Spatial Variability and Influencing Factors of Cr, Ni, Cu, Zn and Pb in Five Mangroves Reserves of South China

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Abstract. In order to study the spatial variability and influencing factors of heavy metals contents in five mangroves in South China, surface sediments and plant canopy new leaves of mangroves were sampled to determine Cr, Ni, Cu, Zn, Pb contents. Moreover, the corresponding pollution load index of sediments and bioconcentration factors of leaves were evaluated. Meanwhile, the physical and chemical properties, organic carbon and nitrogen of surface sediments and new leaves were measured. It was found that the average content of heavy metals in the surface sediments of Futian Reserve was the highest with a pollution load index of 2.61, classified as strongly polluted. The particle size distribution of the five sites was mainly silt-sand, and the pH was slightly acidic. Multivariate analysis of variance indicated that both sampling locations and vegetation types had significant influences on heavy metal contents in surface sediments and leaves as well as bioconcentration factors of leaves. In sum, natural factors such as plant species and organic matter contents significantly influence the metal contents and translocation in mangrove forests. However, in strongly polluted areas anthropogenic factors compromise the natural effects and play the major role in metal distribution.

1. Introduction
In the coastal areas of Southern China, the function of mangroves as pollutant sinks is becoming more and more important with the development of the regional economy. Mangrove wetlands can absorb and store heavy metal pollutants from tides, rivers, and rainstorms due to their inherent characteristics, such as high productivity [1], reduction states on account of anoxic condition in sediments [2,3], adsorption and precipitation capacity of particles, and the absorption capacity of heavy metals by mangrove plants [4,5]. Therefore, coastal mangrove wetland has always been an important place for purification and storage of land-based pollutants, especially heavy metal pollutants [6,7].

Studies on heavy metal pollution in mangrove wetlands have been reported frequently. The metal distribution in mangrove plants and sediments were studied mainly in two aspects. Some scientists focused on the mechanisms of metal tolerance by mangroves [8-12]. They planted mangrove plants under heavy metal stress, and then analyzed the physiological responses of the plants and the anatomical structure of the roots. One of the important findings was that the lignification in root played an important role in metal resistance of mangrove plants. These studies provide important insights for understanding the transport and bioconcentration of metals in mangrove plants. On the other hand, some scientists conducted field studies in mangrove forests to evaluate the factors
influencing the metal contents in plants and sediments. These studies often observed one or two mangrove forests and related environmental factors such as inundation frequency, sedimentary organic carbon, local pollution, etc to the spatial variability of metal contents [10-13].

What is missing from the above research is how the factors affecting the distribution of metals change in two or more mangroves with significantly different human activities. Therefore, the significance of this paper is that we conducted field studies of metal distribution in five mangrove reserves over two provinces of Southern China with fundamentally different economic development, but similar environmental factors such as plant varieties. The large area and the wide variety of the study zones provide us sufficient data to comprehensively estimate the factors influencing the metal distribution in mangrove reserves of Southern China. Metal contents such as Cr, Cu, Ni, Zn, and Pb were measured. The span of sampling crossed mangrove reserves in economically underdeveloped areas as well as well-developed areas. The results hopefully reveal the influences of human activities and vegetation types on the metal distribution in mangrove ecosystems.

2. Methods

2.1. Site Description
Mangrove wetland is one of the most important wetland types in coastal areas, mainly distributed in tropical and subtropical intertidal zones. In China, the northernmost mangrove wetland is in Zhejiang Province while the southernmost mangrove wetland is in Hainan Province [14]. Guangdong and Guangxi are the two important districts of mangrove wetlands, of which there are 14670 hm² mangrove wetlands in Guangdong Province and 8375 hm² in Guangxi Province [14,15]. These mangrove reserves were subject to significant different human impacts due to differences in regional economic development and industrial types. For example, GDP in Guangdong Province has been the highest in China since 1989, which was contributed a lot by Shenzhen. The main productions in Guangdong Province are manufacturing food, textile, machinery, household appliances, automobiles, medicine, building materials, and metallurgical industry. On the other hand, Guangxi Province ranked 18th in 2018 and agriculture is the main industry. To investigate the metal distribution in mangrove wetlands, we took both surface sediments and the nearest mangrove plant leaves in five mangrove reserves of Guangdong and Guangxi provinces (Figure 1). From east to west they are Futian Mangrove Reserve in Shenzhen, Guangdong Province (GDFT, sample size: 53 sediments and 53 leaves), Gaoqiao Mangrove Reserve in Zhanjiang, Guangdong Province (GDGQ, sample size: 58 sediments and 58 leaves), Shankou Mangrove Reserve in Hepu, Guangxi Province (GXSK, sample size: 24 sediments and 24 leaves), Golden Bay Mangrove Reserve in Beihai, Guangxi Province (GXBH, sample size: 26 sediments and 26 leaves), and Dangjiang Mangrove Reserve in Hepu, Guangxi Province (GXDJ, sample size: 24 sediments and 24 leaves).

