Extraction and Composition of Dissolved Organic Matter in Poyang Lake and Its Effects on Morphological Changes of Heavy Metals

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Abstract

Dissolved organic matter (DOM) is generally thought to impact the bioavailability of heavy metals. However, the source of wetland DOM and its interaction with heavy metals remain poorly understood. Fluorescence excitation emission matrices (EEMs)-fluorescence regional integration (FRI) coupling techniques and chromophoric dissolved organic matter (CDOM) was used to explore the source of DOM of Poyang Lake including water body, soil and plants, and the effects on morphological changes of heavy metals. The results showed that the best DOM extraction effect can be obtained with the soil-water mass ratio 1:10, centrifuged at 4 000 rpm for 30 min, and the 0.45 μm glass fiber filter by orthogonal test. There were four types of peaks of DOM in water body of Poyang Lake, which was input mainly by land source, while six types of peaks (ACTDBE) were observed in soil. Soil DOM is highly humified with large molecular weight. More types of fluorescent peaks were observed in plant and the content of DOM in plants was higher than that of water body and soil due to the plant proteins. The content of fulvic acid was less than tryptophan in DOM of *Triarrhena lutarioriparia* and *Phragmites communis* in Longkou, while the opposite were in other samples. Furthermore, pot experiment illustrated that DOM had an activation effect on Cd, Cu and Zn and a passivation effect on Cr and Pb with the increase of DOM content. After the addition of exogenous DOM, Cr, Pb and Zn were immobilized by the function together with DOM and plants, while Cd and Cu were activated.

1. Introduction

DOM is an important part of the natural ecosystem, which comes from soil, algae excreta, decomposed residues of plants and animals, and environmental pollutants (emissions from human activities). It is widely distributed in aquatic ecosystems such as rainwater, rivers, swamps, lakes, sediments, pore water and groundwater, and is a key link between organic and inorganic carbon (Vazquez et al., 2011; Yu et al., 2012; Skoog & Arias-Esquivel, 2009). In aquatic ecosystems, DOM not only affects the maintenance and release of nutrients, bioavailability, but is also used to characterize water quality characteristics (Hill et al, 2009; Zhang et al., 2009; De Vittor et al., 2008).

To date, The DOM extraction method has not been unified. In order to quickly and easily compare the concentration of DOM, many scholars have used the spectral characteristics of CDOM to quickly estimate the concentration of DOM, the concentration of CDOM was quantified based on the absorption coefficient at 355 nm (a_{355}) (Gueguen et al., 2005; Zhou et al., 2016). In addition to external conditions that affect the composition and content of the DOM, the structure and functional characteristics of the DOM itself can alter the migration and bioavailability of pollutants in the environment. The hydrophilic part can provide nutrients for microbial growth, and a strong ability to complex with heavy metals, while the hydrophobic part has a stronger ability to bind other pollutants (Bolan et al., 2011; Chantigny et al., 2014). Therefore, there are urgent requirements for the characterization of DOM. For example, EEM_S separates different fluorescence peaks to characterize the DOM's fluorescent material in the biogeochemical law and its spatial and temporal distribution characteristics, covering a wide range of fields, including rivers, lakes, and oceans in freshwater (Carstea et al., 2016; Wei and Jin, 2015; Timko et al., 2015). Common fluorescent indicators such as the fluorescence index (FI), biosource index (BIX) (characterizing the proportion of newly generated DOM), the humification index (HIX) and $R_{A / C}$ (can be used as indicators to judge the source of DOM in the polluted river water body) was generally used to characterization of DOM (Li et al., 2019;Yu et al., 2012). Fluorescence regional integration (FRI) is another method for analyzing fluorescence spectra, which can identify and characterize overlapping objects of fluorescence spectra in a multi-component system (Song et al., 2018).

Previous studies have shown that the chemical speciation, bioavailability, modification, and toxicity of heavy metals can be affected by DOM in aquatic or soil environments (Li et al., 2013; Dong et al., 2014; Li et al., 2019). In the aquatic ecosystem, human active sewage discharge, land cover, and landscape pattern changes all may cause DOM changes. Nason et al. (2012) found that rainwater runoff DOM can reduce the toxicity of Cu^{2+} by forming a complex with Cu^{2+}, which is easier than Zn^{2+} to integrate with DOM of rainwater runoff (Gnecco et al., 2008). More studies showed that stable complexes formed by DOM and heavy metals, is attributed to the rich functional groups (e.g. hydroxyl, carboxyl and carbonyl groups) in DOM (Impellitteri et al., 2002). Chai et al (2012) combined three-dimensional fluorescence spectroscopy studies to find that humic acid has a strong binding force with Hg^{2+}, and mercury will preferentially bind to trace amounts of thiols and other sulfur-containing groups in organic matter (Ravichandran et al., 2004). In soil, DOM can be used as a carrier of pollutants, adding different organic fertilizers (e.g. urea, liquid nitrogen, lime application) to the soil lead to the increase of pH, which may cause the dissolution of organic matter. The pollutants heavy metals or organic substances, at the same time, will be dissolved from the soil (Johnson and Amy, 1995; Kaschl, 2002; Karlik, 1995). When biochar is applied to contaminated soil, the hydrophobic substance in DOM will increase Cu toxicity, and individual fluorescent substances and functional groups in DOM have different binding characteristics with specific heavy metals (Huang et al., 2019).

Poyang Lake, as the largest freshwater lake in China, has great ecological functions in water resource supply, climate regulation and biodiversity maintenance, playing an important role in flood control and reduction for the middle and lower Yangtze River (Wang et al., 2014; Yang et al., 2016). Due to the extreme fluctuations of seasonal water levels, the lake presents a typical annular hydrophilic plant distribution, with an annular gradient along the bottom (Wang et al., 2019). Its typical riparian zone is between the aquatic and terrestrial ecosystems, which has special vegetation, biology, soil, and hydrological characteristics. The problem of heavy metals in Poyang Lake has been much studied due to non-ferrous metal mines (e.g. Dexing copper Mine, Dayu tungsten Mine) in the Poyang Lake Basin, was long-term mined that affect the ecological environment (water bodies, sediments) of the mining area (Zhang et al., 2018). Concentrations of Arsenic, chromium and mercury in soil was determined in the Poyang Lake region by Jiang et al. (2019), which showed that the average concentrations of these metals significantly increased from 10.72, 63.25, and 0.10 mg kg^{-1} in 1983 to 12.14, 84.06, and 0.13 mg kg^{-1} in 2010, respectively. In addition, previous studies have reported the related work such as eutrophication of Poyang Lake, fluorescence characterization of colloidal DOM, and the impact of DOM on methanogenesis (Li et al., 2020; Yan et al., 2018; Wang et al., 2016). However, the source analysis of Poyang Lake DOM and its impact on heavy metals are rarely reported.

