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Original Research

Research on Ecological Remediation of Water Bodies by Zeolite and Shale Ceramsite (ZSC) Combined with *Vallisneria natans* (L.)

Jian Sun, Shiyan Cai, Qing Li, Mei Wang, Qi Liu, Lei Zou*, Haiyan Liu

Central and Southern China Municipal Engineering Design & Research Institute Co., Ltd., Wuhan 430010, China

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Abstract

Enriched sediment can release nutrients to water and is not conducive to the ecological restoration of submerged macrophytes. Sediment capping with zeolite and shale ceramsite (ZSC) before *Vallisneria natans* (L.) (*V. natans*) planting was set to research the water quality and characteristics of plant and microbial communities of sediment. The results showed that covering ZSC with *V. natans* could effectively maintain the low content of total phosphorous (TP) (0.02~0.06 mg/L), total nitrogen (TN) (0.23~1.62 mg/L), ammonia (NH_3-N) (0.14~0.63 mg/L), chemical oxygen demand (COD) (6.15~74.83 mg/L), and chlorophyll a (Chl.a) (0.04~0.56 mg/L) in overlying water. Covering ZSC can effectively alleviate the environmental stress of sediment on *V. natans* in the performance of biomass, height, and chlorophyll of *V. natans* increasing, the content of ascorbic acid (AsaA), malondialdehyde (MDA), and protein in *V. natans* decreasing. Covering ZSC creates anaerobic conditions in the sediment, increasing the relative abundance of *Levilinea* and *Longilinea*, which may promote the waste anaerobic digestion of sediment. These results, which covered ZSC with *V. natans,* benefit ecological remediation. It is helpful to offer managers useful information on selecting ZSC and submerged macrophytes for ecological restoration.

Keywords: Zeolite and shale ceramsite, *Vallisneria natans* (L.), water quality, plant characteristics, microbial community

Introduction

Internal pollution sources (enriched sediment) can release nutrients into water, which is the main driver of freshwater eutrophication [1]. Therefore, numerous approaches have been employed for sediment treatment, including sediment dredging [2, 3], hypolimnetic aeration [4], sediment capping [5, 6], phytoremediation [7, 8], and chemical reagents [9, 10].

Sediment capping, placing adsorption materials over sediment, has been supposed to be an effective and low-cost way to mitigate the release of pollutants. It is crucial to select the adsorption materials [11, 12]. Up to now, there have been many research studies of materials on Nitrogen (N) or Phosphorous (P) absorption. Among these, zeolite and shale ceramsite are famous for

*e-mail: 10027238@qq.com
Tel.: +86-027-82631888
Fax: +86-027-82426036.

Table 1. Physical properties and chemical compositions of surface sediment.

Parameters	Water content (%)	ORP (mv)	pH	Organic matter (%)	TP (mg/g)	TN (mg/g)	NH_3-N (mg/g)
Content	265.23	217.2	7.03	18.59	4.76	4.97	0.12

Table 2. Physical properties of zeolite and shale ceramsite.

Parameters	Specific surface area (cm^2/g)	Porosity (%)	Density (g/cm^3)	Effective grain diameter (mm)
Zeolite	9.89×10^4	22.86	2.45	4.20
Shale ceramsite	0.92×10^4	13.02	1.46	4.28

nitrogen and phosphorus adsorbents, respectively [13, 14]. Zeolite, composed of silica, aluminum, and oxygen, is distinguished by its systematic structure comprising plenty of channels and pore cavities [15]. And zeolite is widespread in terrestrial environments and is a low-cost mineral. As a traditional environmental material, shale ceramsite has the function of chemical adsorption, precipitation, or biological adsorption mechanisms with high metal (Ca, Fe, Al, or Mg) content [16, 17]. In our previous study, we found that zeolite and shale ceramsite mixed at the weight ratio of 2:1 (ZSC) effectively adsorbed sediment nitrogen and phosphorus mainly by physical adsorption and nonspecific chemisorptions and suggested that the combination of ZSC and other ecological methods may be a new way and more beneficial to ecological restoration [18].

Submerged macrophytes, a typical phytoremediation method, have been widely established to maintain ecological integrity and stability, reduce turbidity from sediments, and increase the diversity of the physical habitat in freshwater ecosystems [19-21]. Therefore, reestablishing submerged macrophytes after interruption of external nutrient input has been recognized as an effective measure to restore the clear state of water [22, 23]. However, the survival and propagation of submerged macrophytes are influenced by many external conditions, one of which is polluted sediments. So submerged macrophytes established after sediment capping can not only reduce the risk of nitrogen and phosphorus release from polluted sediments to water but also provide a good environment for macrophyte growth [24].

Therefore, *Vallisneria natans* (L.) (*V. natans*) and ZSC (mixed at the weight ratio of 2:1) were prepared to restore the enriched sediment in situ. The water quality was measured periodically. On account of a few reports on the effects of ZSC on the growth of submerged macrophytes *V. natans*, the physiological and phytochemical effects of ZSC on the growth process of *V. natans* were investigated intensively in the study. Furthermore, the microbial community of sediment was evaluated. Taken together, this study aims to provide theoretical references and technical support for ZSC and submerged macrophytes in the field of ecological restoration.

