

Original Research

Fluorescence Spectral Characteristics of Soil Dissolved Organic Matter in Zhengyangguan, Middle Reaches of Huaihe River Basin

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Abstract

In order to study the spectral characteristics of soil dissolved organic matter (DOM) under agricultural non-point source pollution in the Huaihe River Basin, this study collected the soil from the Pihe River, a first-order tributary of the Zhengyangguan River, and used the parallel factor analysis method (PARAFAC) to obtain two components of humus and protein. Soil dissolved organic carbon (DOC) content was significantly different under different land use types ($P < 0.01$). The soil DOM in the Huaihe River Basin is affected by both terrestrial and microbial sources. The biological index (BIX) of forest soil is significantly larger than that of cultivated land at different depths, which is caused by the rich plant diversity of forest land. The humification degree of cultivated land soil is higher than that of forest land, and the humification effect of cultivated land soil fertility is stronger due to human factors. The humification index (HIX), BIX and fluorescence index (FI) have a good indication of the degree of soil humification. At the same time, the greater the BIX, FI and spectral slope ratio (S_R), the greater the contribution of microorganisms to soil DOM.

Keywords: agricultural non-point source pollution, Huaihe river basin, land use types, parallel factor analysis method, soil DOM

Introduction

Soil and soil health forms the basis of, and is crucial to, agricultural production. Moreover, soil erosion is the main form of agricultural non-point source pollution. In this process, soil sediments enter a receiving water body, and various nitrogen and phosphorus elements adsorbed by the sediments negatively impacts water

quality. Therefore, studying soil polluted by agricultural non-point source pollution is crucial. The Huaihe River Basin contains a large cultivated land area and is a major grain producing area in China. This cultivated land area accounts for about 70% of the basin area. The use of numerous pesticides, fertilizers, and agricultural films, causes serious soil organic matter pollution [1]. DOM is a carrier of organic pollutants, and soil DOM can affect the physical, chemical, and biological processes of organic pollutants [2, 3]. These processes then further affect the surrounding surface water and groundwater under through runoff and infiltration [4-6].

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DOM can be used as an environmental tracer in some ecosystems due to its unique chemical and fluorescence characteristics. Therefore, soil DOM under agricultural non-point source pollution in the Huaihe River Basin can potentially predict the Huaihe River's water quality and provide effective measures for preventing and controlling non-point source pollution.

Ultraviolet and fluorescence spectroscopy techniques have increasingly been used to study DOM in rivers, lakes, and other water bodies. This is mainly because spectral analysis is fast, highly sensitive, strongly selective, and contains a large amount of information [7]. Qi et al. studied the characteristics of soil DOM in four typical land use types of forest land, cultivated land, grassland and bare land in Piliqing River Basin of Northwest China. The results showed that DOM in different land use types was produced by both endogenous and exogenous sources, but the terrestrial characteristics of forest land and bare land were more obvious [8]. Wang et al. studied the optical properties of DOM in farmland and forest land soils in Zhangxi watershed, Ningbo. The results showed that the DOM in Zhangxi watershed was mixed with exogenous and autochthonous sources, and the aromaticity and humification of farmland DOM were higher than those of forest land [9].

In this paper, soil DOM spectral characteristics were studied at the entrance of the Pihe River, a tributary of the Huaihe River. Spectral analysis was used to explore the composition and source of soil DOM, which revealed soil DOM differences under different land use types to provide a theoretical basis for preventing and controlling non-point source pollution in the Huaihe River Basin.

Experimental

Sample Collection and Pre-Treatment

The sampling point was located in Zhengyangguan Town, Shouxian County, Anhui Province. It is located on the eastern bank of the Huaihe River, Ying River, and Pi River confluence. The region is characterised by a subtropical monsoon climate. The Huaihe River flood season is generally between June–August, and because the sampling point occurs near the Huaihe River, the terrain is relatively low-lying, and is thus easily flooded in summer. Points T1–T5 were sampled in cultivated land, wheat is planted in winter and soybeans, corn and other crops are planted in spring. Due to summer flooding, no crop growth occurred during the October sampling period, since only weeds were actively growing. T6–T8 occurs in forest land, while T7 represents a columnar sample that was selected within the Huaihe River transition zone. The sampling point was characterised by a large soil water content, sparse surface vegetation, and the presence of only a few dead branches.