The main objective of this study were thus to i) elucidate factors affecting DOM extraction conditions and sources: considering soil-water ratio, shaking time, centrifugation rate, centrifugation time, filtration membrane and extract; ii) explore the distribution characteristics, composition characteristics, and sources of dissolved organic matter in different areas of Poyang Lake by FRI; iii) analyze the effect of exogenous DOM on the shapes of heavy metal by BCR extraction.

2. Materials And Methods

2.1. Assay design and sampling

Poyang Lake wetland was divided into three ecological micro-regions: Wucheng national nature reserve, Nanji wetland national nature reserve, and Longkou provincial nature reserve for the sampling site. The sampling period was during the flat water period (April) and the high water period (September) (Fig. 1): left of entrance to the lake (N1), right of entrance to the lake (N2), Tuoshan (N3) and Nanji Bridge (N4); three sampling sites in Wucheng: Wangjiazhou (W5), Datouzhou (W6) and Hengzhou (W7); three sampling sites in Longkou: L8, L9 and L10. Water in Poyang Lake was also collected separately in Nanjishan Lake (NJS) and Wucheng Lake (WH).

We here used shovel to collect 0-10cm surface soil and marked the soil sample with “S”, while the sediment was collected with a metal grab sampler, marked with “D”, which was stored in a 100-mesh sieve protected from light after air-drying. The physical and chemical properties of the test samples are shown in Table 1

Table 1

The physical and chemical properties of the test samples

Sample number	Types	pH	CEC/(cmol·kg^{-1})	Amount of dry matter / (%)	TOC/(mg·kg^{-1})
1#-S	Meadow soil	5.22	9.44	97.12	65.68
1#-D	sediment	5.61	8.23	98.17	73.81
2#-S	Meadow soil	4.74	9.49	96.30	64.87
3#-D	sediment	5.50	7.42	97.59	39.64
3#-D	sediment	6.07	9.71	96.65	18.73

2.2. Extraction of DOM

Overlying water DOM extraction was obtained by using a 0.45 μm glass fiber filter membrane for the overlying water (burned at 500°C for 4 h in a muffle furnace). DOM of soil and sediment was extracted by shaking according to soil-water mass ratio 1:10, and centrifuging, the supernatant was filtered through a 0.45 μm glass fiber filter (calcined at 500 °C for 5 h). The obtained filtrate is the soil or sediment DOM extraction. The plant sample DOM was extracted by shaking with the ratio of plants to water 1:40 in a shaking box for 24 hours, and then centrifuged at 8000 rpm for 20 min. Plant DOM extraction was obtained for use after passing through a

0.45 μm glass fiber filter. Samples need to be diluted 50-fold before determination due to the high DOM content in the plant. DOC concentration was determined with an organic carbon (TOC) analyzer (multi N/C 2100, Jena, Germany).

2.3. Orthogonal test

DOM was scanned in the UV-visible spectrum in the wavelength range of 190–600 nm (Beijing Universal Analysis UV-Visible Spectrophotometer TU-1901) to calculate ag (355) and perform a linear fit regression on ag (355) and DOC concentration. When the other conditions are fixed, the effect of the types of extraction solution (H_2O, $CaCl_2$, and KCl), the pore size of the filter membrane (0.45 μm and 0.22 μm), and the shaking time (15 min, 30 min, 1.5 h, 3 h, 5 h, 8 h, 12 h, 15 h, 16 h, 18 h, 19 h, 24 h, 36 h, 43 h, 44 h) on soil DOM extraction are investigated, based on CDOM ag (355). With the CDOM content as the optimization index, L9 (34) orthogonal test was used, to explore the effects of different extraction conditions on the extraction of soil DOM, utilizing water as the extraction solution. The experiment was repeated three times. The DOM sample obtained by the orthogonal test was measured after proper dilution. The soil-water mass ratio of the DOM sample is 10 times dilution of 1: 2, 4 times dilution of 1: 5, and 2 times dilution of 1:10 so that get the theoretical DOM concentration of consistence.

2.4. Spectral analysis

2.4.1. EEMs analysis

Fluorescence spectrophotometer (F-4500 Hitachi, Japan) was utilized to character DOM (Bandpass: Ex = 5 nm, Em = 5 nm; response time: automatic; scanning speed: 2400 nm/min). The excitation wavelength was from 200 to 600 nm at 10 nm increments, and the emission (Em) wavelength was from 200 to 600 nm at 10 nm increments. The temperature of the sample should be kept at 25 °C before measurement.

2.4.2. FRI analysis of DOM

The three-dimensional fluorescence spectrum is divided into five parts according to the excitation / emission wavelength region: Ⅰ) the Ex/Em is (220 ~ 250) / (280 ~ 330); II) the Ex / Em is (220 ~ 250 / (330 ~ 380); III) Ex/Em is (220 ~ 250) / (380 ~ 500); IV) Ex / Em is (250 ~ 280) / (280 ~ 380); V) Ex / Em is (250 ~ 400) / (380 ~ 500)(He et al., 2013). After pre-processing using matlab2016b, the area was divided into blocks, and the area integral calculation is performed in Excel to calculate the specific fluorescence area integral volume (φi), where φi represents the cumulative fluorescence intensity of organic substances with similar properties. Normalized analysis of φi was used to obtain the standard volume of integral of a certain fluorescent region (φi, n). The proportion of the integral of the specific structure organic matter in a certain fluorescent region to the total integral (Pi, n) was finally calculated.

2.4.3 UV spectrum analysis of DOM

The DOM test solution was poured into a 1 cm quartz cuvette, and scanned at full wavelength in a UV-visible spectrophotometer. Wavelength scanning range is 190 nm to 800 nm, step size is 5 nm, medium speed scanning. The sample was calibrated with ultrapure water before measuring.

