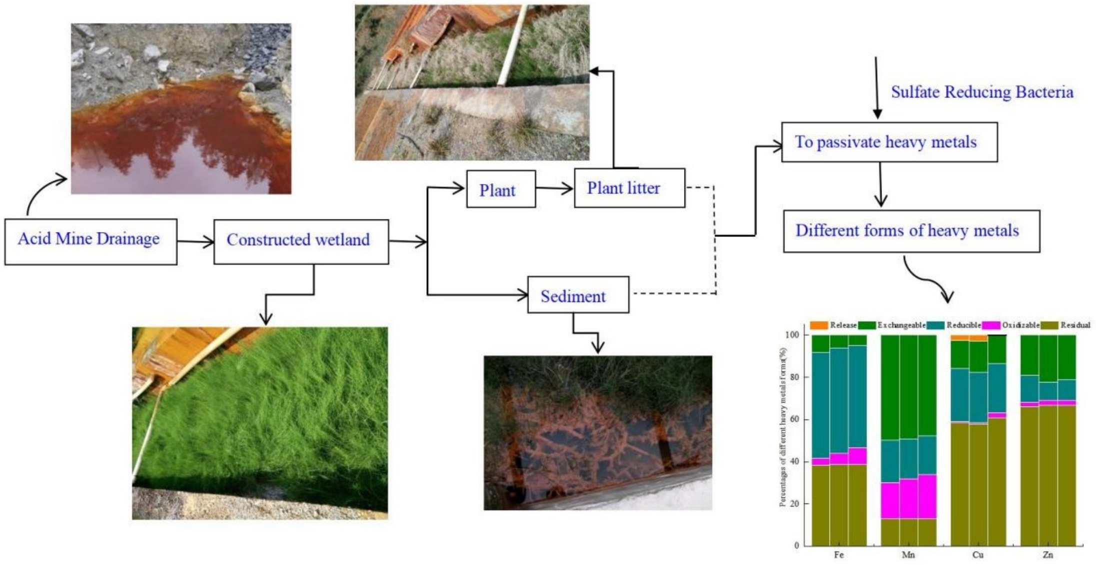
Sulfate-reducing bacteria for bioremediation of sediments from constructed wetlands for acid mine drainage treatment

Graphical Abstract



Sulfate-reducing bacteria for bioremediation of sediments from constructed wetlands for acid mine drainage treatment

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Abstract: Sediment is a source and sink of heavy metal contaminants in wetlands. In this study, sulfate-reducing bacteria (SRB) were used for the bioremediation of sediments from wetlands affected by acid mine drainage. The sediments were inoculated with SRB to study their influence on both the solution and sediments. The changes of pH, electrical conductivity (EC), redox potential (Eh) values, and sulfate (SO_4^{2-}), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) concentrations in the supernatant were monitored over a period of 70 days. Additionally, the variations in exchangeable, reducible, oxidizable, and residual Fe, Mn, Cu, and Zn concentrations. For the supernatant solution, the pH in all experimental groups decreased slightly within the first 14 days, increased gradually thereafter, and finally stabilized during days 35−70. The EC values and Fe and Mn concentrations displayed a similar trend with concentrations initially increasing and then decreasing. In the SRB-inoculated sediments, Fe, Mn, and Cu were gradually transformed from the exchangeable and reducible fractions to the oxidizable fraction. The inoculation with SRB reduced the ability of Fe, Mn, and Cu to migrate in sediment.

Keywords:

1. Introduction

With the rapid development of the global economy, the demand for mineral resources has increased significantly. Acid mine drainage (AMD) resulting from mining processes has become a major source of water pollution worldwide (Park et al.

2019; Tong et al. 2021). Acid mine drainage contains large amounts of pollutants such as iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn), with a relatively low pH. Its discharge poses a serious threat to the natural environment and human health in areas surrounding mining areas (Kefeni et al. 2017). The total discharge of AMD in China is 1.5 billion tons per year, accounting for approximately 30% of the wastewater from the nonferrous metal industry (Luo and Xie 2006; Xu et al. 2018).

Current methods for treating AMD include neutralization, sulfide precipitation, biological treatment, and the creation of constructed wetlands (Tolonen et al. 2014; Skousen et al. 2017; Zhao et al. 2021). Constructed wetlands provide an efficient and low-cost option for the control of heavy metal pollution. Wetland systems can purify water through various pathways, such as coprecipitation and plant absorption (Andrew et al. 2013; Sekarjannah et al. 2019). Both Fe and Mn can form various oxides and hydroxides through oxidation and hydrolysis, respectively (Karathanasis and Johnson 2003; Batty et al. 2002; Woulds and Ngwenya 2004; Sheoran 2017). Plants are among the most important components in constructed wetlands. They add value to the natural landscape and can assist in capturing heavy metals in wastewater.

Studies have shown that wetland systems planted with *Typha orientalis*, *Zizania caduciflora*, and *Scirpus tabernaemontani* can effectively remove pollutants such as Fe and Mn in AMD (Verhoeven and Meuleman 1999; Liu et al. 2012). However, due to rapid plant growth and reproduction resulting in large amounts of biomass, the litter from plants that is generated during the withering stage decays and enters the sediment. This produces a large amount of organic matter and serves as a carrier for organic and inorganic pollutants in wetlands. The organic matter is subjected to a series of reactions, including ion-exchange adsorption, complexation, chelation, and oxidation−reduction, with the metal ions in water, soil, and sediment. This influences the stability of the metal ions (Xu et al. 2019; Du et al. 2007; Tica et al. 2011; Li and Zhou 2010). In previous experiments, it has been observed that the interaction between the dissolved organic matter (DOM) produced during the decomposition of plant litter and iron oxides in the sediment promote the reduction and dissolution of Fe, leading to the release of Fe(II) into the supernatant (O'Louthlin et al. 2010; Xu et al. 2019). This results in secondary pollution. Therefore, it is critical to develop ways to immobilize heavy metals in sediments affected by AMD constructed wetland systems containing large amounts of plant litter.

