Introduction

Zn is an essential substance for the metabolism of marine algae. A low concentration of Zn will limit primary productivity in coastal waters [1]. However, excess Zn is toxic to the embryos of coastal organisms, including sea anemone and sea urchins [2]. It also inhibits phytoplankton reproduction, growth and threatens the integrity of the coastal ecological environment [3]. Monitoring results show that Zn is at a low level in the coastal waters of China, which is not proportional to its flux into the sea [4]. It is believed that Zn may migrate from the water phase into a non-aqueous environment, such as macroalgae, sediments, and suspended particulate matter.

Macroalga is a ubiquitous biological component of coastal seawater. Its metabolism requires the participation of Zn, which may have an important...
impact on the migration and transformation of Zn in seawater. Studies have found that macroalgae have a large specific surface area that can provide numerous binding sites for metal ions and adsorb free Zn ions in seawater [5]. The organic matter produced during the growth and decline of algae can be combined with the free Zn in the seawater, reducing the biological toxicity of Zn and hindering its migration to other non-aqueous environments [6]. Compared with the single adsorption or burial effect of particles and sediments, macroalgae have a more complex impact on the biogeochemical behavior of Zn in seawater. It could be the biological indicator reflecting the migration path and pollution history of Zn in seawater. However, there are limited studies on the Zn adsorption and absorption mechanism by macroalgae. It is an important understanding of the mechanism of the biogeochemical cycle of Zn and the correct assessment of ecological risks.

Ulva lactuca L. is a common macroalga with strong environmental tolerance in the coastal area. It has a wide geographical range, strong sensitivity to heavy metal pollution, and measurable response. It is an important alga indicating Zn pollution in coastal areas. In this study, we investigated the Zn\(^{2+}\) adsorption and absorption behavior of Ulva lactuca L. And, the characteristics of Zn adsorption (externalization) and absorption (internalization) by Ulva lactuca L. in different systems was analyzed. The effect of total organic carbon (TOC) were also investigated.

### Experimental Method

#### Test Materials

Because Zn is a common metal, the cultivation device, sampling bottles, centrifuge tubes, and other experimental equipment used in the experiment were strictly cleaned. The basic process was as follows [7]: the test equipment was first rinsed with tap water five times, then rinsed with 10% Decon90 \(^{®}\) solution three times, and finally rinsed with ultrapure water (>18.2 MQ) five times. The cleaned glassware and devices were soaked in 1% HNO\(_3\) for 72 hours before being taken out, then washed with ultrapure water five times, and finally placed in an ultra-clean bench for air drying. The ultrapure water used in the laboratory was produced by Millipore\(^{®}\) pure water system. The concentration of Zn element stock solution used in the experiment was 10 mg L\(^{-1}\), which was prepared by diluting Zn\(^{2+}\) standard solution (1000 mg L\(^{-1}\), General Research Institute for Nonferrous Metals) in 1% HNO\(_3\). The HNO\(_3\) used in the experiment was obtained by secondary purification of guaranteed reagent grade HNO\(_3\) produced by Fisher Company.

The Ulva lactuca L. algae used in the experiment were collected from Dongying nearshore waters (119°6’30.61”E, 37°52’45.61”N). The brief collection process was as follows [8]: several strong and healthy Ulva lactuca L. algae near the coastal reefs were collected and placed in a plastic bag containing the seawater at the sampling site; another 5 L of coastal seawater was collected and passed through a 0.45-μm filter membrane, then put into a 10-L Teflon-lined polyethylene square box (50 cm × 30 cm × 30 cm) containing filtered seawater for temporary cultivation. Air was introduced during temporary cultivation at 35 μmol·m\(^{-2}\)·s\(^{-1}\) [8]. The 24-h light to darkness ratio was 16:8, and the cultivation duration was one week. After the growth of the algae was stable, healthy algae without transparent spots and with a width of 20 mm and a length of 50 mm were cut [8]. The cut algae were cultivated in filtered seawater and artificial seawater under the same cultivation conditions. The cultivation experiment was carried out in artificial seawater. The recipe was according to the standard ASTM-1190 except no zinc salt was added during the preparation to avoid any interference caused by the addition of Zn.