2.5. Pot experiment

Carex cinerascens collected from Poyang Lake was washed with ultrapure water (Milli-Q, Milli-pore Corporation). The experiment was set up with two groups of planting *C. cinerascens* and no *C. cinerascens*, which included a total of 18 pots and a culture period of 1 month. After the soil collected from Poyang Lake was measured for heavy metal morphology (without planting *C. cinerascens*), DOM was added to the soil that the content of DOM was control (without adding DOM); 0.5g/kg; 1.0g/kg. Heavy metal morphology was analysis one month later. *C. cinerascens* was transplanted and cultivated into the soil in which the heavy metal morphology was determined (planting *C. cinerascens*), which was consistent with the above treatment. The total amount of heavy metals in plants and soil was determined by ICP-OES (ICAP7400, Thermo Fisher).

The morphological extraction of heavy metals in this paper uses the improved three-step extraction method of BCR to extract the Acid extractable form (AEF), oxidizable form (OF), reducible form (RF) and residual form (RESI) (Quevauviller et al., 1997; Li et al., 2019).

2.6. Data analysis

The data was analyzed and processed using Origin8.5, Excel and SPSS software, and the three-dimensional fluorescence spectrum was plotted using Matlab2016. Sampling maps were drawn using Arcgis and Photoshop.

3. Results And Discussion

3.1. Extraction of DOM

3.1.1. Influencing factors of DOM extraction in water-level fluctuation zones of Poyang Lake

The soil and sediment samples collected from 4 sampling points in Nanjishan, Poyang Lake were used to extract DOM according to the method. Part of the TOC value was measured by TOC instrument, and the other part was scanned by ultraviolet spectrum. Combined regression, the relationship between CDOM and DOM concentration is obtained (Table 2). The ag (355) of the five test samples was linearly fitted with the DOM concentration, and the correlation coefficients R were all above 0.99, indicating that the soil / sediment CDOM and the DOM concentration were linearly correlated, which was consistent with the previously reported results (Rochelle-Newall and Fisher, 2002). This shows that the DOM content in soil / sediment samples can be estimated by the CDOM concentration expressed by the absorption coefficient ag (355). Therefore, we selected 1 # -S as the experimental soil sample for the following DOM extraction conditions.

Table 2 Correlation between CDOM and DOM

Sampling	Intercept		Slope		Statistics		
	Value	Standard deviation	Value	Standard deviation	R^2	R	Sum of squared residuals
1#-S	-1.232	0.484	1.665	0.017	0.999	1.000	3.233
1#-D	0.474	1.738	3.774	0.051	0.999	1.000	25.225
2#-S	0.609	0.254	0.212	0.008	0.994	0.998	0.356
3#-D	7.078	1.101	2.964	0.065	0.998	0.999	16.735
3#-D	-0.342	0.144	0.685	0.015	0.998	0.999	0.115

H_2O, KCl solution and $CaC1_2$ solution (molar concentration both 0.5 mol / L) were used as extracts to extract the DOM in the soil, and scan the UV spectrum that was shown in Figure 1a. The ultraviolet absorption curve extracted with $CaCl_2$ was similar to the peak shape of DOM extracted with water. Both of them have an absorption peak at 200 nm and a small absorption platform between 250-300 nm, but the absorbance is significantly lower than that of water extract. When extracted with KCl, a negative absorption peak appeared, indicating that the effect of extracting DOM in wetland soil with KCl is not obvious, which may be related to the content of soil clay and ionic strength. DOM is generally considered to be a continuum or mixture of a series of complex compounds with negative charges (e.g. low molecular weight: free amino acids, carbohydrates, organic acids; large molecular weight: enzymes, polysaccharides, phenols, and humus.) (Kalbitz et al., 2000). Therefore, K^+, Na^+, Ca^{2+}, H^+ and other positive ions will compete with various organic and inorganic ligands in soil solid phase and soil solution. In addition, the negative charges between DOM molecules can be isolated with the increase of ionic strength, which can reduce the electrostatic repulsion between DOM molecules and the mineral surface and between DOM molecules (Shen, 1999). Both of these factors will cause an increase in the amount of soil adsorbed to DOM, which lead to a decrease in the concentration of DOM in the extract. Purification of DOM from water is a prerequisite for the study of its characteristics, superficial behavior and environmental effects (Gnecco et al., 2008).

In order to determine the effect of the pore size of the filter membrane on the extraction of DOM, the absorbance and ag (355) values of 0.22 μm and 0.45 μm glass fiber filter solutions were compared, as shown in Figure 1b. The ag(355) of extraction with 0.45μm filter membrane is ten times more than that with 0.22μm filter membrane. The two extraction trends are resemblance over time, which are both tend to be stable after reaching the highest value at 16h. However, research of Novak et al. (1992) showed that the distribution of water-soluble organic carbon functional groups is very similar and the overall structure of DOM is less affected by the pore size of the filter by using ^{13}C nuclear magnetic resonance (NMR) to compare structure of DOM after filtration with 0.40 μm and 1.20 μm filters membrane.

3.1.2. Optimal extraction conditions for DOM extraction with water by orthogonal test

Influence of pH was not considered in this work based on the study of Weishaar et al. (2003) that when the pH is in the range of 2 ~ 8.6, it basically does not affect the measurement results of the UV absorbance in DOM, due to the pH of the samples are within this range. The effects of water-soil quality ratio, centrifugal rate, filter membrane type, and centrifugation time on the extraction of soil DOM when the extraction is water by orthogonal test were shown in Table 3. A3B2C1D3 is the most suitable extraction process: the highest soil DOM concentration can be extracted on the conditions of the soil-water mass ratio is 1:10, the centrifugal speed is 4 000 rpm, the 0.45 μm glass fiber filter membrane, and the centrifugation time is 30 min. The obtained CDOM reached 132.653 m-1 under the optimal extraction conditions. It has demonstrated that the effect of the soil-water ratio is the largest, followed by the filter membrane, and the centrifugation time is the smallest according to the range (RA> RC> RB> RD). From the trend of the optimization index CDOM content change, the amount of CDOM will significantly increase by increasing the soil-water ratio and increasing the centrifugation time.