Bioremediation is an useful approach to improve the sediment environment in-situ. It involves harnessing the biological activities of plant root systems, microorganisms, or animals, to facilitate the transformation and reduction of pollutants, including heavy metals, in contaminated sediments (Renu et al. 2020). With its advantages of speed, safety, and cost-effectiveness, bioremediation has become the preferred method for in-situ remediation (Li et al. 2016; Xu et al. 2023). Sulfate-reducing bacteria (SRB) are anaerobic microorganisms. They are abundant in underground pipelines, lake sediments, and other hypoxic environments that are rich in organic matter (Su et al. 2022; Gao et al. 2022). Sulfate-reducing bacteria have been used to stabilize heavy metals in contaminated soils, such as lead (Pb) and cadmium (Cd) (Maity et al. 2019; Vogel et al. 2018). They assimilate organic matter to obtain energy and immobilize heavy metals in the form of metal sulfides under anaerobic conditions with sulfate (SO_4^{2-}) or other sulfur compounds acting as electron acceptors (Pagnanelli et al. 2010; Ali et al. 2018). This technique has primarily been applied to the remediation of contaminated water, and the majority of studies have focused on the selection of carbon sources for SRB (Boshoff et al. 2004; Lefticariu et al. 2015; Zagury et al. 2006). However, only a few studies have been conducted to investigate the remediation of wetland sediments using SRB.

In constructed wetlands for treating AMD, the large quantity of plant litter deposited in the sediment increases the sediment organic carbon (OC) concentration and could promote the release of Fe, Mn, and other heavy metals from the sediment. The OC may potentially serve as an effective carbon source for the growth of SRB in the sediment, where the SRB-generated sulfide ions (S^{2-}) could be effectively combined with Fe, Mn, and other heavy metals, which would prevent their release. The potential to use plant litter as a carbon source to support the growth of SRB for the bioremediation of metal-containing wetland sediments has not been investigated. This study therefore investigated the feasibility of using SRB for the bioremediation of metal-containing wetland sediments with plant litter as a carbon source. The effects of SRB on changes in the pH, electrical conductivity (EC), and redox potential (Eh) values and Fe, Mn, Cu, and Zn concentrations in the supernatant were investigated. The long-term changes in the concentrations of different fractions of the four heavy metals in sediments were also investigated. This study developed a fundamental theory to explain the stability and possible environmental risks of heavy metals in constructed wetlands. The research results can provide technical support for the expanded application of SRB remediation technology for treating heavy metals in sediments.

2. Materials and methods

2.1 Experimental materials

Sediment samples were collected in a constructed wetland that had successfully been used to treat AMD from abandoned mines in Chafan, Guiyang, China. The treatment system purified AMD through the coupled effect of plants and microorganisms. With the purification of AMD, a large amount of reddish-brown sediment had formed in the constructed wetland. Sediments were collected from the wetland and then air dried, after which they were ground to pass through a 100-mesh screen (with a corresponding particle size of <0.15 mm). The physical and chemical characteristics of the sediments were as follows: pH = 6.46, EC = 2180 $\mu s \cdot cm^{-1}$, Eh = 208 mV, Fe = 92.11 $mg \cdot g^{-1}$, Mn = 14.03 $mg \cdot g^{-1}$, Cu = 0.07 $mg \cdot g^{-1}$, Zn = 0.089 $mg \cdot g^{-1}$, and OC = 46.55 $mg \cdot g^{-1}$. The plant litter used in this study was prepared from *Equisetum ramosissinum,* which is a vascular plant that is abundant in the constructed wetland. After gently washing and drying in an oven at 65°C, the aquatic part of the plant was ground to pass through a 40-mesh (<0.425 mm) screen as the plant litter material. The total nitrogen, total phosphorus, Fe, Mn, Cu, and Zn concentrations in the plant litter were 8.30, 0.70, 2.62, 0.02, 0.01, and 0.01 $mg \cdot g^{-1}$, respectively. Sulfate-reducing bacteria were enriched from the constructed wetland sediment using the SRB-exclusive Postgate C culture medium, which was composed of the following (in g·L^{-1}): tryptone, 10; Na_2SO_3, 0.5; iron citrate, 0.5; $MgSO_4 \cdot 7H_2O$, 2; $FeSO_4 \cdot 7H_2O$, 0.5; and sodium lactate, 0.4. The pH of the cultures was adjusted to 7 with NaOH. Reducing agents (7.5 g of ascorbic acid and 7.5 g of sodium thioglycolate) were added into the culture medium (Fude et al. 1994). The Postgate C culture medium was sterilized in an autoclave (YXQ-LS-SII, Shanghai Boxun Medical Biological Instrument Corp., China) before fresh sediment was added. The flask was sealed and incubated at 35°C in a shaker incubator (150 r/min). When the solution developed an inky color and a rotten egg smell was emitted from the flask (typically after 7 d), the culture solution was transferred to a fresh liquid culture medium. The same culturing protocol was repeated five times under similar culturing conditions. Enrichments of SRB from the same batch were used in the subsequent experiments.

2.2 Experimental design

Five groups of experiments (three parallel experiments for each group) were devised based on the amount of litter and the volume of SRB inoculum added. The details are given in Table 1. A total of 40 g of sediment was transferred into 400 mL experiment cups. Prior to the experiments, 60 mL of deionized water was added to each cup to reduce the Eh of the different treatments. At the beginning of the experiment, 0 or 100 mL of SRB were inoculated into the sediments and the cups were placed indoors for 1 day. Then, 200 mL of deionized water was added to the CK, L1S0, and L2S0 groups and 100 mL was added to the L1S1 and L2S1 groups to expose the sediments in all groups to similar flooding conditions. After the samples were gently stirred at low speed, all cups were sealed with a film and placed indoors at room temperature. On day 1, 20 mL of supernatant was collected from each treatment group, and pH, EC, and Eh values and the Fe, Mn, Cu, and Zn concentrations were recorded as the initial experimental values. During the experiment, the pH, EC, and Eh values, and Fe, Mn, Cu, and Zn concentrations in the supernatant were monitored on days 7, 14, 21, 35, and 70. At the same time, the supernatant was poured out, and the SRB content in the sediments was monitored in each group using the most probable number (MPN) method (Luo et al. 2014). Additionally, on days 7, 14, 21, 35, and 70, the sediments were dried and screened again with a 100-mesh sieve to remove any undecomposed litter. The dried sediments were used to determine the concentrations of different fractions (i.e., exchangeable, reducible, oxidizable, and residual) of Fe, Mn, Cu, and Zn.