In order to prevent the adsorption of Zn on the wall of the experimental apparatus, the cultivation experiment was carried out in a transparent polyethylene (15 cm × 15 cm × 15 cm) square tank. The basic experimental procedure was as follows (Fig. 1): 1 L of artificial seawater (without additional Zn) was added to a square cultivation vessel, then an appropriate amount of Zn standard solution (Zn\(^{2+}\)) was added to the beaker to allow the Zn\(^{2+}\) concentration in the system to reach 100 μg·L\(^{-1}\). 1 ml of Tris-HCl solution was added to stabilize the pH value in the system at around 8.2 after the beaker was shaken for 2 h at 20°C. A Teflon tweezer was used to carefully pick up rectangular pieces of algae (20 mm × 100 mm), which were placed in the square tank with an adjusted pH. Before being placed in the tank, the surface of the algae was rinsed with artificial seawater five times. The surface of the tank was covered with a sterile light-transmitting film and a PFA (perfluoroalkoxy alkanes) inflatable tube was inserted at the bottom of the beaker. A 0.22-μm syringe filter membrane ( Pall\(^{®}\) ) with a ventilation rate of 15 μmol·m\(^{-2}\)·s\(^{-1}\) was added to the tip of the inflatable tube. The algae were cultivated for 72 h in a dark room. There were 12 groups of cultivation experiments, including three parallel experiments and three blank control experiments.

Due to the need to obtain the Zn content on the surface of the Ulva lactuca L. algae and inside the algae, sampling was performed only once in the cultivation experiment. The sampling times were 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h.
Sampling was performed during active photosynthesis. The experimental light source was from a lamp providing daylight conditions. The illumination time was 24 h with a light-to-darkness ratio of 16:8. When sampling, a pipette was used to accurately transfer 0.5 ml of the solution into a polyethylene centrifuge tube (BD) containing 12 ml of 2 mol/L HNO₃. The HNO₃ used in the experiment was purified twice to prevent interference from the background Zn in nitric acid. The alga body was removed and freeze-dried for storage.

Sample Processing and Measurement

The seawater sample in the centrifuge tube was digested for one week, and then it was stored at a constant temperature (4°C). The freeze-dried algae samples were weighed (accurate to 0.0001 g) and cut into pieces with ceramic scissors. The cut algae were extracted three times with 2 ml of 1% ethylene diamine tetraacetic acid (EDTA) and diethyldithiocarbamate (DDTC) mixed solution. The extracts were combined and digested 3 days after adding 1 ml of concentrated nitric acid and concentrated hydrochloric acid [9]. The sample was air-dried on an ultra-clean bench after which 3 ml of 2 mol/L HNO₃ was added to dissolve the residue. The extracted algae were rinsed with ultrapure water five times, then freeze-dried, transferred to a PFA digestion tube, and treated by adding 2 ml of concentrated nitric acid. The digestion proceeded in a 100°C water bath for 24 h, after which the sample was removed, diluted with ultrapure water to 10 ml, and stored at 4°C. The content of Zn in the sample was measured using an inductively coupled plasma mass spectrometer (ICP-MS, PE Inc) with a Zn detection limit of 0.01 μg·L⁻¹.

Distribution Coefficient

The distribution of Zn on the surface of Ulva lactuca L. algae, the interior of Ulva lactuca L. algae, and the water phase is represented by the distribution coefficient ($K_d$), which is calculated with the following equation:

$$K_d = \frac{C_x}{C_L \times C_U}$$  \hspace{1cm} (1)

where $C_x$ is $C_{i}$, the concentration of Zn$^{2+}$ adsorbed on the surface of Ulva lactuca L., or $C_{i}$, the Zn$^{2+}$ concentration in the Ulva lactuca L. $C_{i}$ is the concentration of Zn$^{2+}$ in the solution. $C_{i}$ is the mass concentration of Ulva lactuca L. in the solution. For convenience, the $K_d$ value is expressed as $log_{10} K_d (L/kg)$.