GDFT is the largest inner-city mangrove forest reserve in China, with a total area of 368 hectares. The main vegetation types in this reserve are Kandelia obovata and Avicennia marina [16,17]. As much terrestrial wastes running through the city and then into Shenzhen Bay and the special geographical location of Shenzhen Bay, this reserve is seriously affected by urban development and environmental pollution. GDGQ is currently the largest natural mangrove reserve in China, with a total area of 19,000 hectares. The main vegetation types are Aegiceras corniculatum and Bruguiera gymnorrhiza. GXSK is one of the five national marine protected areas approved by China with a total area of 8,000 hectares [18]. The main vegetation types are Aegiceras corniculatum and Avicennia marina. GXBH belongs to the Beihai Silver Beach National Tourist Resort, with a total area of 400 hectares[19]. The main vegetation types are Avicennia marina, Kandelia obovata, and Aegiceras corniculatum. The scale of GXDJ is relatively small, and the main vegetation type is Aegiceras corniculatum. The main industries of this reserve are rice cultivation, fish breeding, and shipbuilding.
Figure 1. Sampling sites in Guangdong Province (Top), including Futian National Reserve (GDF); Guangxi Province and borderline (Bottom), from east to west including Gaoqiao National Reserve (GDGQ), Shankou National Reserve (GXSK), Beihai National Reserve (GXBH), and Dangjiang National Reserve (GXDJ). The left-top small panel shows the locations of Guangdong and Guangxi provinces in China.

2.2. Sampling
Surface sediment (upper 5 cm depth) were collected with a plastic spatula, carefully removed branches and leaves, and then sealed in clean polyethylene bags storing in refrigerator at 4°C. All sampling instruments were cleaned with 5% nitric acid solution before use. To examine the accuracy of measurement, samples were collected in triplicates. The fresh leaves at the top of mangrove vegetation closest to the surface sediment samples were collected using pruning shears. Leaves were then sealed in clean polyethylene bags, stored at 4°C, and pretreated within 12 hours.

2.3. Analytical Methods
A series of physicochemical parameters such as grain size composition, bulk density, oxidation reduction potential ($Eh$), pH, total organic carbon (TOC), and total nitrogen (TN) were measured using the following methods. Grain size composition was measured with a Beckman Coulter LS 13320 (Beckman Coulter Inc., Germany). Bulk density was determined by heating the wet sediments at 60°C for 48h, after that sediments were grounded to a fine powder. Sediment pH and $Eh$ were measured by pH meter and Ag $Eh$ meter after mixing sediments with deionized water using mass ration of 1:5. The pretreatment procedures for TOC and TN followed the Chinese GB/T 12763.8-2007. TOC and TN were measured by a Perkin-Elmer 2400 Series II CHNS/O Analyzer (Perkin Elmer Inc., USA). For pH, grain size composition, and $Eh$, all samples were measured in triplicate to check for repeatability and evaluate the measurement precision. For other analyses, selected samples were measured in triplicate resulting in a relative deviation of less than 7%.

According to Yu et al. [20] the following method was used to digest and analyze the contents of Cr, Ni, Cu, Zn, Pb, and Al. We chose these five PTEs based on the origins of pollution in the area from previous studies [20]. Approximately 0.250 g of dried and homogenized sediment powder were mixed with 8 ml of 12 M HCl and 17 M HNO3 (3:1) mixture and then digested using microwave digestion system (DK VELP SCIENTIFICA, Italy) at 190°C for 75 minutes. Metal contents were then determined using Perkin Elmer-Optima 7000DV (Perkin Elmer Inc., USA) inductively coupled plasma optical emission spectrometry. For the analytical quality control, all vials were brand new vials leached with ultrapure nitric acid and rinsed 5 times with deionized water (Milli-Q Element, 18 MΩ). Ultrapure acids and deionized water were used throughout the sampling preparation and analysis procedures. The procedural blanks were measured and corrected for the reported concentration. Standard reference materials (SRM 1646a, Estuarine Sediment, NIST) were digested using the same protocol. The recovery rates for metals in SRM 1646a were higher than 85%.

2.4. Data Analysis
The content of metals was calculated as mg of metal per kg of dry plant tissue or sediment. Organic carbon and total nitrogen contents were calculated as mg of organic carbon/nitrogen per 100 mg of dry plant tissue or sediment, hence presented as weight percentage (100%). Pollution Load Index (PLI) was calculated to evaluate the pollution level of the sampling sites [21]. Multivariate analyses such as single/multivariate analysis of variance (with post hoc REGWF tests), Pearson correlation analysis, and factor analysis were conducted with SPSS.

2.5. Heavy Metal Bioconcentration Factors and Pollution Level Evaluation Method
The pollution level of the specific site can be calculated using equation (1):

$$CF_i = \frac{C_i}{C_{oi}}$$  

(1)

where $CF_i$ was the pollution index of element i, $C_i$ was the absolute value of element i (unit: mg·kg$^{-1}$), $C_{oi}$ was the background value of element i (unit: mg·kg$^{-1}$).

In this study, we used the average value of shale as the background value (Cr=90 mg/kg, Ni=68 mg/kg, Cu=45 mg/kg, Zn=95 mg/kg, Pb=20 mg/kg) [22]. The next step was to calculate the pollution load of a sample with equation (2):

$$PLI = \sqrt[3]{CF_1 \times CF_2 \times CF_3 \times \cdots \times CF_n}$$  

(2)

where PLI was the pollution load index of that sample, and n was the number of elements evaluated. Then, the pollution level of the sample site was calculated by equation (3):

$$PLI_{zone} = \sqrt[3]{PLI_1 \times PLI_2 \times PLI_3 \times \cdots \times PLI_n}$$  

(3)
where PLI was the pollution load level of the sampling site and \( n \) was the number of samples. Typically, PLI values less than 1 were evaluated as not polluted; PLI values between 1 and 2 were evaluated as moderately polluted; PLI values between 2 and 3 were evaluated as strongly polluted; PLI values higher than 3 were evaluated as extremely strongly polluted.