Table 1 Design of the litter-SRB coupling effect experiment

Treatment	Plant litter (g)	SRB (mL)	Treatment	Plant litter (g)	SRB (mL)
CK	0	0	L2S0	5	0
L1S0	1	0	L2S1	5	100
L1S1	1	100			

2.3 Analytical methods

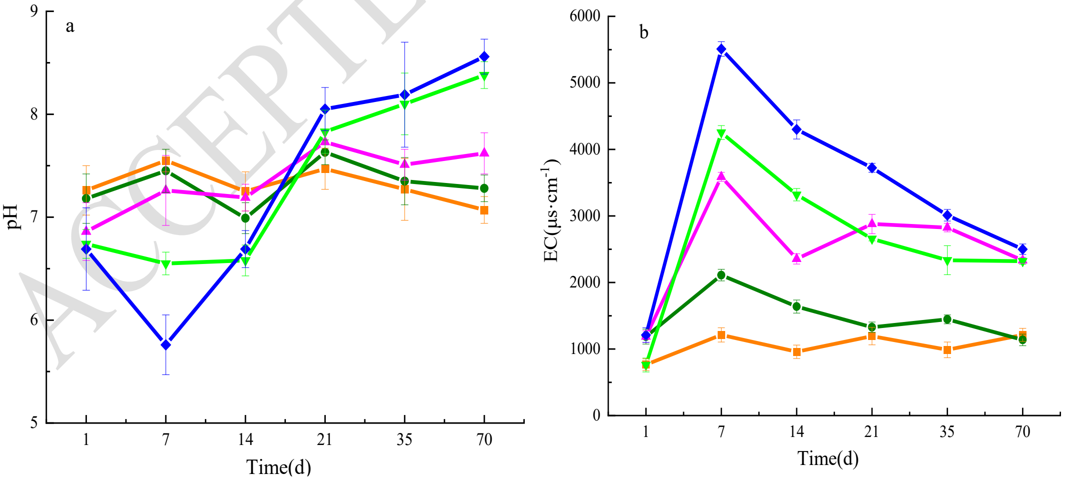
The pH was measured using a PHSJ-3F pH meter (Shanghai, INESA Scientific Instrument Co. Ltd., China). The EC and Eh values were monitored using a conductivity meter (DDS11A, Shanghai Raytheon, China) and an Eh meter (ORP-422, Shanghai SHKY, China), respectively. To determine the Fe, Mn, Cu, and Zn concentrations in the supernatant, it was filtered through a 0.45-μm microporous membrane. A 10% HNO_3 solution was added to obtain a solution pH <2 before an atomic absorption spectroscopy (WFX-110, Beijing Rayleigh, China) analysis was conducted. Sulfate in the supernatant was determined by barium chromate spectrophotometry. The sediment OC concentration was measured according to the literature (Liu et al. 2018). To determine the concentrations of the different fractions of Fe, Mn, Cu, and Zn in the sediments, 1 g of the freeze-dried sediments was taken for a speciation analysis using the modified European Community Bureau of Reference continuous extraction method (Rauret et al. 1999).

2.4 Statistical analyses

All data were analyzed with DPS2000 software and the figures were prepared using Origin 8.5.

3 Results

3.1 Influence of the SRB content on pH, EC, and Eh values and the SO_4^{2-} concentration of the supernatant



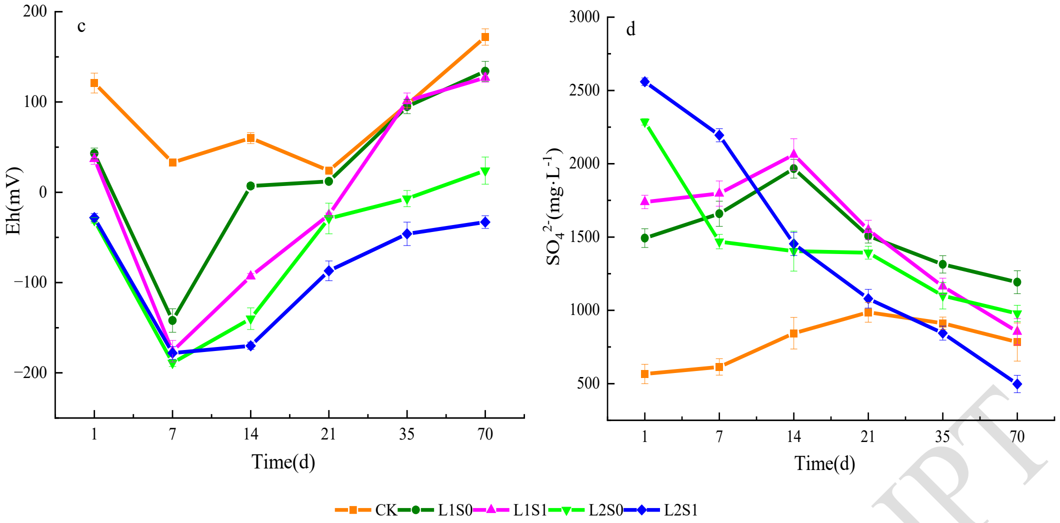


Figure 1. Influence of the SRB content on the (a) pH, (b) EC, (c) Eh, and (d) SO_4^{2-} concentration of the supernatant during the 70 day experiment. Each data point represents the mean value of three experiments. All error bars represent the standard deviation of the three experiments.