The mass concentration of Ulva lactuca L. is obtained from the volume ratio ($V$) of the solution and the wet weight ($M_u$) of the algal body after the excess water is removed from the sample with absorbent paper.

$$C_U = \frac{M_U}{V}$$  \hspace{1cm} (2)

The distribution ratio ($F$) of Zn$^{2+}$ on the surface and inside Ulva lactuca L. is used to measure the Zn biological utilization tendency of Ulva lactuca L. The calculation equation is [10]:

$$F = \frac{K_{d,in}}{K_{d,ex}}$$  \hspace{1cm} (3)

where $K_{d,in}$ is the distribution coefficient of Zn on the surface of Ulva lactuca L. and $K_{d,ex}$ is the distribution coefficient of Zn inside the Ulva lactuca L. organism [10]. If $F>1$, Zn tends to be adsorbed on the surface of Ulva lactuca L. If $F<1$, Zn tends to be used by the organism [10].

Results and Discussion

Zn Mass Balance in the Cultivation System

In order to ensure the reliability of the experimental data, we analyzed the mass balance coefficient of
Zn during the experimental process and corrected the experimental results to obtain more accurate experimental data. The mass balance coefficient of Zn is expressed by the ratio (I) of the initial concentration of Zn (C) to the sum of the concentration of Zn on the surface of Ulva lactuca L. (C), Zn inside Ulva lactuca L. (C), and Zn in the solution (C). If I>90%, there was no significant loss of Zn during the experiment. If 85%<I<90%, Zn was lost during the experiment and the data need to be corrected. If I<85%, the loss of Zn in the experiment was significant and the experiment needs to be repeated. The change in the mass balance coefficient of Zn at different sampling times is shown in Fig. 2. In the blank sample without Ulva lactuca L., the I value varied between 97-100% indicating no significant loss of Zn in the blank sample during the cultivation process. Zn ions did not precipitate in the simulated seawater (pH: 8.2) without organic ligands. Therefore, the container material used in the experiment did not significantly adsorb Zn ions (the hydroxyl complex of Zn in seawater). In the system with Ulva lactuca L. added, the I value fluctuated between 89%-96%. The longer the cultivation time, the smaller the I value. The fluctuation in pH was less than 0.3 over the entire culture process. Therefore, change in hydroxyl was not a factor in the loss of Zn ions in the culture system. Particulate debris or colloidal substances produced during the growth of algae cause heavy metal elements to be present in particulate or complex form through adsorption or complexation [11]. Sedimentation or coagulation loss occurs as the physicochemical conditions of the system change. Therefore, the particulate debris and organic matter produced during the growth of Ulva lactuca L. may be the leading cause of the loss of Zn ions in the cultivation system.

Zn Bio-Adsorption Behavior of Ulva lactuca L.

The kinetic process of Zn bio-adsorption by Ulva lactuca L. algae is an important basis for clarifying the migration and transformation of Zn in seawater. This study found that the Ulva lactuca L. discs in all cultivation systems did not increase significantly during the 72-hour cultivation period. The bio-adsorption capacity of Zn by Ulva lactuca L. can be considered to occur in a system with a fixed amount of adsorbent. Fig. 3 shows the Zn adsorption on the surface of Ulva lactuca L. at different sampling times. During the 0 h–12 h cultivation period, the bio-adsorption capacity of Ulva lactuca L. for Zn increased with an average adsorption rate of about 0.44 μg·(kg·h)^{-1}. The adsorption rate increased initially and then decreased. The adsorption ranged between 5.33±0.05 μg kg^{-1} and 10.65±0.02 μg kg^{-1}. The adsorption capacity parameter fluctuated greatly. During the 12 h–72 h cultivation period, the Zn adsorption capacity on the surface of Ulva lactuca L. changed slightly, between 12.15±0.03 μg kg^{-1} and 13.51±0.04 μg kg^{-1}. The adsorption rate was 0.065 μg·(kg·h)^{-1}, about 1/14 of the 0 h to 24 h stage. The adsorption amount fluctuated slightly while adsorption equilibrium was reached. At the initial stage of cultivation, the amount of Zn adsorbed on the surface of Ulva lactuca L. fluctuated greatly. As the cultivation time was prolonged, the adsorption of Zn on the surface of Ulva lactuca L. gradually became stable. During the 0 h to 24 h cultivation period, the amount of Zn adsorbed by Ulva lactuca L. varied slightly (about 0.17 μg kg^{-1}) from 12 h to 24 h and approached equilibrium. Therefore, to clarify the adsorption process of Zn on the surface of Ulva lactuca L., this paper will discuss the adsorption kinetics of the 0 h–24 h stage and the 48 h–168 h stage.
Because the cultivation did not reach adsorption equilibrium, the adsorption capacities at 24 h and 168 h were used as the equilibrium adsorption capacities to fit the kinetic model. Fig. 4 shows the fitted line of the pseudo-I-order kinetic equation during the cultivation period. According to Fig. 4, the linear correlation index $R^2$ of the pseudo-I-order kinetic equation during the cultivation period was 0.91, with good degree of fit. However, the linear coefficient $R^2$ fluctuated greatly in another cultivation period. Within 0 h to 24 h, $R^2$ decreased from 0.98 to 0.67 and then increased to 0.85. From 48 h to 168 h, $R^2$ initially increased from 0.96 to 0.98 and then decreased to 0.78. Therefore, in the early stage of cultivation (0 h–4 h) and the late stages (48 h–144 h), the pseudo-I-order kinetics showed a higher degree of fit. This phenomenon demonstrated that the adsorption of Zn by *Ulva lactuca* L. at this stage was dominated by surface adsorption and was disturbed at other stages.