The accumulation ability of heavy metals in plants is not only related to the content of heavy metals in soil but also related to the morphology of heavy metals in soil and the demand of plants for elements [10-13,23]. The accumulation capacity of plants to heavy metal elements can be expressed by the bioconcentration factors, which is one of the indicators to evaluate the bioconcentration capacity of plants to heavy metal elements. The greater the enrichment coefficient is, the stronger the bioconcentration factors of plants to this heavy metal element will be [24]. Its calculation equation is shown in equation (4):

\[
BCF_{leaf} = \frac{C_{leaf}}{C_{sediment}} \tag{4}
\]

where BCF is the bioconcentration factors, is the measured value of the heavy metal in the new leaves of the canopy of the plants at the sampling point, and is the measured value of the heavy metal in the soil at the sampling point [8].

3. Results

3.1. The Spatial Distribution of Physicochemical Parameters and Heavy Metals in Sediments and Bioconcentration Factors in Plants

The physicochemical parameters including grain size composition, pH, Eh, Salinity, and organic matter contents of surface sediments of five sampling sites were summarized in Table 1. Fine size particles (less than 63μm) dominant the surface sediment which was commonly observed in the intertidal environments. The surface sediments were in relatively acid and reduced status with pH ranged from 5.6 to 7.3 and Eh ranged from 200 to 500 mV. Since mangrove wetlands were commonly found in the higher zone of the intertidal environments the salinity of surface sediments were generally less than 6 ppt. With high productivity in mangrove trees, the surface sediments were typically rich in organic matter. Here we found that the organic carbon contents in surface sediments of the sampling sites were between 0.31% and 9.33% while nitrogen contents were between 0.03% and 0.61%.

| Sampling sites | Grain size composition | Physicochemical parameters | Organic matter contents (weight percentage) |
|----------------|------------------------|-----------------------------|---------------------------------------------|
|                | <4μm | 4μm-63μm | >63μm | pH  | Eh (mV) | Salinity (ppt) | Organic carbon | Nitrogen |
| GDFT           |      |          |       | 28%±18 | 6.63±0.29 | 318.92±50.8 | 3.17±1.0 | 4.16±1.7 | 0.28±0.1 |
| GDG            | 15%±5% | 72%±18% |       |       | 36 |  6 | 0 | 1 |
| Q              | 24%±4% | 100%±0% | 0%±0% | 6.47±0.20 | 313.08±48.9 | 3.01±0.9 | 3.30±1.2 | 0.19±0.0 |
| GXSK           | 27%±5% | 99%±6% | 1%±6% | 6.57±0.29 | 358.68±67.5 | 2.64±0.9 | 2.08±0.8 | 0.14±0.0 |
| GXB            | 18%±6% | 88%±26% | 12%±26 | 6.70±0.38 | 313.99±41.1 | 2.26±0.6 | 1.07±0.9 | 0.09±0.0 |
| H              | 25%±4% | 100%±0% | 0%±0% | 6354±0.2 | 327.89±22.3 | 1.53±0.4 | 2.02±1.5 | 0.13±0.0 |
The Cr, Ni, Cu, Zn, and Pb contents in surface sediments and leaves were summarized in Table 2. In general, Zn content is the highest in the surface sediments, followed by Cr, Cu, Pb, and Ni. The average content of Cu and Zn in the new leaves of the mangrove canopy was the highest, while the average content of Ni was the lowest. The bioconcentration factors of Cu and Zn are the largest (Table 2), while that of Pb is the smallest. And the average bioconcentration factors of five heavy metals in *Kandelia obovata* was higher than other plants. In sum, significant differences in metal contents and bioconcentration factors were observed among sampling sites as well as mangrove species. Analysis of variances will be performed later to investigate the patterns of metal contents in various sites and species.

**Table 2.** The contents of Cr, Ni, Cu, Zn, Pb and bioconcentration factors in the surface sediments of five sites and six mangrove plants.

| Sampling sites | Heavy metals contents (ppm) | Pollution evaluation |
|----------------|----------------------------|----------------------|
|                | Cr  | Ni  | Cu  | Zn  | Pb  | PLI | Level        |
| GDFT           | 64.73±10.04 | 33.23±4.34 | 76.94±13.36 | 348.51±57.98 | 46.97±4.84 | 2.61 | Strongly polluted |
| GDGQ           | 41.36±10.67 | 14.68±3.82 | 16.42±4.33 | 70.26±16.09 | 16.90±4.29 | 0.85 | Not polluted |
| GXSK           | 34.16±9.54 | 13.61±4.14 | 13.43±3.92 | 57.03±16.66 | 16.98±4.78 | 0.74 | Not polluted |
| GXBH           | 13.72±7.15 | 4.93±2.83 | 5.36±3.07 | 20.02±11.88 | 3.13±3.02 | 0.22 | Not polluted |
| GXDJ           | 42.82±14.73 | 17.18±4.71 | 20.29±6.32 | 100.78±26.21 | 22.97±5.23 | 1.09 | Moderately polluted |

| Mangrove plants | bioconcentration factors |
|-----------------|--------------------------|
|                 | Cr  | Ni  | Cu  | Zn  | Pb  |
| *Avicennia marina* | 0.73±0.51 | 1.22±1.00 | 2.02±2.07 | 3.29±3.47 | 0.61±0.57 |
| *Bruguiera gymnorrhiza* | 0.71±0.31 | 1.10±0.61 | 0.91±0.34 | 1.51±0.71 | 0.49±0.19 |
| *Kandelia obovata* | 1.10±0.41 | 1.93±0.70 | 3.58±1.90 | 5.66±3.01 | 1.05±0.47 |
| *Aegiceras corniculatum* | 0.79±0.28 | 1.08±0.37 | 1.02±0.61 | 1.73±1.00 | 0.52±0.19 |
| *Sonneratia apetala* | 1.02±0.29 | 1.68±0.71 | 2.92±1.90 | 4.96±3.09 | 0.96±0.40 |
3.2. Correlation Analysis Between Metal Contents and Physicochemical Parameters