Sulfate-reducing bacteria can use SO_4^{2-} as an electron acceptor to oxidize organic compounds and produce sulfide, which increases the alkalinity (Shan et al. 2019; Zheng et al. 2021). The pH value of the supernatant in each treatment group is shown in Figure 1-a. Throughout the experiment, the pH value in the CK group did not vary significantly, but for the other treatment groups, the pH values decreased and then increased. On day 1, the pH values in the L1S0 and L2S0 groups without SRB were 7.18 and 6.74, respectively. The pH values of each group later decreased to 6.99 and 6.59, respectively. Subsequently, the pH in each group gradually increased and at the end of the experiment, the pH values in the two groups were 7.28 and 8.38, respectively. The SRB inoculation resulted in a decrease in the supernatant pH from days 1 to 7, but a subsequent inoculation with SRB promoted an increase. At the end of the experiment, the pH values of L1S1 and L2S1 increased to 7.62 and 8.56, respectively.

The changes in the supernatant EC values are shown in Figure 1-b. At the beginning of the experiment, the EC value in each group increased, then slowly declined, and finally reached a relatively stable value. In comparison with the CK group, the EC values in the treatment groups were all higher than in the CK groups. The EC in the five groups reached peak values of 1213 (CK), 2111 (L1S0), 3588 (L1S1), 4254 (L2S0), and 5510 μs. cm^{-1} (L2S1) on day 7 and then gradually declined. The EC values in the L2S1 group had a significantly larger range of decrease than in the other groups.

The Eh values in all groups displayed similar trends during the experiment (Figure 1-c). In all groups, the Eh declined by a large margin from days 1 to 7, and the more plant litter was added to the sediment, the lower the Eh value in the supernatant. By day 7, the Eh values in the L2S0 and L2S1 groups had fallen to -189 and -178 mV, respectively. As the experiment proceeded, the Eh values were significantly increased. By the end of the experiment, the Eh value in the L2S1 group had increased to -33 mV, but it remained in a strong reducing state, while the Eh value in the CK group had increased to 177 mV.

Sulfate-reducing bacteria are considered to be one of the dominant microorganisms involved in the degradation of sulfate. At the beginning of the study, the greater the amount of plant litter added, the higher the SO_4^{2-} concentration in the supernatant. On the first day, the SO_4^{2-} concentrations in the L2S1 and L2S0 groups reached their maximum values of 2560 and 2230 $mg \cdot L^{-1}$. The SO_4^{2-} in the L2S1 group inoculated with SRB was higher than that in the L2S0 group. This was mainly due to the addition of deionized water to restore biological activity before the experiment began. The organic matter in the sediment was rapidly decomposed by aerobic microorganisms, resulting in a decrease in the Eh values. The results of previous studies have shown that the wetland sediment was mainly composed of quartz, gypsum, and goethite (Xu et al. 2019) and SRB metabolites could promote the decomposition of sulfate minerals (such as barite, sardinianite, and gypsum) (Schröder et al. 2018; Li et al. 2006; Kong et al. 2017). Therefore, in the early stage of the experiment, a large amount of SO_4^{2-} was released due to the dissolution of sulfate minerals. Later, in the L1S1 and L2S1 groups inoculated with SRB, the SRB used SO_4^{2-} as an electron acceptor, causing a sharp decrease in the SO_4^{2-} concentration.

3.2 Influence of the SRB content on heavy metals in the supernatant

The Fe concentration in the supernatant is shown in Figure 2-a. Throughout the entire experiment, the Fe concentration in the CK group was notably lower than in the other groups. In the treatment groups, the Fe concentration first increased and then decreased until it reached 0 $mg \cdot L^{-1}$. As shown in Figure 2-a, the Fe concentration in the L2S0 group reached a maximum of 17.33 $mg \cdot L^{-1}$ on day 14, and declined slightly to 14.17 $mg \cdot L^{-1}$ on day 21. Then, a rapid decrease was observed from days 21 to 35, reaching 0 $mg \cdot L^{-1}$ at the end of the experiment. In comparison with the L2S0 group, the Fe concentration in the L2S1 group was reduced significantly. The Fe concentration in the L2S1 group initially increased and then decreased, reaching a maximum of 11.23 $mg \cdot L^{-1}$ on day 14. Then, the Fe concentration in the L2S1 group declined at a faster rate than in the L2S0 group. The Fe concentration in the L2S1 group declined to 0 mg·L^{-1} on day 21.

The Mn and Fe concentrations had similar characteristics, displaying a trend of first increasing and then decreasing. The Mn concentration in the supernatant increased with the increase in plant litter. In the early stage of the experiment, litter decomposition, and SRB inoculation promoted an increase in the Mn concentration of the supernatant. On day 7, a maximum value of 9.00 mg·L^{-1} was reached in L2S1. After day 7, there was a sharp decrease in the Mn concentration. At the end of the experiment, the Mn concentration in each group decreased to 0 mg·L^{-1} except for L2S0, which still had a relatively high concentration.

Litter decomposition and SRB inoculation affected the Cu concentration in the supernatant. As shown in Figure 2-c, the Cu concentration in the L2S0 group reached a maximum value of 0.83 mg·L^{-1} on day 21. Subsequently, it decreased to 0.44 mg·L^{-1} during days 21−35, and there was no significant change in concentration during days 35−70. In the L2S1 group the Cu concentration was maintained at a low level. No Zn was detected in the supernatant during the entire experiment.