Because the degree of fit of the pseudo-I-order kinetic model was not high, we used a pseudo-II-order kinetic model to fit the adsorption process and further explore the Zn adsorption process on the surface of *Ulva lactuca* L. $R^2$ of the fitting model during the cultivation period was 0.998 and the adsorption process conformed to the pseudo-II-order kinetic model. In order to clarify the Zn adsorption process of *Ulva lactuca* L., the pseudo-II-order adsorption kinetic models for the 0 h to 24 h stage and the 48 h to 168 h stage were also investigated. It can be seen from Table 1 that the $R^2$ of the fitting model for the 0 h to 24 h adsorption stage increased continuously from 0.958 to 0.995. The degree of fit was generally consistent with the pseudo-II-order kinetic model. In the 48 h to 168 h stage, all of the $R^2$ values of the fitting model were 0.997. Therefore, the adsorption of Zn by *Ulva lactuca* L. during the cultivation period basically conformed to the pseudo-II-order kinetic model. The adsorption was controlled mainly by chemical adsorption and was affected by the electron sharing or electron transfer between the adsorbent and the adsorbate [12].

From the above discussion, it can be concluded that the surface of *Ulva lactuca* L. has obvious bio-adsorption for Zn ions in seawater, and the adsorption process is mainly controlled by electron transfer or electron donation. This mechanism may be closely related to the adsorption sites on the surface of *Ulva lactuca* L. or the polysaccharides secreted by *Ulva lactuca* L. [13]. *Ulva lactuca* L. alga has a large specific surface area, providing multiple electron adsorption sites for Zn. The surface of *Ulva lactuca* L. is also wrapped by its own secreted polysaccharide substances, which can also provide electrons for empty metal orbitals [14]. The polysaccharides on the surface of *Ulva lactuca* L. can enter seawater in the form of soluble organic matter. This organic matter can be in a free state or agglomerate to form colloids that react with the Zn$^{2+}$, reducing the chances for Zn$^{2+}$ empty orbitals to combine with the polysaccharides on the surface of *Ulva lactuca* L. However, these large Zn complexes are subject to the intermolecular repulsion of hydrophobic groups, which ultimately hinders the adsorption of Zn on the surface of *Ulva lactuca* L. [15]. Therefore, at the initial stage of Zn$^{2+}$ entering the cultivation system, a large amount of Zn$^{2+}$ is adsorbed on the surface of *Ulva lactuca* L. However, over the metabolism process of *Ulva lactuca* L., polysaccharides are gradually released into the solution, the adsorption process is gradually inhibited, and the increasing trends of the adsorption capacity and adsorption constant slow down.
The Bio-Absorption Behavior of Zn by *Ulva lactuca* L.