Table 3 Intuitive analysis of orthogonal design in CDOM experiment

Test number	soil-water ratio (A)	Centrifugation rate (B)	filter membrane (C)	centrifugation time (D)	CDOM/m^{-1}
1	1	1	1	1	18.194
2	1	2	2	2	0.691
3	1	3	3	3	1.382
4	2	1	2	3	7.600
5	2	2	3	1	0.691
6	2	3	1	2	1.382
7	3	1	3	2	27.406
8	3	2	1	3	132.653
9	3	3	2	1	2.303
K1	20.267	53.200	152.229	21.188	
K2	9.673	134.035	10.594	29.478	
K3	162.362	5.067	1.555	141.634	
A1	6.755	17.733	50.743	7.063	
A2	3.224	44.678	3.531	9.826	
A3	54.120	1.689	9.826	47.212	
Range	50.896	42.989	47.212	40.149	

3.2. Source and structure of DOM in water-level fluctuation zones of Poyang Lake

All the lake water samples in the three sampling areas showed similar fluorescence patterns, showing four peaks of A, C, B and T (Fig. 3). Fluorescence peaks A and C belong to humic acid-like fluorescence, representing DOM that is more difficult to degrade, mainly from terrestrial input (Kalbitz et al., 2000). The EEMs of Poyang Lake water showed humic acid A and C peaks with high fluorescence intensity, indicating that the lake water is affected by less human activity pollution, and the lake mainly comes from terrestrial input. Peaks B and T are weaker than peaks A and C, but also has a certain intensity. Peaks B (Tyrosine-like substances) and peaks T (Tryptophan-like substances) are considered to be related with microbial activities and tryptophan-like peaks can indicate the presence of bioavailability organic matter (natural or artificial) reported by Hudson et al. (2008), which indicated that some organic matter enters the Poyang lake through exogenous and microbial activities. In the soil sampling area, it can be observed that there are four peaks of ABCT, two peaks of E and D in the soil of NJS, peak D in WC soil, and peak E was more obvious in LK soil. Peak D is the fluorescence peak of tyrosine, which generally appears in DOM with a high degree of humification or a large molecular weight and very sensitive to photodegradation, representing the fluorescence groups of some aromatic protein structures produced by microbial degradation (Williams, 2000). Peak E can be generated by the decomposition of fulvic acid-like substances by microorganisms. Compared with humic acid, it is not easy to combine with organic pollutants in the environment to form toxic substance, due to humic acid has a strong ability to complex and adsorb cationic groups. Humic acid-like peak in soil, compared with the water body, is more obvious, and the protein-like peak is less obvious, indicating that the humification and molecular weight in soil are higher than that of the water body. The three-dimensional fluorescence spectra of plants (*Carex cinerascens, Phragmites australis, Triarrhena lutarioriparia, Daucus carota*) were observed to be similar to water bodies and soils, but there were more types of peaks than water bodies and soils. These four plants are rich in fulvic acid-like substances and humic acid-like substances. There are many adsorption sites on the surface of humic acid, which can form insoluble complexes with heavy metals in Poyang Lake (Ren et al., 2017; Bai et al., 2019). During the high water period, the lake water will immerse these plants that may provide humic acid to the water body after they die and decomposition.

The parameters reflecting DOM characteristics were shown in Table 4. The fluorescence index (FI) is defined as the ratio of the spectral intensity of the excitation wavelength between 450 nm and 500 nm, while excitation wavelength is 370 nm, which is used to evaluate the source of humic (especially fulvic acid) (Zhang et al., 2018). The FI values of terrestrial DOM and biological source DOM are 1.4 and 1.9, respectively. When FI> 1.9, the DOM source is mainly microbial and algae activities (endogenous), and the autogenic characteristics are obvious, while FI <1.4, the endogenous contribution that mainly from external input, is relatively low (Jaffé et al., 2004).The FI of water, soil and plant were 1.64 ~ 1.76, 1.70 ~ 1.98 and 1.52 ~ 2.15 respectively, and the average values were all close to 1.9, indicating that DOM originated from the activity of bacteria and algae, showing the characteristics of spontaneous source. BIX is generally used to evaluate the relative contribution rate of DOM derived from microorganisms in the sample (Ylla et al., 2012). When BIX is between 0.6 and 0.8 that indicate there is less autogenous contribution in the sample. When it is between 0.8 and 1.0, it indicates that there are more authigenic DOM; and when BIX> 1.0, it indicates that DOM is mainly derived from authigenic sources and organic matter is newly generated (Zhang et al., 2018).The BIX of the water sample is between 0.85 and 0.93, indicating that it had the characteristics of recent autogenous source. The BIX in the soil was between 0.69 and 3.24. Except for sample L10 in LK, the soil BIX at all other sampling sites is greater than 1, indicating that the soil DOM is highly bioavailable and has significant autogenous characteristics, which contributes to the soil microbial community formation, and thereby leaded to higher protein-like components in soil DOM. The $R_{A/C}$ of Poyang Lake water is between 0.89 and 2.00, and the $R_{A/C}$ of soil DOM is distributed between 2.50 and 8.59, with a higher average value degree..The $R_{A/C}$ value reflects the degree of change of DOM affected by light radiation and development of humic components in DOM (Gao et al., 2015; Chen et al., 2016), thus, this may be related to the photochemical behavior of DOM that the humus-like peak C is more likely to occur photolysis than the peak A (Helms et al., 2009). The $R_{T/C}$ in water body and soil were basically less than 2.0, which was illustrated that they were less affected by human emissions due to the previous studies has shown that $R_{T/C}$ is the ratio of protein-like fluorescence to humic acid-like fluorescence, which can evaluate the proportion of endogenous contribution, and $R_{T/C}$ of DOM affected by anthropogenic emissions is usually greater than 2.0 (Gnecco et al., 2008; Galapate et al., 1998). Except for *T. lutarioriparia* and *P. australis* in LK, the $R_{A/E}$ and $R_{C/E}$ of other samples were all greater than 1. This showed that the content of fulvic acid-like substances in DOM is greater than that of tryptophan-like substances, while *T. lutarioriparia* and *P. australis* in LK were opposite.