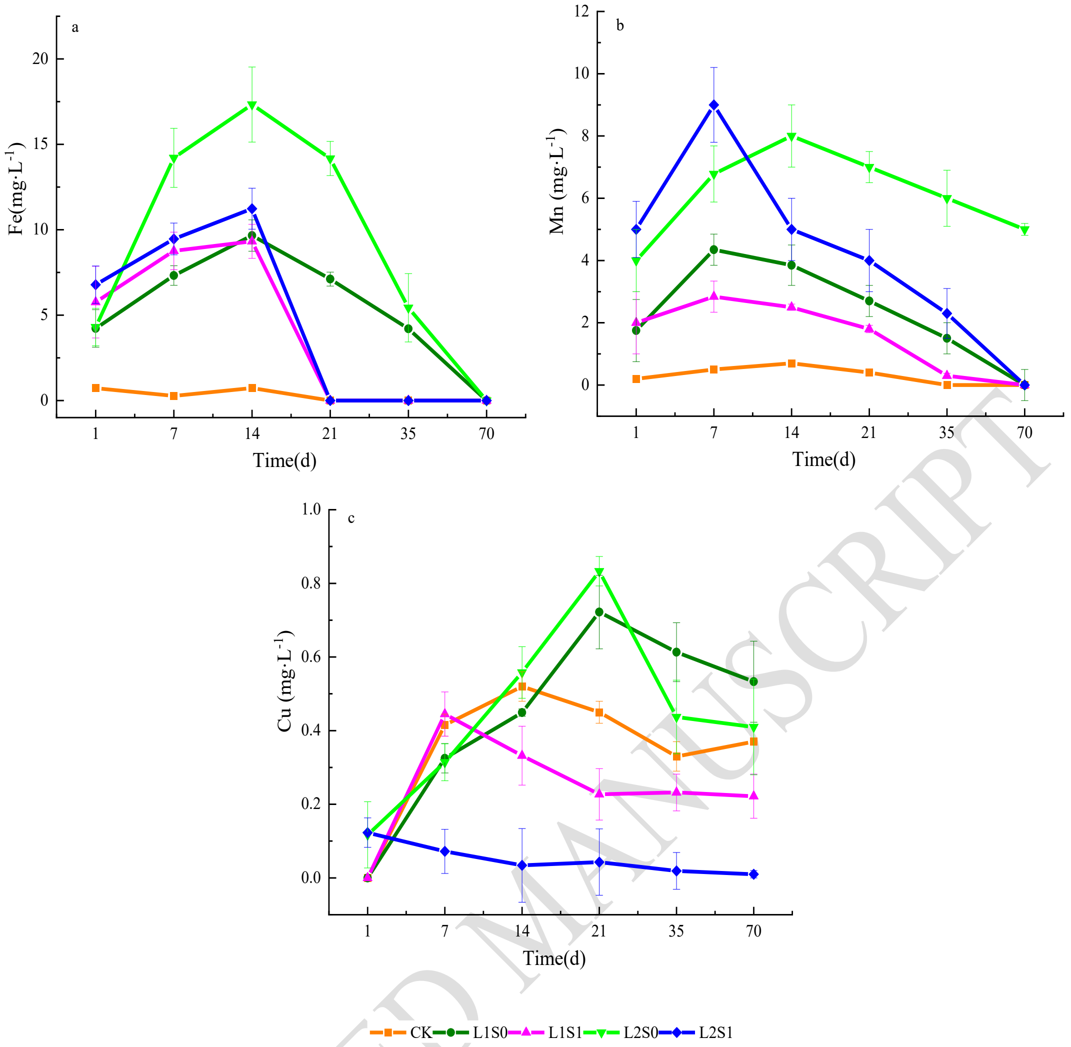


Figure 2. Influence of SRB on the Fe, Mn, and Cu concentrations in the supernatant during the 70 day experiment. Each data point represents the mean value of three experiments. All error bars represent the standard deviation of three experiments.

3.3 Influence of the SRB content on the OC in the sediments

In the sediments, the reduction of sulfate to sulfide by SRB is a key process in the anaerobic oxidation consumption of OC (Wijsman et al., 2001). The changes in the OC concentration in sediments during the experiment are shown in Figure 3. The OC concentration in the L2S0 group increased and reached a maximum value of 5.57 mg. g^{-1} on day 21. The OC concentration remained relatively stable afterward, with only a minor decrease toward the end of the experiment. More notable and continuous decreases were observed in the L1S1 and L2S1 groups. The decline was most rapid in the L2S1 group, reaching a value of 37 mg. g^{-1} on day 70.

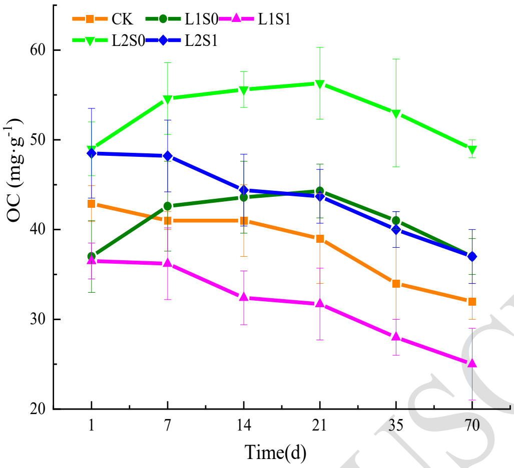
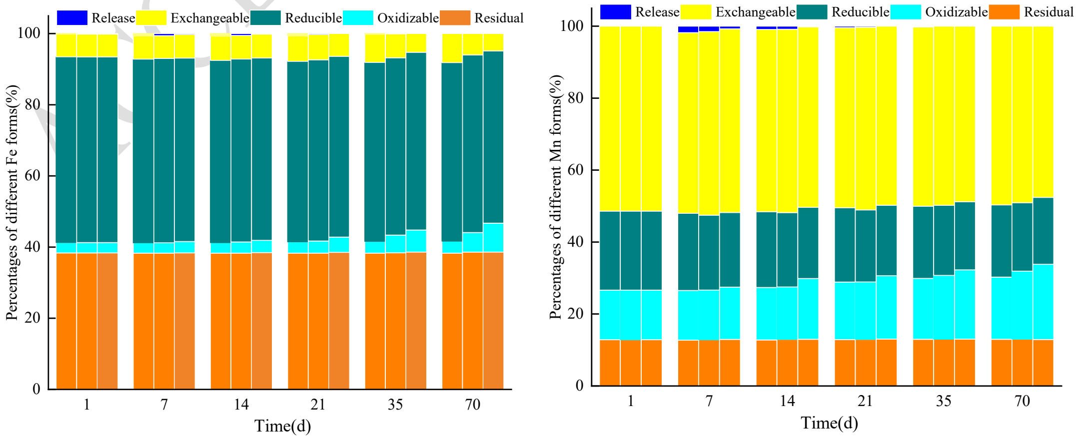
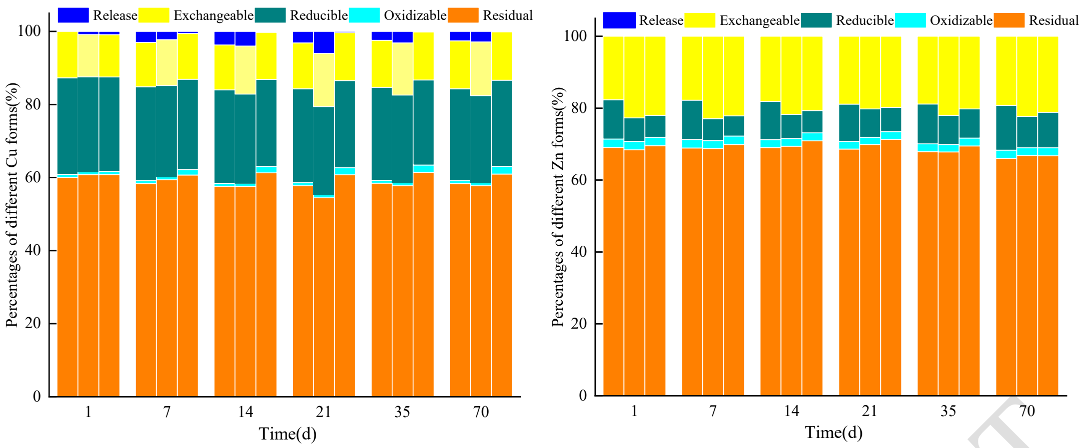