Fig. 5 shows the changes in the amount of absorbed Zn and the distribution coefficient of *Ulva lactuca* L. during the cultivation period. It can be seen from the curve in Fig. 5a that *Ulva lactuca* L. exhibited an apparent absorption process for Zn. During the cultivation period, the absorption of Zn by *Ulva lactuca* L. consisted of two processes, i.e., the steady absorption stage of 0 h to 24 h and the rapid absorption stage of 24 h to 168 h. During the 0 h to 24 h period, the average Zn absorption rate of *Ulva lactuca* L. was 0.041 μg·(g·h)^{-1} and the change in the absorption amount was 0.99±0.02 μg·g^{-1}. During the 24 h to 168 h stage, the average Zn absorption rate *Ulva lactuca* L. was 0.63 μg·(g·h)^{-1}, and the change in the absorption amount per 24 h was 1.52±0.04 μg·g^{-1}. At the end of the cultivation, the Zn content in *Ulva lactuca* L. increased by about 10.35 μg·g^{-1}, which accounted for about 20% of the total Zn. The change of the distribution coefficient conformed to the change in the absorption amount (Fig. 5b). The final distribution coefficient was 3.95.

In order to better understand the Zn absorption kinetics of *Ulva lactuca* L., we investigated the piecewise linear fitting of the absorption kinetics change. Fig. 6 shows the linear fitting results of Zn absorption by *Ulva lactuca* L. for 0 h to 24 h and 48 h to 168 h. The linear fitting parameters of 48 h to 168 h were considerably better than those of the 0 h to 24 h stage. In both stages, R^2 showed a decreasing trend after an initial increase. Studies have found that algae affect photosynthesis by absorbing glycosides formed by free Zn and that the absorption of Zn shows a linear trend [16]. However, the results of this study revealed

---

Table 1. Fitting parameters of the pseudo-II-order kinetic equation of Zn adsorption by *Ulva lactuca* L. during the cultivation period.

<table>
<thead>
<tr>
<th>Cultivation period</th>
<th>Fitting equation</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–168 h</td>
<td>y = 0.45 + 0.11x</td>
<td>0.998</td>
</tr>
<tr>
<td>0–4 h</td>
<td>y = 0.19 + 0.12x</td>
<td>0.958</td>
</tr>
<tr>
<td>0–12 h</td>
<td>y = 0.19 + 0.13x</td>
<td>0.971</td>
</tr>
<tr>
<td>0–24 h</td>
<td>y = 0.17 + 0.13x</td>
<td>0.993</td>
</tr>
<tr>
<td>48–120 h</td>
<td>y = 1.00 + 0.11x</td>
<td>0.997</td>
</tr>
<tr>
<td>48–144 h</td>
<td>y = 1.19 + 0.10x</td>
<td>0.998</td>
</tr>
</tbody>
</table>

Fig. 4. Fitting of the pseudo-I-order kinetic equation of Zn adsorption by *Ulva lactuca* L. during the cultivation period.
that the degree of linear fitting for Zn absorption by *Ulva lactuca* L. did not completely conform to the linear model. Furthermore, the absorption process was obviously disturbed.

Due to the controlled and closed system used in this study, the factors interfering with the absorption of Zn by *Ulva lactuca* L. must come from the initial Zn content and *Ulva lactuca* L. itself. Although Zn is an essential element for the biological metabolism of algae, when a specific concentration is exceeded, Zn can also become a substance harmful to organisms [17]. In order to obtain more accurate measurements, the initial Zn concentration used in the experiment far exceeded the Zn concentration level in normal seawater. Therefore,
within the 0 h to 24 h period, free Zn may have had a toxicological effect on *Ulva lactuca* L. and inhibited the absorption of free Zn by *Ulva lactuca* L. In addition, the growth and metabolism of *Ulva lactuca* L. produces polysaccharides, which can easily form complexes with free Zn ions after being dissolved in seawater. These complexes can inhibit the toxicity of free Zn ions and promote the absorption of Zn by *Ulva lactuca* L. However, the complex behavior between the large molecular weight organic matter with Zn may hinder the absorption by *Ulva lactuca* L. The concentration of TOC was used to represent the polysaccharide complex in the system. No organic matter was added in the initial stage of the cultivation, and the seawater was sterilized by ultraviolet (UV). The correlation between TOC and Zn absorption by *Ulva lactuca* L. was analyzed. Fig. 7 shows the correlation coefficient analysis. As shown in Fig. 7a), there was a significant correlation ($R^2 = 0.982$) between TOC and Zn absorption by *Ulva lactuca* L. TOC apparently affected Zn the absorption behavior.

Fig. 7. The linear relationship between the total organic carbon content in the cultivation system and the amount of Zn absorbed by *Ulva lactuca* L.