Previous studies have shown that in surface sediment physicochemical parameters influence heavy metal contents [8,12]. Meanwhile, linear correlations were often observed between various metals, implying certain interrelationships (Tables 3-7). This result is consistent with the results of Tam et al. [25] in Hong Kong Tingjiao and other mangrove areas. However, in the Wailingdingyang area where marine sediments dominate [26], the correlation between heavy metals in sediments is better than the results of this study. This shows that the accumulation process of heavy metals in the sediments is affected by a combination of many factors, not just related to the physical and chemical properties of the sediments [27]. On the other hand, due to the differences in economic development near the mangrove reserves, the interrelationships between the above-mentioned values likely differ among the five sampling sites. Hence, we performed correlation analysis on physicochemical parameters and metals in the five sampling mangroves respectively (Tables 3-7). Significant linear correlations were observed among various parameters. For example, organic carbon content was always positively correlated with nitrogen content. There is always a significant positive correlation between Cr, Ni, Cu, Zn, and Pb. On the other hand, there were some different interrelationships among five sampling sites. For example, pH values were negatively correlated with most metals in GXBH and GXDJ, while in other sites no similar trends were observed. Eh was not correlated with any parameters except for samples at GDGQ where Eh was negatively correlated with Cu, Zn, Pb, OC, Salinity, and N. Salinity was positively related to Cr, Ni, Cu, Zn, Pb, OC, and N in GDGQ, GXSK, GXBH, and GXDJ, however, no similar correlations was observed in GDFT. OC and N were positively correlated with Cr, Ni, Cu, Zn, and Pb at GDGQ, GXSK, GXBH, and GXDJ, while such correlations were not observed at GDFT. Comparing the five sampling sites, fewer correlations were observed at GDFT, while similar interrelationships among physicochemical parameters and metals were observed at the other four sites.

Table 3. Correlation coefficient matrix of physicochemical parameters and metal contents at GDFT.

| Clay  | pH     | Eh   | Salinity | Cr   | Ni   | Cu   | Zn   | Pb   | OC  |
|-------|--------|------|----------|------|------|------|------|------|-----|
| pH    | .178   |      |          |      |      |      |      |      |     |
| Eh    | .111   | .186 |          |      |      |      |      |      |     |
| Salinity | -.059 | .079 | .054     |      |      |      |      |      |     |
| Cr    | .133   | -.100| -.019    | -.101|      |      |      |      |     |
| Ni    | .026   | .004 | -.116    | .045 | .818**|      |      |      |     |
| Cu    | -.157  | -.214| .028     | -.081| .669**| .567**|      |      |     |
| Zn    | -.151  | .014 | -.095    | -.034| .504**| .591**|.807**|      |     |
| Pb    | .002   | -.106| .048     | -.084| .253 | .242 | .664**| .644**|     |
| OC    | -.526**| -.158| -.222    | .087 | -.076| -.127| .068 | .003 | .008|
| N     | -.467**| -.128| -.235    | .113 | -.029| -.131| .124 | .049 | .038| .965**|

**represent p<0.01, *represent p<0.05

Table 4. Correlation coefficient matrix of physicochemical parameters and metal contents at GDGQ.

| Clay  | pH     | Eh   | Salinity | Cr   | Ni   | Cu   | Zn   | Pb   | OC  |
|-------|--------|------|----------|------|------|------|------|------|-----|
| pH    | .001   |      |          |      |      |      |      |      |     |
| Eh    | .336** | -.246|          |      |      |      |      |      |     |
| Salinity | -.281*| -.152| -.317*   |      |      |      |      |      |     |
| Cr    | -.230  | .053 | -.204    | .478**|      |      |      |      |     |
| Ni    | -.213  | -.004| -.252    | .518**| .914**|      |      |      |     |
| Cu    | -.255  | -.025| -.327*   | .583**| .764**| .919**|      |      |     |

**represent p<0.01, *represent p<0.05
Due to the differences in absorption and utilization of metals, the metal contents, as well as the interrelationships between metals, would probably be different in diverse mangrove plants. Hence, we performed a correlation analysis of Cr, Ni, Cu, Zn, and Pb contents in plant leaves for the five mangrove species respectively (Table 8). Unlike sediments, less significant linear correlations were...
observed in the metal contents of leaves. For example, there were no statistically significant correlations between metals in *Sonneratia apetala* and *Rhizophora stylosa* leaves. We observed the most correlations in *Avicennia marina*, in which Cr, Cu, Zn, Pb were positively correlated with each other. In *Bruguiera gymnorrhiza* leaves, Cr was positively correlated with Ni, and Zn was positively correlated with Pb. In *Kandelia obovata* leaves, the positive correlations were observed between Cr and Cu as well as Zn and Cu. *Aegiceras corniculatum* was similar to *Kandelia obovata*. Besides Cr&Ni and Cu&Zn, Cu was also positively correlated with Cr.