Table 4 Fluorescence parameters of DOM (water, soil and plants) in Poyang Lake

Water sample	FI	BIX	$R_{A/C}$	$R_{T/C}$	Soil sample	FI	BIX	$R_{A/C}$	$R_{T/C}$	Plant sample	FI	BIX	$R_{A/E}$	$R_{C/E}$
N1	1.76	0.93	1.52	0.42	N1	1.95	1.88	2.50	0.58	N1 *C. cinerascens*	1.82	0.48	3.24	2.38
NJS	1.71	0.89	0.89	0.19	N2	1.86	1.27	4.67	1.05	N1 *P. australis*	2.15	0.73	4.11	3.57
N2	1.68	0.90	1.71	0.49	N3	1.97	3.24	2.29	2.41	W5 flowers	1.90	0.66	38.18	26.13
N3	1.68	0.88	1.06	0.18	N4	1.69	1.61	4.26	1.28	W5 *D. carota*	1.79	0.48	5.41	3.86
N4	1.70	0.88	1.76	0.49	W5	1.83	1.16	3.33	0.90	W5 *C. cinerascens*	1.60	0.56	10.82	10.28
W5	1.64	0.87	1.24	0.20	W6	1.70	1.28	3.30	1.03	W5 *P. australis*	1.89	0.81	2.56	2.06
W6-1	1.69	0.90	2.00	0.47	W7	1.75	1.37	6.67	1.52	L8 *C. cinerascens*	1.52	0.38	6.51	5.75
W6-2	1.71	0.88	1.29	0.21	L8	1.79	1.27	8.59	1.90	L9 weed	1.61	0.19	11.62	16.01
WH	1.72	0.85	1.94	0.42	L9	1.98	1.04	2.52	0.70	L9 *T. lutarioripa-ria*	1.88	0.60	0.88	0.56
W7	1.66	0.86	1.37	0.21	L10	1.81	0.69	4.06	0.28	L10 *P. australis*	2.09	1.54	0.86	0.23
L8	1.70	0.85	1.67	0.27										
L9	1.67	0.91	1.49	0.26										
L10	1.71	0.90	1.74	0.60										

The FRI can be used to express the relative content of certain types of compounds that is based on the five regions of the EEM fluorescence spectra presented in fig. 4(chen et al., 2017). $P_{(III, n)}$ accounted for the highest proportion of water samples (23% ~ 39%), followed by $P_{(II, n)}$ (17 ~ 32%). Due to the characteristics of the wetland ecological structure, the concentration of organic matter was relatively stable. The proportion of aromatic protein in water is between 25% and 50%, indicating that the water body is affected by a certain degree of human activities such as ferry and fishing. $P_{(III, n)}$ of soil was the highest, which was more than 60% in the N4, W5, W6 and L9, indicating that the soil humification intensity is greater. $P_{(I, n)}$ and $P_{(II, n)}$ were observed have a higher proportion in N1, N2, N3, W7 and L8 due to those regions were large number of residents, and greatly affected by human activities, while N4 was the opposite. The content of soluble microbial by-product-like and humic acids in plants is relatively high, and the proportions of *C. cinerascens* in three ecological micro-regions were similar. The *P. australis* of N2 and W5 had similar proportions, but are not the same as L10 which had high content of tryptophan-like, may be due to the affected by anthropogenic emission factors. In addition, planting of different kinds of plants will greatly affect the distribution of DOM in soil and water.

3.3. Effect of DOM on the changes of heavy metal forms in soil

Heavy metal form was determined after transplanting the *C. cinerascens* (dominant species) into the soil with the determined heavy metal form and planting for 1 month (Fig. 5a). Among these five heavy metals, RESI-Cr accounted for more than 50%, and AEF-Cr was basically undetectable, which was shown that biotoxicity of Cr in soil was low. Under the influence of plants, AEF-Pb, RF-Pb, AEF-Zn, RF-Zn and RF-Cr were decreased, and more RESI appeared. Studies have shown that in the classification of the morphology of heavy metals, the AEF is the most active form that can be easily absorbed by plants (Meng et al., 2017), The RF is the effective form, which was strong bioavailability with high ratio. Therefore, the bioavailability of the three heavy metals became smaller, indicating that planting *C. cinerascens* provided a certain immobilization on Pb, Zn and Cr. Zn was the most effective that AEF-Zn decreased 14.1% and RESI-Zn increased 24.0%. On the contrary, planting *C. cinerascens* provided activation on Cd and Cu due to the decreased 18.4% of RESI-Cd and 4.3% RESI-Cu.

The change in the distribution ratio of heavy metals in the different fractions after adding to DOM (0.5 g/kg～1 g/kg) alone of soil were presented in fig. 5b. The RESI in Cd, Cu and Zn was decreased with the increase of DOM of soil. Furthermore, it is obvious that the RESI of Cd and Cu transformed into OF and RF, while that of Zn transformed into AEF. The RF-Cr decreased by 6.18 mg/kg (0.5 g/kg DOM) and 6.96 mg/kg (1.0 g/kg DOM) respectively (Table 6) and the RESI-Cr increased by 8.08 mg/kg (0.5 g/kg DOM) and 9.11 mg/kg (1.0 g/kg DOM). In terms of Pb, the maximum decrease rate after treatment was 13.6% (1.0 g/kg DOM) based on the sum of the AEF-Pb and RF-Pb with relatively higher risk for the plant. Thus, adding DOM in the soil provided activation on Cd, Cu and Zn and a certain immobilization on Cr and Pb.

After adding DOM (0.5g/kg～1g/kg) to the soil planted with *C.cinerascens*, the morphological changes of heavy metals were shown in Fig. 5c. The RESI in Cr, Pb and Zn increased, while RESI in Cd and Cu decreased,indicating that plant and DOM combined effect provided an immobilization on Cr, Pb and Zn, and activated Cd and Cu, which was consistent with planting *C. cinerascens* alone. The AEF- Cd increased by a maximum of 6.71% (Planting *C.cinerascens* +1.0 DOM), and its original AEF content accounted for more than 60%, which was more harmful to the soil of Poyang Lake. Previous studies has demonstrated that due to the affinity of Cu to organic acids that contributes to the formation of humic acid and the organic fractions was found to be well correlated with humic acid (Dias et al., 2010). It might be high content of humic acid in DOM that led to the transformation of RESI of Cu into a reducible state RF. In addition, the decrease of AEF-Zn was basically transformed into the RESI-Zn. This was might because there are several oxygen-containing functional groups, such as carboxyl, phenol hydroxyl, as well as C=O in DOM that can cooperate with the metal ions to form more active AEF heavy metals (Albrecht et al., 2011)

Table 5 Total amount of heavy metals with different treatment

Total amount of heavy metals (mg/kg)	Cd	Cr	Cu	Pb	Zn
Control	2.08±0.20	133.49±1.10	101.29±9.18	46.05±2.40	204.77±8.73
Planting *C.cinerascens*	1.69±0.22	93.23±5.02	97.43±15.97	51.44±1.85	218.43±31.33
0.5 g/kg DOM	2.11±0.21	91.54±1.68	92.26±13.34	53.08±7.62	245.82±1.90
1.0 g/kg DOM	1.90±0.35	91.27±0.05	77.93±7.87	54.33±7.40	207.34±21.78
Planting *C.cinerascens* +0.5 g/kg DOM	1.85±0.05	91.73±1.30	87.67±3.90	50.14±2.30	212.17±12.67
Planting *C.cinerascens* +1.0 g/kg DOM	1.85±0.29	96.36±0.03	89.64±6.36	54.61±2.07	194.79±17.64