Fig. 8. The adsorption and absorption behaviors of *Ulva lactuca* L. for Zn$^{2+}$. a: The trend of change in the distribution ratio F value of Zn on the surface and inside of *Ulva lactuca* L.; b) The linear relationships between the amounts of Zn adsorbed and absorbed by *Ulva lactuca* L. and the concentration of Zn in the solution.
of Ulva lactuca L. As shown in Fig. 7b), there were significantly different linear relationships between TOC and Zn absorption stages of Ulva lactuca L. During the 0 h to 24 h period, the linear relationship between TOC and Ulva lactuca L. was poor and the R^2 was 0.776. As the cultivation time increased, the linear relationship increased gradually and R^2 increased to 0.922. Therefore, polysaccharides or other organic substances produced in the metabolism of Ulva lactuca L. interfere with the adsorption and absorption of Zn by Ulva lactuca L. However, the mechanism of the influence of polysaccharides on the biogeochemical behavior of Zn is still unclear, and further research is needed.

Discussion on the Biological Indicator Effect of Ulva lactuca L. on Zn in Seawater

The analyses discussed in 2.1-2.3 show that the biogeochemical processes affecting Zn after entering the offshore environment where Ulva lactuca L. exists can be preliminarily explained. When the free Zn^2+ meets Ulva lactuca L., it first undergoes adsorption on the surface of Ulva lactuca L. From 0 h to 48 h, the ratio of Zn on the surface and inside Ulva lactuca L. (F) was about 1.15-1.18, indicating that Zn^2+ was mainly adsorbed on the surface of Ulva lactuca L. (Fig. 8a). As the cultivation period of Ulva lactuca L. increased, F decreased. At 144 h, F was less than 1 and the absorption and utilization of Zn by Ulva lactuca L. became the dominant process. Therefore, free Zn in coastal waters may first be adsorbed on the surface of algae, and then absorbed and utilized by the algae. The polysaccharides generated during the growth of Ulva lactuca L. will also affect the form of Zn in seawater and interfere with the biogeochemical behavior of Zn^2+ in the coastal area [18]. Fig. 8b) shows the relationship between the concentration of Zn in seawater and the amount of Zn absorbed by Ulva lactuca L. in the cultivation system. The change in the concentration of Zn in the solution was the same as that of the Zn absorbed by Ulva lactuca L. (R^2 = 0.952). Therefore, Ulva lactuca L. can be used as an important indicator to monitor the biogeochemical behavior of Zn in coastal waters [19]. However, due to the complexity of real-world coastal environments, it is necessary to further investigate the effects of pH, salinity, temperature, organic matter concentration, and types of organic matter on the adsorption and absorption of Zn by Ulva lactuca L.

Conclusions

By analysis of the kinetics and distribution coefficients of the adsorption and absorption of Zn by Ulva lactuca L., we found a path that Ulva lactuca L. affect the Zn biogeochemical cycle in seawater. The free Zn^2+ in seawater may first interact with the surface of Ulva lactuca L. and then enter Ulva lactuca L. through biological utilization. The polysaccharides secreted during the growth of Ulva lactuca L. form complexes with the Zn^2+ in the seawater, inhibiting or promoting the adsorption and absorption of Zn by Ulva lactuca L. In addition, there was an obvious relationship between the amounts of Zn adsorbed and absorbed by Ulva lactuca L. Therefore, Ulva lactuca L. can be used to indicate of Zn content in seawater and an important factor for monitoring Zn geochemical behavior. However, the fundamental processes that control the geochemical behavior of Zn in Ulva lactuca L. are still unclear. The interaction between the expansion and extinction of the algae and the Zn should be further studied.

Acknowledgments

Scientific research was financed by the Conservation and restoration of bio-resources and habitats in typical coastal zones in Shandong province CF-MEEC/ER/2016-03 for statutory activity.

Conflict of Interest

The authors declare no conflict of interest.

References

2. CUNNINGHAM B., TORRES-DUARTE C., CHERG G., ADAMSA N. Effects of three zinc-containing sunscreens on development of purple sea urchin (Strongylocentrotus purpuratus) embryos. Aquatic Toxicology. 218, 105355, 2020.
8. TURNER A, PEDROSO S.S, BROWN M.T. Influence of salinity and humic substances on the uptake of trace metals