Figure 3. Influence of SRB on the OC concentration in the sediments during the 70 day experiment. Each data point represents the mean value of three experiments; All error bars represent the standard deviation of the three experiments.

3.4 Influence of the SRB content on the different fractions of heavy metals in the sediments





(Note: The histograms from left to right represent the CK, L2S0, and L2S1 treatment groups, respectively)

Figure 4. The influence of SRB on changes in the fractions of Fe, Mn, Cu, and Zn in the sediments; Each data bar represents the mean value of three experiments.

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During the experiments, large quantities of a black substance formed on the inner walls of the plastic cups. The reducible fraction of Fe was observed to be the main form in the original sediments. The effect of inoculation with SRB on the Fe fractions in the sediment are shown in Figure 4-a. In the CK group, the amount of exchangeable Fe displayed a gradually increasing trend during the experiment, increasing from an initial value of 6.41% to 8.15% at the end of the experiment. In the L2S0 group, the exchangeable Fe reached a maximum content of 7.09%, and then declined to 6.04% on day 70. In the L2S1 group, the exchangeable Fe content increased to 6.71% on day 14, then substantially declined to 4.85% on day 70. The amounts of reducible Fe in all three groups gradually decreased as the experiment proceeded, with the rate of decrease in the L2S1 group being greater than in the CK and L2S0 groups. The reducible Fe content in the L2S1 group declined to 48.41% on day 70. The amounts of oxidizable Fe in the three groups did not change notably at the beginning of the experiment. As the experiment proceeded, the oxidizable Fe contents in the three groups gradually increased. By the end of the experiment, the oxidizable Fe content in the L2S1 group had increased to 8.14%.

The influence of inoculation with SRB on the Mn fractions in the sediment is shown in Figure 4-b. Exchangeable Mn was the main Mn fraction in the sediments, with a consistent trend of decreasing abundance observed across all three groups. At the end of the experiment, the exchangeable Mn contents in the CK, L2S0, and L2S1 groups were 49.68, 49.06, and 47.58%, respectively. The amounts of reducible Mn exhibited similar trends in all three groups, namely, a gradual decrease. Furthermore, the decreases in the amounts of reducible Mn in the CK and L2S0 groups were smaller than in the L2S1 group, in which the amount of reducible Mn was 18.59% at the end of the experiment. During the experiment, the amounts of oxidizable Mn in the three groups displayed an increasing trend, and a higher increase was observed in the L2S1 group than in the other two groups.

During the experiment, there was an increase in the exchangeable Cu content in each group, although this was not always obvious. The combined effect of litter and SRB resulted in a gradual decrease in the reducible Cu content in the sediment. The decrease was greater in the SRB treatment group than in the litter group. As the experiment continued, the oxidizable Cu content in the L2S1 group increased. On day 70, the oxidizable Cu content in the L2S1 group had increased to 2.1%, which was greater than in the L2S0 group. The SRB inoculation resulted in the transformation of reducible Cu into oxidizable Cu in the sediment.

During the experiment, the main Zn fractions in each group varied. Compared with the CK, the exchangeable Zn content in the L2S0 and L2S1 groups significantly increased, and on day 7, the exchangeable Zn contents in the CK, L2S0, and L2S1 groups were 16.85%, 22.92%, and 22.13%, respectively. Compared with the CK, on day 7, the reducible Zn content in the L2S0 and L2S1 groups decreased by 41.94% and 46.24%, respectively. Subsequently, the continuous increase in reducible Zn in the two treatment groups may be due to the action of litter in reducing the Eh in the system, causing the release of reducible Zn and the subsequent adsorption by iron oxides in the sediment, resulting in a further increase in its content. The changes in the oxidizable Zn fraction in each group were not significant.

4 Discussion

The decomposition of wetland plants affected the pH, EC, and Eh values of the supernatant. The decomposition of plant litter could be separated into two stages. First was a rapid decomposition stage, in which the litter decomposition rate was very fast. This was followed by a stable decomposition stage, during which the mass loss of litter was relatively stable. In the early stage of litter decomposition, the anaerobic decomposition of organic matter produced low molecular weight organic acids, leading to a decrease in the pH of the supernatant (Yadav et al. 2010; Cao et al. 2015). According to the principles of thermodynamics, iron-reducing bacteria in sediments should metabolize OC before SRB. However, in sediments rich in organic matter, Fe and SO_4^{2-} reduction can occur simultaneously, and SO_4^{2-} reduction may even occur before Fe reduction (Cao et al. 2015; Roden and Wetzel, 1996). Alkalinity was generated during SO_4^{2-} reduction, with 4 mol HCO_3^- generated per 1 mol SO_4^{2-} reduction (Cao et al. 2008). Additionally, the base cations released by litter decomposition could increase the pH (Zhang et al. 2018), and the decarboxylation of organic anions during organic matter decomposition could also cause an increase in pH (Wu et al. 2019).