4. Conclusion

This work has demonstrated that Soil DOM extracted with H_2O can obtain the best effect due to its CDOM has the largest UV absorption coefficient. The optimum condition of extracting DOM is that soil-water mass ratio is 1:10, the optimal centrifugal speed is 4 000 rpm, the centrifugation time is 30 min and pass the 0.45 μm glass fiber filter membrane by orthogonal test. FIR-EEMs was used to character DOM in Poyang lake, which can obtain that aromatic proteins in the water of Poyang Lake occupy a higher concentration (25%~50%), indicating that the water body is affected by a certain degree of human activities. The proportion of III substances in soil is higher and plants have a higher proportion of IV and V substances which has illustrated higher levels of soluble microbial metabolites and humic acids. The experiment of the effect of DOM on the change of soil heavy metal morphology showed that the combined effect of plant and DOM was consistent with that of planting *C. cinerascens* alone, which provided an immobilization effect on Cr, Pb and Zn, and an activation effect on Cd and Cu. When DOM was added alone, Cd, Cu and Zn were activated, while Cr and Pb were immobilized and the effect of that in Cr and Pb were stronger with the increase of adding DOM.

Declarations

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors Contributions

Yuanhang Li: Conceptualization, Data curation, Writing-original draft. Zhaoying Shen: Methodology, Formal analysis, Writing-review & editing. Xiaofeng Gong: Conceptualization, Supervision, Funding acquisition. Huiqing Zeng: Investigation. Chunli Chen: Software. Ru Zhang: Supervision, Visualization.

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Figures

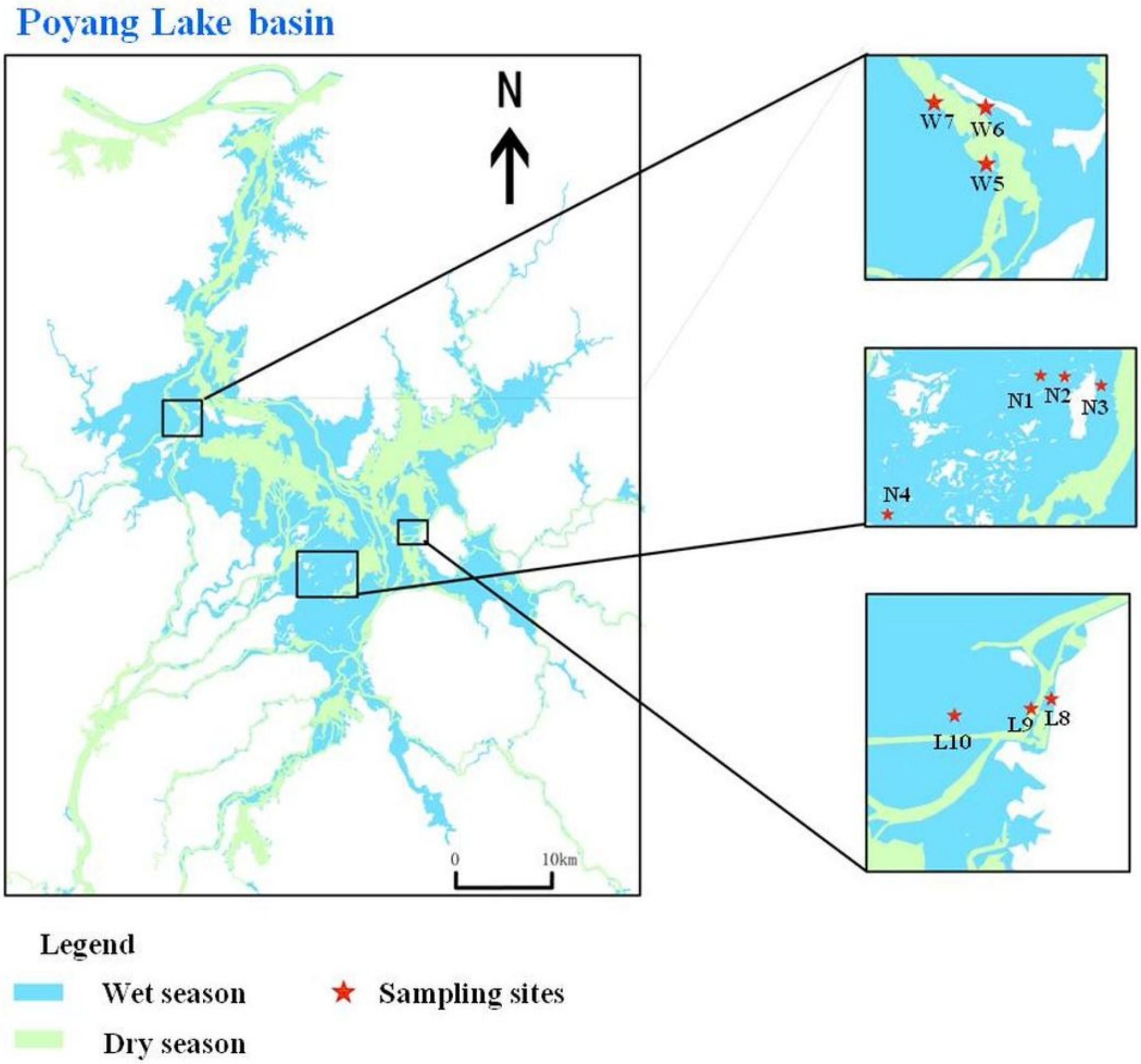

Figure 1

The sampling sites of Poyang Lake basin. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