In October 2021, soil samples were collected at Zhengyangguan in the Huaihe River Basin. Eight points were collected along the Huaihe River, and one columnar sample, as well as one to three surface samples, were collected at each point, including cultivated and forest land. After collection, samples were loaded into a self-sealing bag according to a specific sequence number. Soil samples were dried in the dark, whereafter roots and gravel were removed before grinding, and the samples were again stored in the dark after passing through a 100-mesh sieve. The distribution of sampling points was shown in Fig. 1 and the geographical location of sampling points was shown in Table 1. Specifically, T1–T5 occurs in cultivated land and T6–T8 occurs in forest land.

Extraction of Soil DOM

Soil DOM was extracted using the soil-water shaking method. A total of 5 g pre-treated soil was weighed in a conical flask, and 50 mL of ultrapure water was added [10–12]. The flask was placed in a constant temperature oscillator for 24 h at a temperature of 20°C and rotation speed of 200 r/min. Hereafter, the soil was centrifuged at 4000 r/min for 20 min, and the supernatant passed through a 0.45 µm glass fibre filter membrane to obtain the DOM solution. The solution was stored in the dark at 4°C for testing, and the other analyses were completed within five days. Soil DOM concentrations are expressed as DOC mg/kg, and data are expressed as mean±standard deviation (SD).

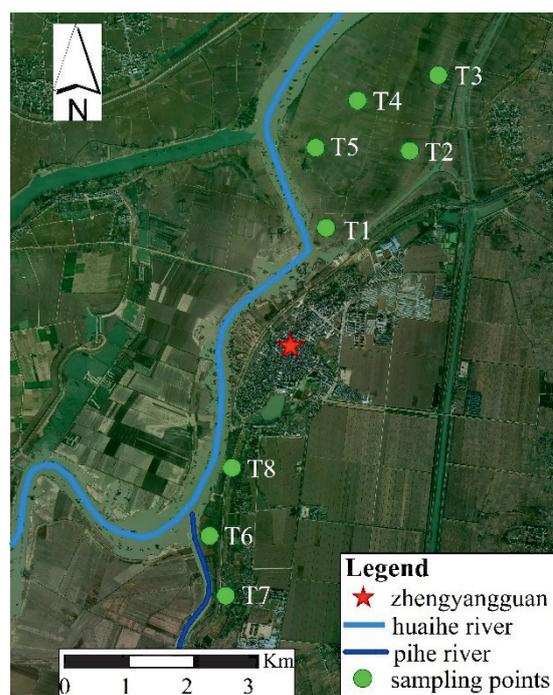


Fig. 1. The distribution of sampling points.

Table 1. Latitude and longitude of the sampling points.

Sampling point	Longitude (E)	Latitude (N)
T1	116.52803421	32.49456044
T2	116.53953552	32.50368153
T3	116.54399872	32.51352547
T4	116.53335571	32.51106458
T5	116.52597427	32.50397107
T6	116.51275635	32.46139822
T7	116.51361465	32.45270741
T8	116.51584625	32.47008819

Spectral Scanning and Analysis

Ultraviolet-visible absorption spectra were measured using a spectrophotometer (N5000Plus, Shanghai, Youke), with ultrapure water as the blank, and a scanning wavelength range of 200-800 nm and interval of 1 nm. The DOM fluorescence spectrum was measured by a Hitachi F-4600 fluorescence spectrophotometer. The scanning speed was 1200 nm/min, the excitation wavelength (Ex) scanning range 200-500 nm, and the emission wavelength (Em) scanning range 200-550 nm.

Data Processing and Drawing

The components of soil samples were identified by parallel factor analysis. The PARAFAC model was run and validated following by the execution of the package “staRdom” in R (4.2.1). The data were analyzed with reference to the method of Scholar Pucher [13]. The first is to remove Raman and Rayleigh scattering, and then identify abnormal samples. Secondly, A two- to seven-component PARAFAC model was fitted to the data to determine the correct number of components. Finally, the number of components was verified by split-half analysis. Difference of DOC content in cultivated soil and forest soil by Two-way ANOVA. Correlation analysis in SPSS 27.0 was used to analyze the correlation between different indicators. Using Origin 2021 and Arcgis 10.2 to draw the figures.

