and Zn. The distributions of the ten layers were the same as those of #6, suggesting that the variations of the heavy metals were the same as those of #6. In the midstream, the potential factors of heavy metal pollution were As, Cd, Cu, Cr, Hg, and Zn, whose concentrations were relatively higher in the 0–60 cm layer than those in the 60–200 cm depth range.

For site #8 (Fig. 4h), PC1 (59.28%) contained Cu, Zn, Cd, and As, and PC2 (18.73%) with Pb. These heavy metals mainly occurred in the surface layer, as the scores in the top layer were the highest. For site #9, PC1 (47.63%) contained Cu and Hg, and PC2 (34.51%) Zn, Cd, and As. The trend of these heavy metals was similar to that at site #8.
For site #10, PC1 (69.29%) had As, Cd, Hg, and Zn, whose trend was similar to sites #8 and #9. Downstream, the potential factors of heavy metal pollution were the same as the midstream, which contained As, Cd, Cu, Hg, and Zn. Diversely, the distributions of the heavy metals were higher at 0–20 cm than those at 20–200 cm.

3.4. Ecological risk assessment

3.4.1. Ecological risk of the single heavy metal

The $E_r^i$ values of the heavy metals in the sediments were obtained using formula one. According to the grading standards (Table 2), the risk grade of a single heavy metal can be deduced from its $E_r^i$. For the As (Fig. 5a), Grade II was exhibited at 40-100 cm at site #1, where a moderate risk occurred. Grade III exhibited at 0-20 cm at site #6, at 0–40 cm at site #9, and at 20–40 cm at site #10 where the high risk occurred. Grade IV was observed at 0–20 cm at site #10, where a very high risk occurred. For Cd (Fig. 5b), Grade V in the upstream occurred at 0–200 cm at site #2, at 0–20 cm, 40–120 cm, and 160–180 cm at site #1, and at 0–20 cm and 80–200 cm at site #3, where disastrous risk was recorded. Grade V in the midstream occurred at 0–20 cm at site #4, at 0–40 cm, and 80–100 cm at site #5, in 0–20 cm, 40–60 cm, and 80–100 cm at site #6, and at 0–40 cm and 100–120 cm at site #7. The risk levels decreased with the depth from 120 cm to 200 cm at sites #4 to #7, which indirectly indicated that the sediment depth could be approximately 120 cm in the midstream. Grade V in the downstream occurred at 0–20 cm at site #8, at 0–40 cm and 80–100 cm at the site #9, and at 0–20 cm and 40–100 cm at site #10. The trend of the risk levels with the depth was similar to that of the midstream (Fig. 5b). For Cr, all $E_r^i$ values reached Grade I at all sites, where a low risk occurred (Fig. 5c). For Cu in the upstream (Fig. 5d), the sediment profile of site #1 showed the disastrous risk level (Grade V) at 60–80 cm, and high risk (Grade III) at 40–60 cm. The sediment profile of site #2 was high risk at 100–120 cm, and moderate risk at 0–100 cm and 120–140 cm. The sediment profile of the site #3 was low risk at 0–200 cm. For the midstream (Fig. 5d), a moderate risk occurred at 0–20 cm at sites #4
to #7, except for the site #6 which showed high risk. For the downstream (Fig. 5d), all sediment profiles were low risk.

Fig. 5. Spatial distributions of the grades deduced from the $E_r^f$ of the heavy metals in Xihe River.