**Table 8.** Correlation coefficient matrix of Cr, Ni, Cu, Zn, and Pb contents in plant leaves for six mangrove species--spearman correlation.

| Avicennia marina | Cr   | Ni   | Cu   | Zn   | Bruguiera gymnorrhiza | Cr   | Ni   | Cu   | Zn   |
|------------------|------|------|------|------|-----------------------|------|------|------|------|
| Ni               | .183 |      |      |      | Ni                    | .180 |      |      |      |
| Cu               | .741**| .034 |      |      | Cu                    | .193 | -.368|      |      |
| Zn               | .630**| -.131| .655**|      | Zn                    | .603*| -.176| .263 |
| Pb               | .502**| .019 | .359**| .394**| Pb                    | .387 | .841**| -.517*| .057 |

| Kandelia obovata | Cr   | Ni   | Cu   | Zn   | Aegiceras corniculatum | Cr   | Ni   | Cu   | Zn   |
|------------------|------|------|------|------|-----------------------|------|------|------|------|
| Ni               | .253 |      |      |      | Ni                    | .461**|      |      |      |
| Cu               | .545**| -.063|      |      | Cu                    | .464**| .184 |      |      |
| Zn               | .259 | -.142| .543**|      | Zn                    | .209 | .086 | .455**|      |
| Pb               | .143 | -.379*| .144 | .225 | Pb                    | .103 | .144 | -.108| -.085|

| Sonneratia apetala | Cr   | Ni   | Cu   | Zn   | Rhizophora stylosa | Cr   | Ni   | Cu   | Zn   |
|--------------------|------|------|------|------|-------------------|------|------|------|------|
| Ni                 | .327 |      |      |      | Ni                | -.739**|      |      |      |
| Cu                 | .291 | -.357|      |      | Cu                | .644**| -.600*|      |      |
| Zn                 | .400 | -.179| .119 |      | Zn                | .717**| -.734**| .755**|      |
| Pb                 | .523 | -.144| .414 | -.162| Pb                | -.130 | -.090| -.255| .160 |

**represent p<0.01, *represent p<0.05**

In sum, statistically significant linear correlations were observed between physicochemical parameters such as grain size composition, pH, Eh, salinity, and Cr, Ni, Cu, Zn, and Pb contents in surface sediments, indicating certain interrelationships between various parameters and metals. On the other hand, the interrelationships were different among the five sampling sites, likely owing to the differences in local anthropogenic activities such as pollution levels and land use. Moreover, correlation analyses were performed on metal contents in mangrove leaves and different interrelationships were observed among various species. Detailed explanations of these observations will be presented later in the discussion session.

3.3. Distribution Characteristics of Heavy Metals in Sediments and Leaves
There were multiple factors influencing the metal contents in sediments and leaves, hence we performed a multivariate analysis of variance (MANOVA) in order to investigate the distribution characteristics of heavy metals in surface sediments and mangrove leaves, respectively. First, a full
factorial MANOVA was performed on the Cr, Ni, Cu, Zn, and Pb contents in surface sediments for all five sampling sites, totaling 5 variables and 199 samples. Two fixed factors, “location” with five levels and “vegetation type” with six levels were investigated in this test (Table 9). Results showed that location had a significant effect on the overall variance of metal contents in surface sediments (F4, 198=265, p<0.001) and explained 89.2% of the total variance with an observed power of 1.000. The factor vegetation type also significantly affected the overall variance of metal contents in surface sediment (F5, 198=20.041, p<0.001) and explained 38.4% of the total variance with an observed power of 1.000. Moreover, a Multiple-Stage REGWF test gave information about the orders of individual metal content at different factor levels. For example, GDXF had the highest Cr, Ni, Cu, Zn, and Pb contents of all five sampling sites, while GXBH had the lowest Ni, Cu, Zn, and Pb contents. For the factor vegetation type, it significantly influenced Cu, Cr, Ni, and Zn contents in surface sediments while had no statistically significant effects on surface Pb contents. Furthermore, the effects of vegetation types on surface Cu and Zn contents were similar, it followed the order of Bruguiera gymnorrhiza≈Rhizophora stylosa≈Aegiceras corniculatum<Avicennia marina<Sonneratia apetala<Kandelia obovata, indicating that surface Cu and Zn contents were the highest with Kandelia obovata surrounded (Table 9).

Table 9. MANOVA results of between-subjects effects. P values less than 0.05 indicated that the factor had a statistically significant effect on the variable. Partial Eta Squared values presented the contribution of the factor to the variable. In Multiple-Stage Test (REGWF) results column, “<” indicated statistically significantly smaller, “≈” indicated no statistical significance.

| Variables in surface sediments | Factor       | df | F    | p     | Partial Eta Squared | Observed Power | Multiple-Stage Test (REGWF)                |
|--------------------------------|--------------|----|------|-------|---------------------|---------------|-------------------------------------------|
| Cr**                          | Location     | 5  | 30.713 | <0.001 | 43.3%               | 1.000         | GXBH<GXSK≈GDGQ<GDFT                      |
| Ni**                          | Location     | 5  | 85.709 | <0.001 | 68%                 | 1.000         | GXBH<GXSK<GDGQ<GDJ<GDFT                 |
| Cu**                          | Location     | 5  | 239.030 | <0.001 | 85.6%               | 1.000         | GXBH<GXSK<GDGQ<GDJ<GDFT                 |
| Zn**                          | Location     | 5  | 235.807 | <0.001 | 85.4%               | 1.000         | GXBH<GXSK<GDGQ<GDJ<GDFT                 |
| Pb**                          | Location     | 5  | 146.205 | <0.001 | 78.4%               | 1.000         | GXBH<GDGQ<GXSK<GDJ<GDFT                 |