Results and Discussion

Cluster Analysis

The pH, DOC concentration, and spectral characteristics cluster analysis results showed that all sampling points were divided in two clusters (Fig. 2). The first cluster included sites T1, T2, T3, T4, and T5, and the second cluster included sites T6, T7, and T8. The land use types of T1, T2, T3, T4, and T5 are cultivated land, while the land use types of T6, T7, and T8 are forest land.

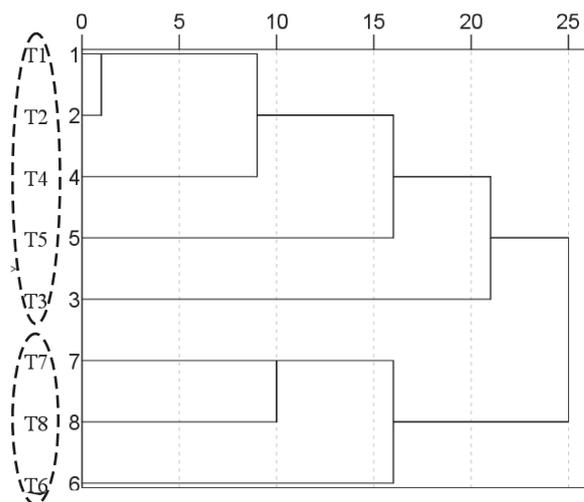


Fig. 2. Cluster analysis results of soil physicochemical and fluorescence indices.

Differences in DOC Concentrations at Different Regional Depths

The average DOC concentration in cultivated land was the highest in 0-10 cm layer, reaching 170 mg/kg, and the lowest in the 20-30 cm layer (Fig. 3). The average DOC concentration in forest land was the highest in the 10-20 cm layer, reaching 204 mg/kg, and the lowest in the 20-30 cm layer. Soil DOC content was significantly different under different land use types ($P < 0.01$) according to a Two-way analysis of variance. Forest soil DOC content was significantly higher compared to cultivated land. This is because forest plant diversity is higher compared to cultivated land, and the influence of human activities, together with alternations between wet and dry periods, leads to organic matter oxidation in cultivated land [14, 15]. Specifically, the DOC content at T7 was less than T6 and T8. This may be due to a higher soil water content for T7, which is

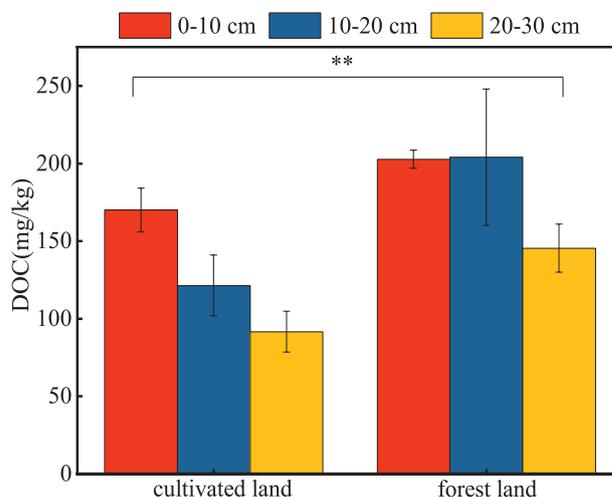


Fig. 3. Vertical distribution of DOC.