For Hg (Fig. 5e), Grade V was found in whole sediment profiles upstream, where the disastrous risk occurred. In the midstream, Grade V was found within the sediment profile of site #1 (except for 20-60 cm), at 0–40 cm and 60–100 cm of site #5, at 0–100 cm and 120–140 cm of site #6, at 0–60 cm and 140–160 cm of site #7. In the downstream, Grade V was found at 0–60 cm and 80–160 cm of site #8, at 20–140 cm at site #9, and at 0–80 cm and 160–200 cm of site #10. The ecological risk levels of the heavy metals in the sediments were higher in the upstream than those in the midstream and downstream. For Ni (Fig. 5f), Grade III was only found at 80–100 cm of site #1, Grade
II only at 60–80 cm of site #1 and at 0–20 cm of site #2, and Grade I widely existed in the other layers. For Pb (Fig. 5g), Grade I could be completely enriched within the whole sediment profiles at all sites. For Zn (Fig. 5h), Grade II occurred at 60–100 cm of site #1, at 140–160 cm of site #2, and at 0–20 cm of sites #6 and #7. Based on the above analyses, the ecological risk levels of Cd and Hg were far higher than those of the other heavy metals. Furthermore, the ecological risk levels of Cd, Cu, Ni, and Zn were higher in the upstream than those in the midstream and downstream, while risk level of As was higher in the downstream than those in the upstream and midstream.

3.4.2. Ecological risks of the whole heavy metals

The PERI of the heavy metals could be calculated using formula one. According to the grading standards (Table 2), the ecological risks of the heavy metals in each sediment layer can be deduced from its PERI (Fig. 6). At almost all sampling sites (except site #4), surface sediments (0–40 cm) were defined as being a very high ecological risk. This may indicated that the pollution is a consequence of recent anthropogenic pollution. All sediment layers upstream were defined as being a very high ecological risk, except for 180–200 cm of site #1, where a high ecological risk occurred. Very high ecological risk was found at approximately 0–100 cm in the midstream sites, high ecological risk at 100–140 cm, and moderate and low risks at 140–200 cm. The trend of the ecological risks downstream was similar to that in the midstream. These indicated that the ecological risk levels were much higher in the upstream than those in the midstream and downstream. This indirectly proved that the heavy metals in the sediments should be derived from upstream anthropogenic activities.
Fig. 6. Distributions of the ecological risks of the heavy metals in Xihe River.

Abbreviations: No. 1 = 0–20 cm; No.2 = 20–40 cm; No.3 = 40–60 cm; No.4 = 60–80 cm; No.5 = 80–100 cm; No.6 = 100–120 cm; No.7 = 120–140 cm; No.8 = 140–160 cm; No.9 = 160–180 cm; No.10 = 180–200 cm.

4. Conclusions

The average concentrations of As, Cd, Cu, Hg, and Zn in all the sediments were significantly higher than their background values in soils in the region. These heavy metals mainly gathered in the top 0–120 cm of sediments in the upstream areas, in 0–60 cm midstream, and 0–20 cm downstream, indicating that these metals were derived from the upstream region where a large quantity of effluents from the wastewater treatment plants entered the river. Ni, Pb, and Cr were close or slightly higher than their background values. The decreasing order of the average concentrations of As was downstream > midstream > upstream. The trend of Cd, Cr, Cu, Ni, and Zn was opposite to that of As. The average concentration of Hg was the highest in the midstream, followed by the upstream and downstream regions, indicating that rural domestic sewage has a stronger influence on Hg pollution than industrial pollutants. The potential factors of heavy metal pollution were Cd, Cu, Hg, Zn, and As, whose ecological risks...
were much higher than those of the Ni, Cr, and Pb, especially Cd and Hg. The ecological risk levels of all heavy metals were much higher upstream than those in the midstream and downstream. The results showed that efficient solutions must be found to protect the environmental quality of urban rivers.

Abbreviations
CCA: Canonical correlation analysis; PCA: principal component analysis; PC: principal components; PERI: potential ecological risk index; No. 1 = 0–20 cm; No.2 = 20–40 cm; No.3 = 40–60 cm; No.4 = 60–80 cm; No.5 = 80–100 cm; No.6 = 100–120 cm; No.7 = 120–140 cm; No.8 = 140–160 cm; No.9 = 160–180 cm; No.10 = 180–200 cm.

Declarations
Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Availability of data and materials
The datasets obtained and analyzed in the research can be available from the first author on reasonable request.

Competing interests
The authors declare no conflict of interest.

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Authors’ contributions
D.L. carried out the experiments, analyzed the data and wrote the manuscript; H.Y. and H.G. analyzed the data and modified the manuscript; W.X. wrote the manuscript and
improved the language of the manuscript. All authors have read and agreed to the published version of the manuscript.

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