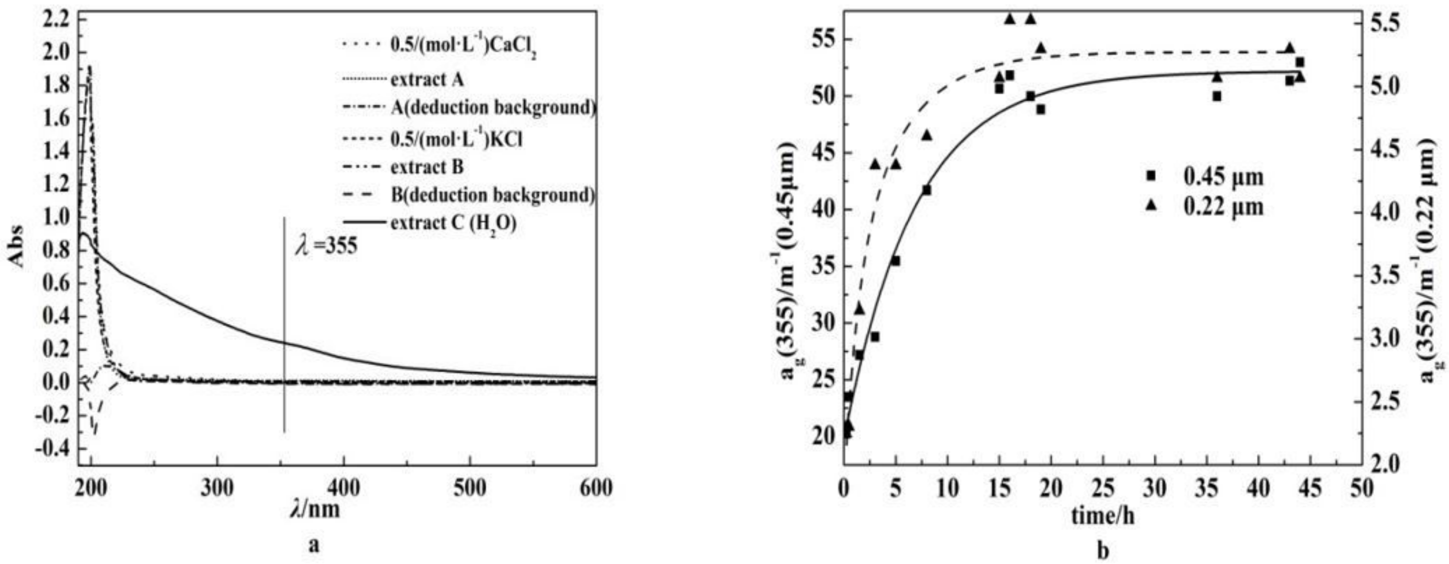

Figure 2

Effect of different factors on DOM extraction

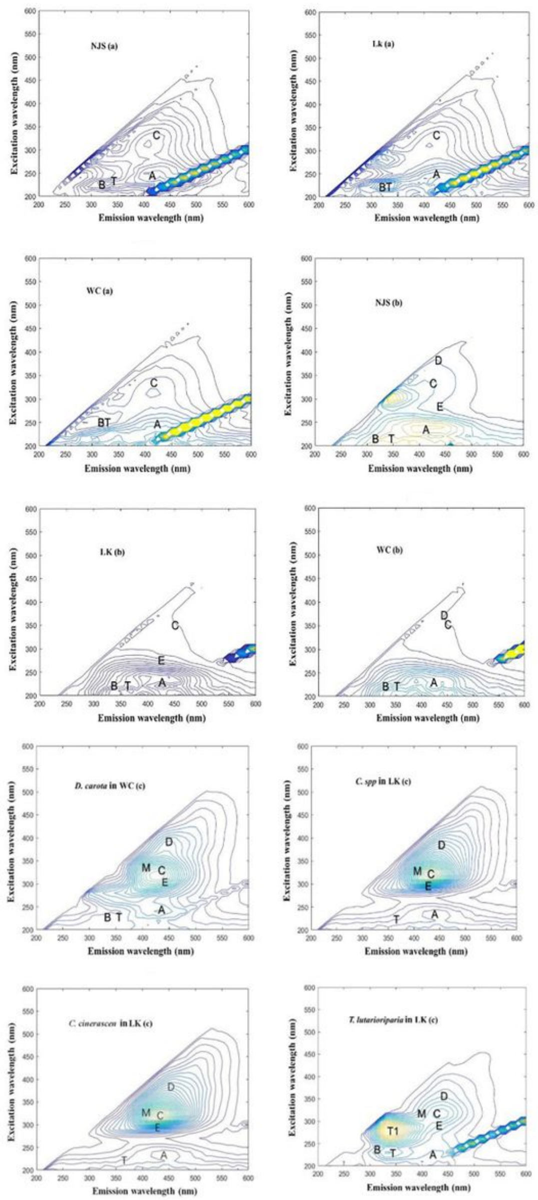

Figure 3

Three-dimensional fluorescence spectra of DOM derived from Nanjishan (NJS), Wucheng (WC) and Longkou (LK) in three areas of Poyang Lake. Lake water was expressed with (a); Soil was expressed with (b); Plant was expressed with (c).