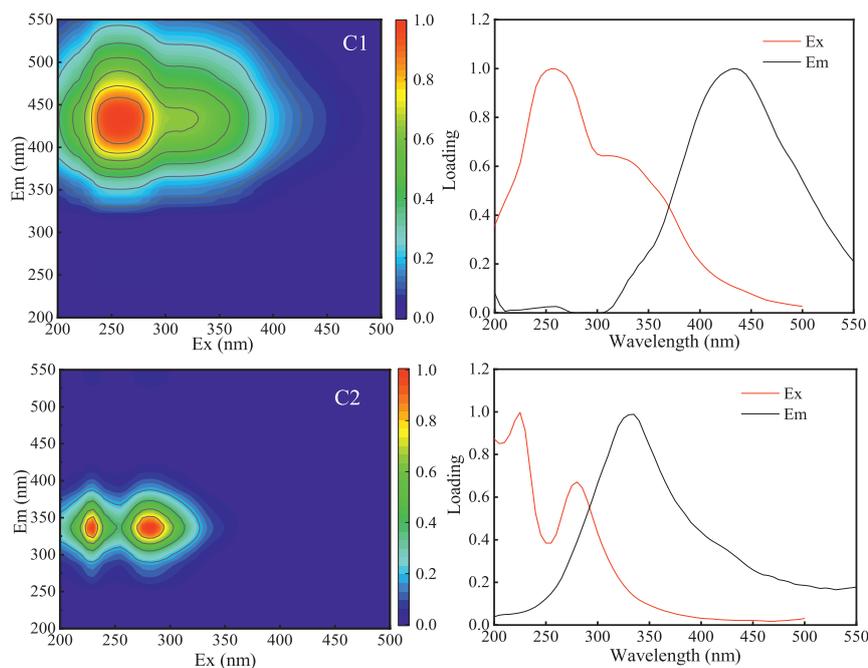


Fig. 4. Fluorescent component diagram of soil DOM.

in close proximity to the river. Long-term flooding causes plant death, and consequently sparse vegetation, after water withdrawal, which is not conducive to DOC accumulation. Surface layers contain the maximum DOC content for cultivated soil [16]. The reason for this is that, since sampling occurred in October, the surface layer was characterized by numerous dead weeds, the contents of which leached into the soil layers during rainfall events, thereby providing sufficient nutrients for microorganisms. The maximum DOC content appeared in the 20-30 cm soil layer, since more root exudates occur in this layer due to deep tree roots. The higher root zone soil organic carbon content results from crop root exudates, as well as small amounts of dead root material that contribute to soil organic carbon input [17]. The herbaceous plant layer occurring beneath the tree layer is dense and species rich, and its deciduous nature leads to an increased amount of dead branches. Furthermore, plant diversity can enhance downward DOM transport [14]. As a consequence, the surface layer becomes nutrient enriched, and rainfall events cause these nutrients to enter the middle soil layer, thereby promoting soil development, improving soil fertility, and providing a suitable living environment for soil microorganisms.

Soil Fluorescence Components

PARAFAC was used to analyse fluorescence data from soil samples, and two fluorescent components (C1 – C2) were obtained (Fig. 4). The main peak of C1 (Ex/Em = 250/450 nm) represents humic-like components found in wastewater, wetlands, and agricultural environments [18, 19]. C2 has two peaks at 230/330 nm and 280/330 nm, representing a tryptophan-like substance and a biodegradable protein-like substance [10, 20] (Table 2).

Fluorescence Indices Analysis

FI is the ratio of the emission wavelength at 450 nm and 500 nm with an excitation of 370 nm, and FI is often used to distinguish between native (FI>1.9) and remote (FI<1.4) DOM sources [21]. The soil DOM FI values in this study ranged between 1.51-1.92 (mean = 1.69), indicating that soil DOM was affected by both terrestrial and microbial sources [22]. Soil DOM FI values in small agricultural watersheds generally range between 1.4-1.9, and is affected by both autogenic and terrestrial sources. One-way analysis of variance showed that FI values differed significantly between cultivated and forest land ($P<0.05$). Soil DOM FI changed with depth (Fig. 5a). In addition to T1,

Table 2. Spectral components identified by the parallel factor method.

Component	Ex/Em (nm)	Substance	Reference
C1	Ex/Em = 250/450 nm	Humic substances	[18, 19]
C2	Ex/Em = 230/330 nm Ex/Em = 280/330 nm	Proteinoid	[10, 20]