The more intense the SRB activity, the greater the amount of sulfide that would be generated via SO_4^{2-} reduction, and the larger the amount of H^+ that would be consumed. Previous studies have shown that the ions that significantly contribute to EC include H^+, Na^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, and Fe^{2+} (Mccleskey et al. 2012), whereas EC is relatively sensitive to the chemical form of dissolved matter in solution. In this study, the plant litter generated considerable amounts of DOM during the decomposition process. The DOM could complex or chelate heavy metals in the sediments. Consequently, heavy metals would be released into the water, thereby affecting the EC of the supernatant.

The oxygen consumption and production of reductive substances resulted in Eh decreases (Wu et al. 2007). The water-soluble substances in plant litter decomposed first during the decomposition process, and the biological degradation of these organic substances consumed a large amount of oxygen (Yang et al. 2008). Therefore, the Eh declined sharply at the beginning of the experiment. In the L1S1 and L2S1 groups inoculated with SRB, increasing amounts of reductive substances would be generated when SRB reduced SO_4^{2-}, and therefore the Eh decline rates in the two groups were notably higher than in the L1S0 and L2S0 groups.

Both Fe and Mn are highly sensitive to the oxidation−reduction environment and can be strongly affected by Eh. In this study, the sediment was kept under a water layer such that oxygen was not being supplemented; thus, the Eh in the sediment declined sharply. Furthermore, Fe(III), which acts as an electron acceptor, was gradually reduced to Fe(II) and released into the supernatant. At the same time, the decomposition of plant litter generated reductive substances that exchanged electrons with Fe and Mn oxides in the sediment. This resulted in the dissolution of reductive Fe and Mn. This process was the main reason why the Fe and Mn concentrations increased sharply at the beginning of the experiment (during the first 7 days). This result was in agreement with the results of previous studies (Zhang et al. 2014). Cao (2012) reported that low molecular weight organic acids are the preferred carbon sources for SRB. In the present study, low molecular weight organic matter generated during the initial stage of litter decomposition provided a sufficient carbon source for SRB, and promoted SRB activity in the treatment groups. The SRB content in sediments was affected by factors such as sediment depth, salt concentration, pH, organic matter content, and oxygen content(Ma et al. 2022; Ding et al. 2016; Chen et al. 2012). The content of OC in the L2S1 group was more than the other treatment groups(Figure. 3), in which SRB could more effectively convert SO_4^{2-} into S^{2-} and generate precipitates with Fe(II) that were released from sediments. Thus, the inoculation of sediments with SRB could effectively prevent Fe and Mn from being released into the supernatant. Therefore, the inoculation of sediments with SRB would exert an active protective effect to immobilize the released Fe and Mn, and thus reduce the amount of secondary pollution.

When naturally occurring plant litter was present in the wetland system, the OC concentration in the sediment increased gradually (Figure 3). Sulfate-reducing bacteria are heterotrophic microbes, and the abundance of carbon sources resulted in an increase in the quantity of SRB in the sediment. The SRB consumed a large quantity of the OC during the growth process. Thus, the OC concentration in the sediment gradually decreased, and the rate of OC decrease in the L2S1 group was higher than in the L2S0 group, indicating that SRB subsisted on the OC generated from plant litter-derived carbon sources. Low molecular weight organic acids generated during the initial litter decomposition stage in the L2S1 group were first used by SRB as carbon sources to promote their growth. Thus, the SRB content increased rapidly during days 7−14. As the litter and SRB environment stabilized, the SRB growth rate gradually decreased, possibly because in the later stage of the experiment, celluloses, hemicelluloses, and xylogens in the litter started to decompose (Tang et al. 2005). However, the decomposition rate was low and the availability of carbon sources was gradually reduced. Thus, the SRB growth rates were notably lower than those during days 7−14. The activity of SRB was influenced by SO_4^{2-} and OC, and therefore the ratio of OC to SO_4^{2-} should be considered.

Iron was mainly present in its reducible form in the raw sediment. In the CK group, litter decomposition promoted a decrease in the amount of reducible Fe and an increase in the amounts of exchangeable and oxidizable Fe. Reducible Fe is formed via Fe oxidation or is present on the surface of sediment granules (Colombo et al. 2014). Reducible Fe is characterized by its poor stability under reducing conditions and high vulnerability to being reduced and released into the environment. In this study, plant litter decomposition consumed oxygen in the water and resulted in reduced Eh levels that were lower than -100 mV (Figure 1-c). In a reducing environment, the reducible Fe in sediments would be dissolved, resulting in decreasing reducible Fe contents in sediments. Reduced and released Fe would enter the supernatant and some would then be readsorbed onto the surface of sediments (Macdonald et al. 2011), thereby resulting in an increase in the amount of exchangeable Fe in sediments. The results indicated that litter decomposition could enhance Fe activity in the sediment. In the L1S1 and L2S1 groups inoculated with SRB, SO_4^{2}- was reduced under reductive conditions and formed S^{2-}, which resulted in the formation of FeS with dissolved Fe. Fan et al. (2008) indicated that when Cd-contaminated soil was inoculated with SRB, the exchangeable Cd concentration gradually decreased, and the stability of Cd was enhanced. The formation of FeS in the sediment increased the oxidizable Fe content, which could only be released under strong oxidizing conditions (He et al. 2003; Qin et al. 2012). Oxidizable Fe and Mn are relatively stable forms of Fe and Mn in the sediment (Li et al. 2015). Therefore, inoculation of the sediment with SRB exerted a positive effect on Fe and Mn immobilization. Given the reduction and dissolution of Mn in the sediment, the Mn ions were dissolved, after which the ions remigrated and were distributed in the sediment. The exchangeable Mn content gradually decreased in the L2S0 group, possibly because the low molecular weight organic acids generated during the litter decomposition process promoted the release of exchangeable Mn. The changes in the Mn contents were similar to those in the Fe contents. The amounts of reducible Mn in the L1S1 and L2S1 groups inoculated with SRB gradually decreased, whereas the amount of oxidable Mn gradually increased.