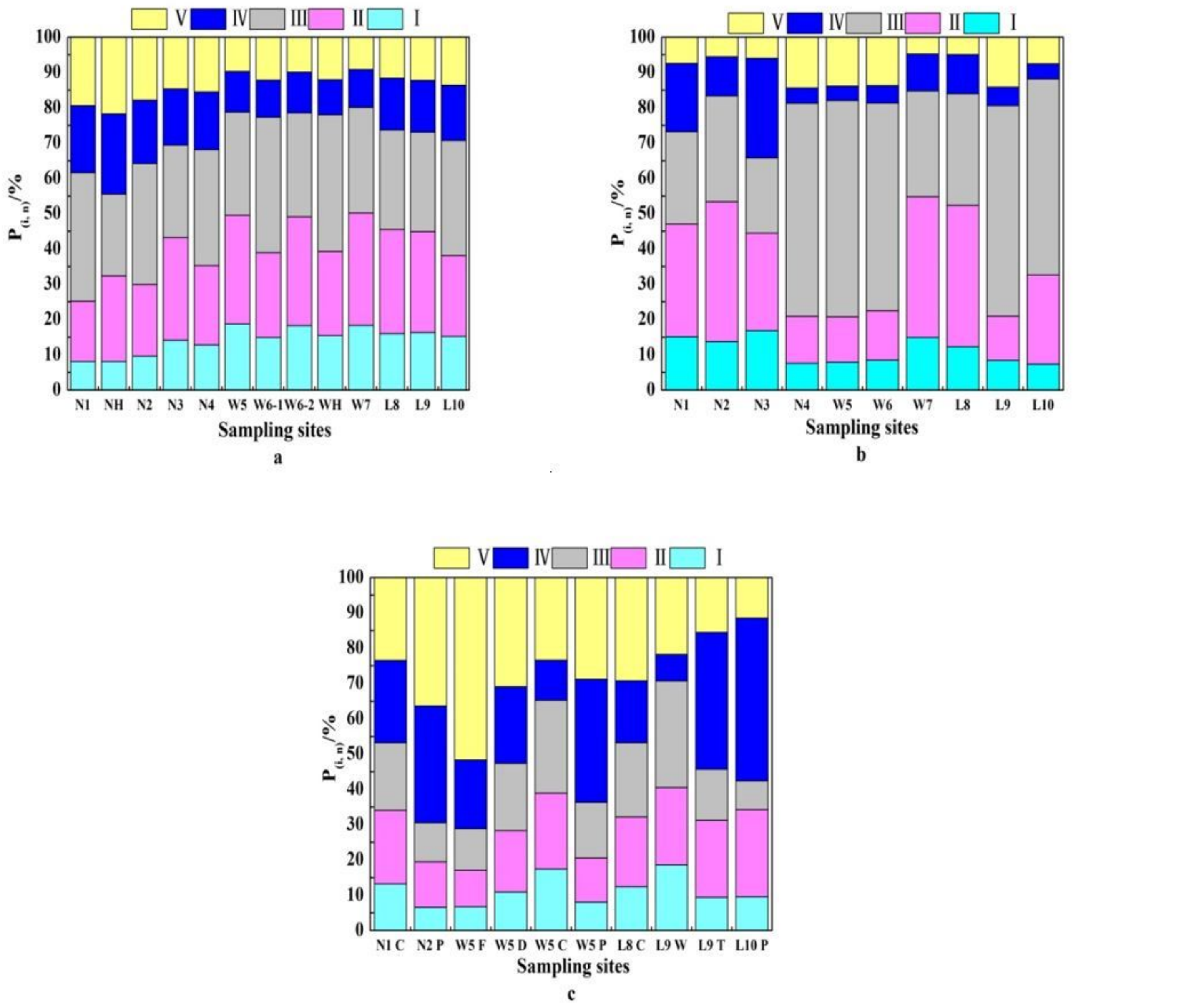

Figure 4

The EEM spectra could be divided into five regions, which were I, II, III, IV and V. Peaks in each region indicate the presence of tyrosine-like (I), tryptophan-like (II) proteins, fulvic acid-like (III), soluble microbial by-product-like (IV), and humic acid-like (V) substances. Water, soil and plant sample were expressed with a, b and c, respectively. Letters appeared in abscissa of plant samples (C, P, F, D, W, T) represented C. cinerascens, P. australis, flowers, D. carota, weed and T. lutarioriparia, respectively.

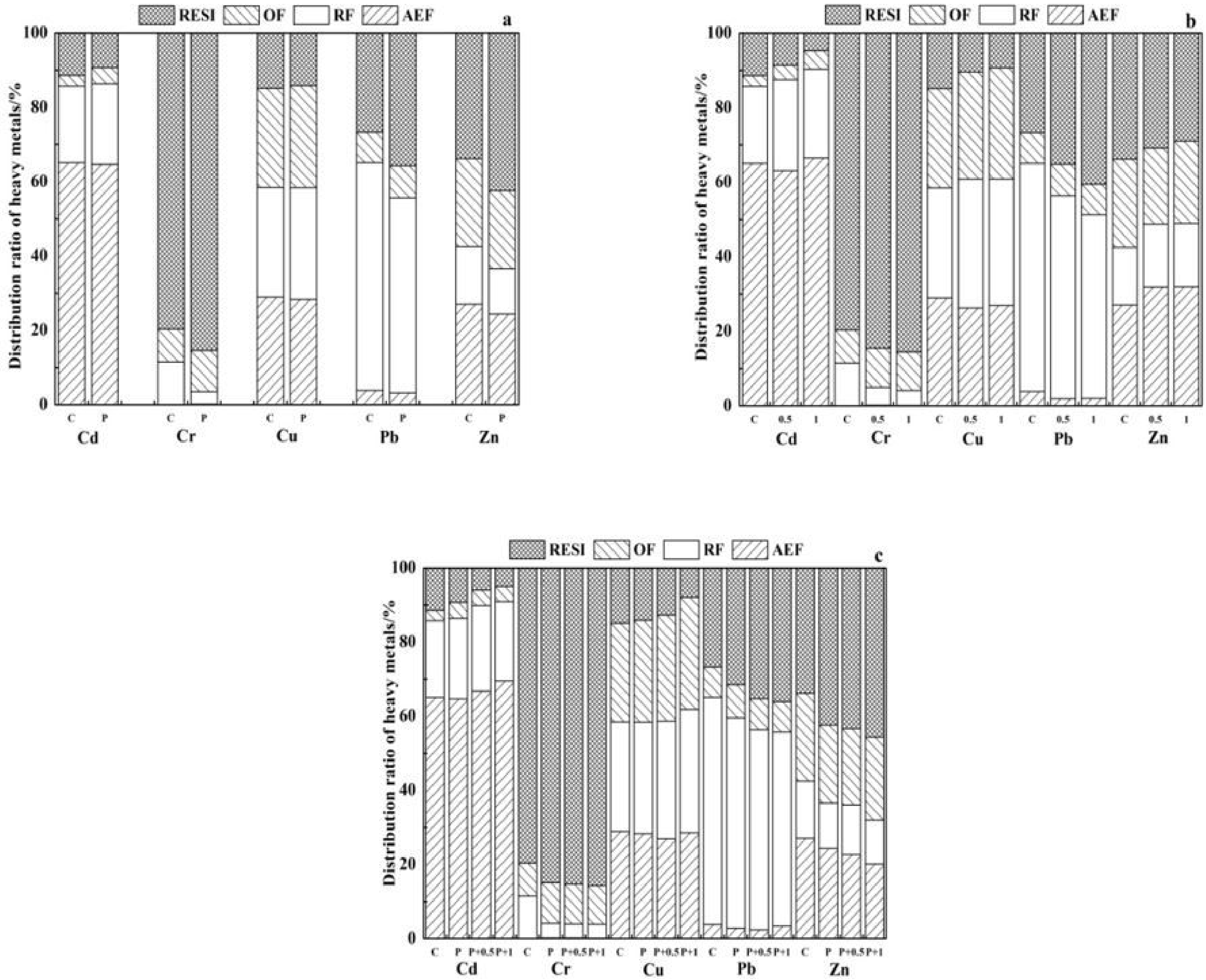

Figure 5

The heavy metals can be distributed into four forms with different treatment. The ‘C’, ‘P’ were represented for ‘control’ and ‘planting C. cinerascens’.