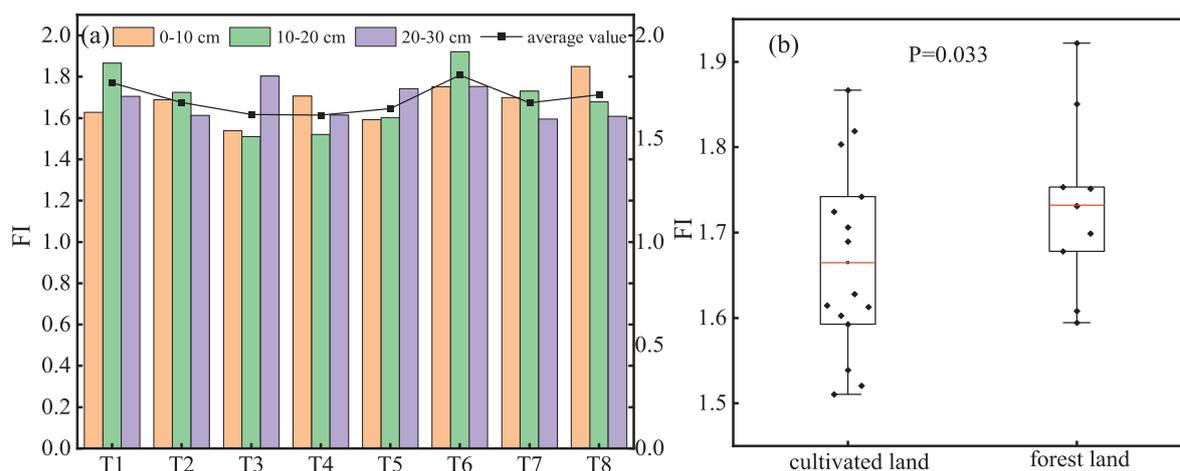


Fig. 5. Fluorescence index (FI) vertical distribution characteristics.

the average FI value of forest land was significantly higher compared to cultivated land. This is because forest communities have more vegetation types and are richer in biodiversity, and microbial sources therefore account for a large proportion. The soil moisture content of T1 is suitable since it occurs close to the river. Soil moisture changes can directly affect the abundances and activities of soil autotrophic microorganisms, which explains the larger FI values [23]. The FI value of T7 was smaller compared to T6 and T8 because of its small biodiversity, and because it was covered by river water for an extended time period, since sampling occurred in October. The vegetation at this point is dry, the soil moisture is larger than other points, and the microbial activity is weak, all of which are not conducive to organic matter accumulation [24].

BIX is described as the ratio of the fluorescence intensities of the emission wavelength at 380 nm and at 430 nm when the excitation wavelength is at 310 nm [25]. BIX represents the size of the soil DOM autochthonous contribution rate, as well as the level of bioavailability. Larger values indicate greater proportions of autochthonous contribution, with values greater than 1 indicating microbial activity as the main source [8]. BIX at different depths ranged between 0.65-1.37, with averages shown in Table 3. Irrespective of cultivated or forest land, the 0-10 cm layer values were significantly less compared to lower depths. The forest soil biological index was significantly higher compared to cultivated land at different depths,

especially at 20-30 cm. This is because tree roots are much deeper than grass roots, and the microorganisms in the root growth areas are more active, resulting in higher soil organic carbon content. However, cultivated land is greatly affected by humans, resulting in a small proportion of DOM autogenic components and a small BIX value [26]. Grass roots are generally distributed between depths of 10-20 cm, which is why cultivated land BIX values are largest at these depths. Forest land BIX values changed greatly with depth, which was consistent for T6 and T8; that is, the biological index increased with increasing depth. The value of T7 was much smaller compared to T6 and T8 because no living vegetation occurred at this point, and the soil moisture content was compared to other points, which is not conducive to microbial survival. The trends for T1 and T2 were similar, namely, both initially increased and then decreased, and were larger compared to other cultivated land samples. Reasons for this may include a close river proximity, suitable soil moisture, high organic carbon content, and a large microbial activity [23]. The trends for T3, T4, and T5 were consistent, namely a slight increase followed by a decrease; specifically, the rate of change was very small. This is because grass roots generally grow to a depth of 10 cm; therefore, more root exudates occur at this depth, which provide nutrients to microorganisms and are conducive for microbial survival.

HIX equals the fluorescence intensity integration at the emission wavelength of 435-480 nm divided

Table 3. Mean values of BIX and HIX fluorescence indices in different land types.