During the experiment, a large amount of black matter was generated in the L1S1 and L2S1 groups. This was accompanied by the odor of H_2S, indicating that the quantity of SRB increased. The newly-generated H_2S reacted with Cu to form sulfide precipitates, which were extracted in an oxidizable state in the experiment. This significantly reduced the available Cu content and thus reduced the toxic effect of Cu ions on microorganisms. There was no significant change in the oxidizable Zn content in the L2S1 group, which was mainly related to the solubility product constant of heavy metal sulfides: CuS (Ksp = 6 × 10^{-37}) ＜ZnS (Ksp = 2 × 10^{-25}) (Kiran et al. 2017). The smaller the solubility product constant, the easier it is for sulfides to form. Additionally, the SRB reduction of SO_4^{2-} caused other coexisting ions to preferentially precipitate during the precipitation of heavy metals.

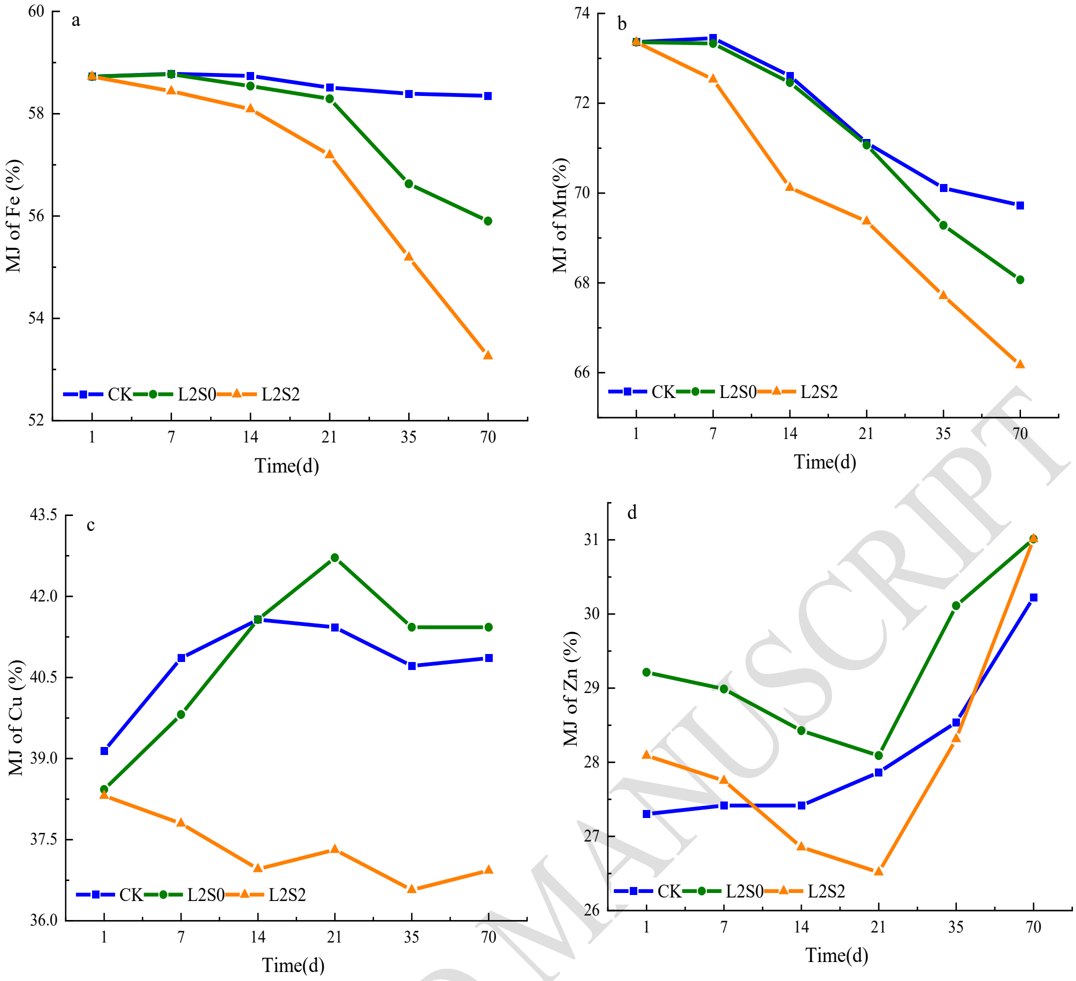

Figure 5. Changes in the (a) Fe, (b) Mn, (c) Cu, and (d) Zn migration coefficients in the sediment

Zhao et al. (2013) employed a migration coefficient (MJ: the ratio of the sum of exchangeable and reducible heavy metals in the sediment to the total metal content) to represent the migration abilities of different heavy metals in sediments. The higher the value of MJ, the stronger the migration abilities of heavy metals. In contrast, heavy metals would be more stable at lower MJ values. The MJ values of the three groups in this study are shown in Fig. 5. The inoculation of the sediment with SRB significantly reduced the migration abilities of Fe and Mn, which previously exhibited a substantial migration ability in the sediment. Therefore, inoculation with SRB had an active immobilization effect on Fe and Mn. The MJ of Cu displayed a similar trend to that of Fe and Mn, namely, inoculation of the sediment with SRB reduced the mobility of Cu. The MJ values of Zn initially decreased and then increased, and the stability of Zn decreased in the later stage of the experiment. The increased stability of Fe, Mn, and Cu resulted in a reduced risk of secondary releases from the sediment.

5. Conclusions

(1) The pH in all treatment groups increased gradually, with the L2S1 group exhibiting the largest increase. During the experiment, the Eh in the supernatant initially decreased and then increased, while the EC initially increased and then decreased.

(2) Inoculation of the sediments with SRB had a negative effect on the release of Fe, Mn, and Cu into the supernatant, but had no effect on the Zn concentration in sediments.

(3) Each of Fe, Mn and Cu gradually changed from their exchangeable and reducible forms into their oxidizable forms in SRB-inoculated sediments. Bioremediation using SRB significantly enhanced the stability of Fe, Mn, and Cu and substantially reduced the secondary risk from the sediments.

(4) When using SRB for the ecological remediation of heavy metals, the ratio of organic carbon to SO_4^{2-} should be monitored to improve the remediation effect.

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