| Variables in surface sediments | Factor       | df | F    | p     | Partial Eta Squared | Observed Power | Multiple-Stage Test (REGWF)                |
|--------------------------------|--------------|----|------|-------|---------------------|---------------|-------------------------------------------|
| Cr**                          | Vegetation   | 5  | 3.667 | <0.01  | 10.2%               | 0.922         | Avicennia marina<Bruguiera gymnorrhiza<Aegiceras corniculatum<Avicennia marina<Sonneratia apetala<Kandelia obovata |
| Ni**                          | Vegetation   | 5  | 5.902 | <0.001 | 15.5%               | 0.994         | Bruguiera gymnorrhiza<Aegiceras corniculatum<Avicennia marina<Sonneratia apetala<Kandelia obovata |
| Cu**                          | Vegetation   | 5  | 15.539 | <0.001 | 32.5%               | 1.000         | Bruguiera gymnorrhiza<Avicennia marina<Avicennia marina<Sonneratia apetala<Kandelia obovata |
| Zn**                          | Vegetation   | 5  | 8.734 | <0.001 | 21.3%               | 1.000         | Bruguiera gymnorrhiza<Avicennia marina<Avicennia marina<Sonneratia apetala<Kandelia obovata |
| Pb                             |              |    | 1.482 | 0.199 | 4.4%                | 0.511         |                                           |

| Metals in mangrove leaves      | Factor       | df | F    | p     | Partial Eta Squared | Observed Power | Multiple-Stage Test (REGWF)                |
|--------------------------------|--------------|----|------|-------|---------------------|---------------|-------------------------------------------|
| Cr**                          |              | 5  | 34.928 | <0.001 | 46.3%               | 1.000         | GDXJ≈GXBH<GDGQ<GXSK<GDFT                 |
A similar full factorial MANOVA was performed on the Cr, Ni, Cu, Zn, and Pb contents and their BCF values in mangrove leaves for all five sampling sites, totaling 199 samples. Location with five

**represent p<0.01, *represent p<0.05
levels and vegetation type with six levels were both the fixed factors. Results showed that the factor location had a significant effect on the metal distribution in mangrove leaves (F4, 198=48.856, p<0.001) and explained 60.3% of the total variance with an observed power of 1.000. The factor vegetation type also significantly affected the metal distribution in mangrove leaves (F5, 198=65.529, p<0.001) and explained 66.9% of the total variance with an observed power of 1.000. Moreover, results of the Multiple-Stage REGWF test indicated that for individual metal contents of mangrove leaves Cr, Ni, Cu, BCFCr, BCFNi, and BCFZn were statistically significantly influenced by the factor location, however, the outcomes were different (Table 9). For example, Ni and BCFNi were the highest in GXSK. Cu in leaves were the highest in GDFT while BCFZn were the highest in GDFT and GXSK. On the other hand, vegetation type significantly influenced Cu, Zn, Cr, Ni in mangrove leaves, and location significantly influenced BCFCr, BCFNi, BCFZn with totally different performances. For example, Cr, Cu, and Zn contents in leaves were the highest in Sonneratia apetala, while BCF of Ni, Zn, and Cr were all highest in GXSK. The detailed orders were presented in Table 9.

In sum, the MANOVA test provided information about whether the location and vegetation type affected metal distribution in surface sediments and mangrove leaves and how location and vegetation type influenced the individual variables. This information will help to investigate the controlling factors on metal distribution in the five mangrove reserves.

4. Discussion

4.1. Influencing Factors on Sedimentary Metal Contents

We listed metal contents in surface sediments of our five sampling sites and other typical mangrove wetlands in China and abroad (Table 10) to compare the levels of metal contents between our and previous studies. Based on pollution load index values, the five sampling sites in this study were separated into two groups, GDFT was strongly polluted with a PLIzone value of 2.61 while the rest were relatively not polluted (Table 1). GDFT site was in the city center of Shenzhen with several sewage discharge rivers running through. Therefore, a higher metal-polluted situation was expected, which was consistent with previous studies [7]. Compared with Singapore, India, and other regions [28-35], the heavy metal contents are generally high in mangroves of Hong Kong and Shenzhen in China (Table 10). For example, much higher Cr contents were observed in mangroves of Hong Kong, Shenzhen, Pearl River, Haikou of China [29-32], and Sundarban of Bangladesh [10]. Higher Ni contents were reported in mangroves of Pearl River and Shenzhen of China, Sundarban of Bangladesh, and Kerala of India [10,20,33]. Higher Cu values were observed in mangroves of Hong Kong of China, Kerala of India, and Farasan Island of Saudi Arabia [8,28,33]. Comparable and higher Zn contents were reported in the mangroves of Hong Kong and Shenzhen in China [7,28]. Higher Pb values were observed in mangroves of Qinzhou, Quanzhou of China, and Kerala of India [30-31,33]. These high values of metals in sediments were all observed in mangrove wetlands with severe anthropogenic activities. For example, Hong Kong and Shenzhen were both highly advanced cities in China with developed industry and likely discharged industrial wastewater containing heavy metals into mangroves and further causing elevated metal levels in mangrove sediments. Moreover, other cities such as Kerala in the reference was also notorious for it is the most densely populated state in India [33]. Hence, the observed high levels of metals were reasonably attributed to human effects.