Fluorescence index Soil layer (cm)	BIX		HIX	
	Cultivated land	Forest land	Cultivated land	Forest land
0-10	0.73±0.05	0.84±0.09	0.69±0.10	0.64±0.11
10-20	0.78±0.16	0.86±0.13	0.64±0.13	0.67±0.08
20-30	0.74±0.01	1.09±0.31	0.68±0.07	0.56±0.15

by the sum of the fluorescence intensity integration at 300-345 nm and 435-480 nm when the excitation wavelength is at 254 nm [27]. HIX characterises the soil DOM humification degree. HIX values at the different depths ranged between 0.41-0.80, and were thus less than 4, indicating that local sources largely contributed to DOM [28]. The humification degree of cultivated soil was greater compared to forest soil in both the upper and lower layers, and was slightly lower than forest soil in the 10-20 cm layer, thereby indicating weak humus characteristics and obvious endogenous characteristics (Table 3). The forest land has more surface vegetation and litter, higher organic carbon content, stronger microbial activity, lower humification, and a higher autochthonous source contribution. Cultivated land is greatly affected by humans, thereby resulting in higher soil fertility. This may be due to continuous fertilization and cultivation. During the ripening process, soil physical and chemical properties are changed, soil organic matter is continuously degraded into fulvic acid, and fulvic acid is converted into humic acid, this leads to enhanced soil humification. Mineral-bound organic carbon content is higher in forest soil, which leads to stronger mineralisation, and weaker humification [29-31].

Correlation Analysis of Fluorescence Indices

HIX was significantly and negatively correlated with BIX, FI and S_R , and the correlation coefficients were -0.825, -0.816, and -0.824, respectively (Fig. 6). The degree of soil humification was negatively correlated with autochthonous source contribution, which is consistent with the above results. Forest land autochthonous source contribution was greater than cultivated land, but the humification degree was less than cultivated land due to human disturbance and soil mineral adsorption. BIX was significantly and positively correlated with FI and S_R [32], and the correlation

coefficients were 0.825 and 0.759, respectively. Greater autogenic BIX indices indicate greater microorganism contributions, and a greater fluorescence index FI indicates more local sources; that is, a greater microorganism contribution. Greater BIX values indicate greater S_R value, and the S_R value is inversely proportional to molecular weight. Thus, greater S_R values indicate smaller soil DOM molecular weights; that is, the greater the microorganism contribution rate, the smaller the molecular weight, indicating that, under the action of microorganisms, soil has a tendency to be transformed into small molecular substances [33]. There was a significant positive correlation between FI and S_R , and the correlation coefficient was 0.748. Larger fluorescence index FI values indicate increased microorganism contribution. The larger the S_R value, the smaller the molecular weight. This also shows that microorganisms slow down soil humification and reduce its molecular weight, which is not conducive to maintaining DOM stability [24]. According to the above analyses, HIX, BIX, and FI represent soil humification accurately, and BIX, FI, and S_R can represent the contribution of soil microorganisms to a large extent.

Conclusions

The soil in the Huaihe River basin includes two fluorescent components, namely a humic-like substance and a protein-like substance. Significant differences existed in soil DOC content under different land use types, and overall forest land performed greater than cultivated land ($P < 0.05$). Furthermore, fluorescence indices analysis showed that soil DOM was affected by both terrestrial and microbial sources, and that forest land plant diversity was significantly greater than cultivated land. Thus, the autochthonous characteristics were more obvious than cultivated land, while cultivated land soil was greatly affected by human beings, and its humification degree in the upper and lower layers was greater compared to forest land. The fluorescence index correlation analysis showed that HIX, BIX, and FI were good indicators of soil humification, and that BIX, FI, and S_R could all characterise the contribution of microorganisms in soil to a large extent.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

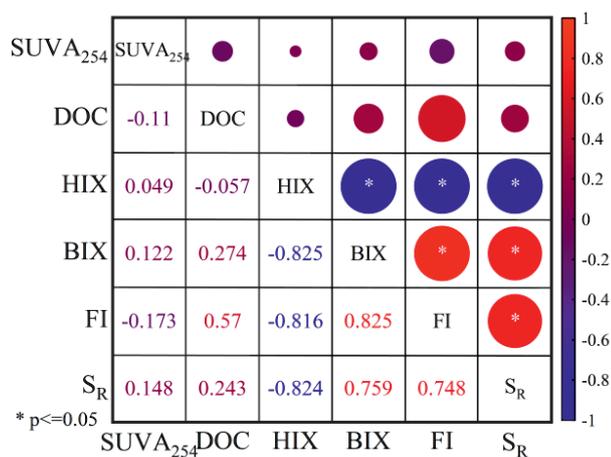


Fig. 6. Correlation analysis between fluorescence index and DOC.

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