| Location | Cr     | Ni     | Cu     | Zn     | Pb     | Reference |
|----------|--------|--------|--------|--------|--------|-----------|
| GDFT     | 64.73±10.04 | 33.23±4.34 | 76.94±13.36 | 348.51±57.98 | 46.97±4.84 | This study |
| GDGQ     | 41.36±10.67 | 14.68±3.82 | 16.42±4.33 | 70.26±16.09 | 16.90±4.29 | This study |
| GXSK     | 34.16±9.54  | 13.61±4.14 | 13.43±3.92 | 57.03±16.66 | 16.98±4.78 | This study |
On the other hand, metal contents in our relatively unpolluted sites were comparable to other unpolluted sites in China and aboard (Table 10). Other than the anthropogenic factor significantly influencing metal contents in mangrove sediments, natural factors such as grain size composition, vegetation types, organic carbon contents, sedimentary pH, and salinity also affected the metal contents. For example, due to the low surface area, the accumulation ability of heavy metals in coarser sediments would be lower than finer sediments, resulting in lower metal contents in sandy sediments than clay sediments [25]. This was consistent with the observation that relatively low metal contents were reported in Techeng Island, Zhanjiang, Guang Dong, China due to its sandy sediment textures in the mangrove wetland [32], while mangrove wetland in Quanzhou Bay with fine size particles dominated presented comparable metal contents with our strongly polluted site of GDFT (Table 10).

Also, organic matter is typically positively correlated with heavy metals due to their complexation. Hence, the high organic matter contents are typically associated with elevated metal contents [7].
GDFT had higher values in both metal contents and organic carbon (Table 1). This observation is seemingly caused by the complexation between metals and organic matter. However, there were no statistically significant linear correlations between organic carbon and metals (Table 3). Together, we concluded that the enriched organic matter in GDFT was likely one of the reasons causing the elevated metal contents, while anthropogenic input of industrial wastewater from Shenzhen also contributed significantly to the metal pollution. Moreover, we observed significant linear correlations between salinity, N, and metals in all sites except for GDFT. Among the unpolluted sampling sites, GXDJ has the dominant anthropogenic activities of fishery and aquaculture. GXBH is a wetland park with the lowest average content of heavy metals Cr, Ni, Cu, Zn, and Pb, and the least pollution. GDGQ and GXS have established natural protected areas in the early stage, far away from the urban area, and the main human activity is tourism. These observations together indicated that natural factors dominated the metal distribution in unpolluted study areas in South China (Tables 3-7).

In sum, two types of factors influenced metal contents in surface sediments, anthropogenic factor, and natural factor. Metal contents in surface sediment of unpolluted sites were typically controlled by natural factors such as salinity, grain size composition, and organic matter contents. On the other hand, the controlling factors on the polluted sites were likely the combination of natural and anthropogenic factors. Moreover, the overwhelming anthropogenic impact probably concealed the influence of natural factors, leading to the lack of some normal trends in the metal distribution of surface sediments.

4.2. Influencing Factors on Metal Distribution and Translocation in Mangrove Plants

Metal distribution and translocation in mangrove plants had been studied widely with series of metals and in various mangrove species. Some studies focused on investigating the uptake and tolerance of heavy metals in mangroves planted under a controlled environment with heavy metal stress [32,36-38]. Other studies conducted field studies to explore the distribution and translocation of metals in mangroves in the natural environment [8,11,37]. The studies under controlled conditions lacked consideration of the complexity of the real environment, while the field studies normally sampled one mangrove reserve and were likely fragmentary. Our study sampled five mangrove reserves in South China, hoping to comprehensively consider the impact of complex environmental factors on the distribution and translocation of heavy metals in mangrove plants. For example, MANOVA results indicated that different locations had a significant influence on metal contents in mangroves, where a highly polluted site such as GDFT also had higher metal contents in both surface sediment and mangrove leaves (Table 9). In addition to the impact of sampling location on the content of heavy metals in plant leaves, the type of mangrove plants also has an extremely significant impact on the content of Cr, Ni, Cu, and Zn in leaves (Table 9). The content of Cr, Cu, and Zn is the highest in Sonneratia apetala. The Ni content is the highest in Bruguiera gymnorrhiza and the least in Sonneratia apetala. The samples of Sonneratia apetala and Bruguiera gymnorrhiza came from GDFT and GXDJ, and some samples of Sonneratia apetala came from GXGQ. This is consistent with the results we observed in Table 1.

Studies have found that the most direct and important reason that affects the levels of heavy metal elements in mangrove plants is the corresponding heavy metal content in understory sediments [39]. The basic health of understory sediments directly affects the health of mangrove vegetation. Studies have shown that the three rhizome plants (Rhizophora stylosa, Bruguiera gymnorrhiza, Kandelia obovata) always show higher tolerance to the three species of pioneer plants (Aegiceras corniculatum, Avicennia marina, Acanthus ilicifolius) [36]. For example, Cheng et al. [40] found that three rhizome species (Rhizophora stylosa, Bruguiera gymnorrhiza, Kandelia obovata) have the thickest qualitative exocytosis and the “closest barrier” in the ROL spatial model, and therefore have the most resistance to Zn. From this study, it can be found that, except for Kandelia obovata, the average content of Zn in the crown leaves of the Bruguiera gymnorrhiza and Rhizophora stylosa are significantly lower than the other three mangrove vegetation. The average contents of Cr and Ni in new canopy leaves of Sonneratia apetala, Bruguiera gymnorrhiza, and Kandelia obovata are higher than those of Aegiceras corniculatum, but the average content of Cu, Zn in new canopy leaves of Avicennia marina and
**Aegiceras corniculatus** is higher than that of *Rhizophora stylosa* (Table 2). It is inferred that these observations may be caused by the different tolerances of different places to different mangrove vegetation. The samples of *Rhizophora stylosa* were collected from GDGQ and GXSK, and the average content of heavy metal species in the surface sediments nearby is low (Table 1). Zhang et al. [23] pointed out that the absorption and enrichment ability of soil elements by plants is related to the plant's demand for elements. Zn is an essential element for plants, and Cu is an essential element for the synthesis of chlorophyll and red yeast. The mobility of Pb is very small because Pb entering the soil is easily fixed by the soil and difficult to transfer to the plant parts on the ground. It can be seen from Table 2 that the content of Pb in plant leaves is low and BCFPb is the smallest.

Our samples crossed five mangrove reserves with different anthropogenic impacts, hence, observations of this study can make a very good summary of the distribution characteristics of heavy metals in mangrove plants in natural environments. It can be observed that BCFCu and BCZF in the mangrove plants in five locations are significantly higher than those of the other three heavy metals (Table 2). In particular, the bioconcentration factors of *Avicennia marina*, *Kandelia obovata*, and *Sonneratia apetala* are much higher than those of *Bruguiera gymnorrhiza*, *Rhizophora stylosa*, and *Aegiceras corniculatum*. Usman et al. [8] found that based on the values of BCF were considered too high (>1), *Avicennia marina* is regarded as a high-efficiency plant that accumulates heavy metals especially with Cu and Cr. Comparing with the *Avicennia marina* data in this study, it can be found that the average content of the five metals in the new leaves of the *Avicennia marina* canopy is at a moderate level, and the bioconcentration factors of the five species of heavy species are always lower than that of *Kandelia obovata* and *Sonneratia apetala* (Table 2). When taking the bioconcentration factors of the new canopy leaves as the research variable, two-factor analysis of variance showed that the *Avicennia marina* showed strong adsorption ability for Zn, Pb, and Ni (Table 9). Therefore, *Avicennia marina* can be regarded as a high-efficiency plant that accumulates heavy species with BCF generally higher than *Kandelia obovata* and *Sonneratia apetala*, which was consistent with previous findings [8]. However, in this study, the bioconcentration factors of the five heavy metals in the *Avicennia marina* are all smaller than those of *Kandelia obovata* and *Sonneratia apetala*. This shows that mangrove plants have strong self-regulation ability, and when the concentration of heavy metals increases, they can reduce their ability to accumulate heavy metals to avoid injury [27]. Moreover, the ability of *Avicennia marina* to accumulate metals may be lower than that of *Kandelia obovata* and *Sonneratia apetala*. This may be because *Kandelia obovata* and *Sonneratia apetala* are both rhizome plants, and *Avicennia marina* is a pioneer plant. The pioneer plants are intolerant, so the bioconcentration factor’s capacity is not as strong as rhizome plants.

In short, the contents of heavy metals in leaves are affected by comprehensive factors such as sampling locations, vegetation types, and plant requirements for elements. Different plants have different adsorption capacities for heavy metals. In addition, it is not that the higher the content of heavy metals in the sediments, the higher the bioaccumulation ratio. Sometimes sediments with higher heavy metals contents do not cause a corresponding increase in bioconcentration factors but will reduce its bioaccumulation capacity [27].

### 5. Conclusion

The purpose of this study is to explore the spatial variability of Cr, Ni, Cu, Zn, and Pb in different mangroves in South China and the factors affecting the distribution of heavy metals. We found that there are significant differences in the average content of heavy metals in the five mangrove reserves, the order is GXBH < GXSK < GDGQ < GXDJ < GDFT. GDFT was evaluated as strongly polluted due to the highest pollution load index of 2.61. Through the correlation analysis between physicochemical parameters and heavy metals contents in surface sediments and leaves, we observed that the main factors affecting the distribution of heavy metals in unpolluted sites are natural factors, such as organic matter, salinity, and Eh. However, no similar correlations were observed in severe polluted site GDFT, likely indicating that natural factors play little role in the metal distribution in the polluted area.
Moreover, it was found that sampling locations and vegetation types have significant effects on the content of heavy metals in surface sediments and leaves through the MANOVA test. Different sampling locations, different natural environments, different economic levels, and different levels of human activities have different effects on mangrove pollution. The controlling factors on the polluted sites were likely the combination of natural and anthropogenic factors. Meanwhile, the surface sediments of different sampling locations can directly affect the health of mangrove vegetation. Different mangrove vegetation type has different adsorption abilities for heavy metals. And the bioconcentration factors are not only related to the soil pollution level but also related to the vegetation's demand for elements. The Avicennia marina and Bruguiera gymnorrhiza show strong adsorption ability for Zn, Pb, and Ni, but the bioconcentration ability of heavy metals may be lower than that of Kandelia obovata and Sonneratia apetala. It was deduced that when the concentration of heavy metals increases, plants can reduce the bioconcentration ability to avoid injury.

In sum, this study presented comprehensive and systematical analysis to examine the spatial variability and influencing factors of Cr, Ni, Cu, Zn, and Pb in different mangrove reserves. It contributed more data and facts for metal distribution and translocations in the mangrove forests under both natural and anthropogenic influences, likely provided a more complex perspective to future related studies. However, the influence of time on the content of heavy metals in the mangrove reserve was not considered. Therefore, future studies should discuss the characteristics and influencing factors of mangrove metal contents in combination with time changes and seasonal changes.

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