Materials and Methods

Experimental Materials

Sediment Sampling

The Peterson grab sampler collected surface sediments (0-10 cm) from Huangxiaohe River, Wuhan City, China (114º28′E, 30º62′N). The samples were then transported to the laboratory at under 4 ℃ for further experiments. The surface sediments' physical properties and chemical compositions were determined after drying in an oven at 55 ℃ to constant weight and sieving with a stand 100-mesh sieve, as shown in Table 1.

Zeolite and Shale Ceramsite (ZSC)

Zeolite (2-4 mm in diameter) was purchased from Runjia Environmental Protection Co., Ltd., and Shale ceramsite (2-4 mm in diameter) was purchased from Xinjiayuan Material Co., Ltd. Tap water and ultrapure water were used to wash zeolite and shale ceramsite 3 times. Then zeolite and shale ceramsite were dried under 105±5 ℃. The physical properties of zeolite and shale ceramsite are shown in Table 2. Zeolite and shale ceramsite were mixed at a weight ratio of 2:1.

Vallisneria Natans (V. natans)

V. natans, as the typical species of submerged macrophytes, was collected from East Lake, Wuhan City, China (30°53′ N, 114° 38′ E). Before the experiment, running tap water was used to flush away marl and periphyton coverage on the macrophytes. The well-grown macrophytes were picked out and then flushed away with running tap water to remove marl and periphyton coverage on the macrophytes.

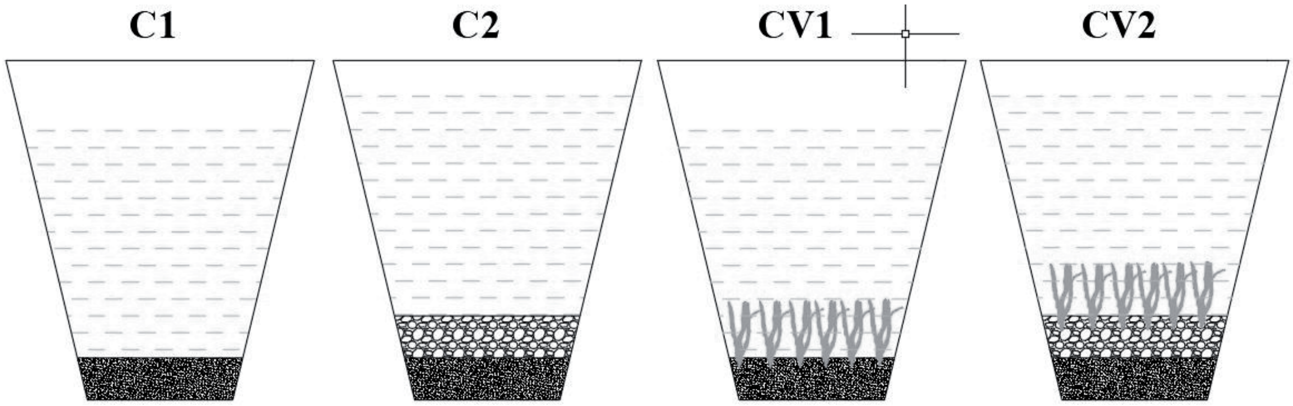

Fig. 1. Schematic graph of the experiment design.

Experiment Designed

The experiment was carried out in 200 L PVC tanks and divided into four treatments: (1) only sediments (C1); (2) sediments with ZSC (C2); (3) sediments planted with *V. natans* (CV1); (4) sediments with ZSC planted *V. natans* (CV2). The height of sediments (C1-C4) and ZSC (C2, CV2) was 10 cm. Each treatment had three replicates. The height and weight of *V. natans* were 25 cm and 180 g in CV1 and CV2. The water depth in each tank was 50 cm (Fig. 1). The experiment lasted for 84 days.

Sampling and Analysis

Water Quality

Water in the tanks was sampled once a week between 10:00 and 11:00. Total nitrogen (TN), total phosphorous (TP), ammonia (NH_3-N), and chemical oxygen demand (COD) were analyzed according to standard procedures [25]. Chlorophyll a (Chl. a) was measured using Whatman GF/C glass filters (0.45 mm) to filter water samples, and then the filters were extracted with 90% acetone for 24 hours.

Morphological and Physiological Parameters of V. natans

The morphological characteristics of *V. natans*, including the plant biomass and height, were determined using electronic scales and a measuring ruler.

The physiological parameters of *V. natans* included chlorophyll a (Chl.a), chlorophyll b (Chl.b), carotenoids, protein, malondialdehyde (MDA), and ascorbic acid (AsaA). The leaves' Chl.a, Chl.b, and carotenoid contents were analyzed using the 95% alcohol extraction spectrophotometric method [26]. The protein contents of leaves were measured using the Coomassie Brilliant Blue G-250 dyeing method [26]. The MDA content was determined by ion-pairing high-performance liquid chromatography [27]. The AsaA contents were measured using the 5% trichloroacetic acid extraction spectrophotometric method [27].

Microbial Communities of Sediments

The sediments of each experimental treatment were taken and placed in an EP tube to be frozen with liquid nitrogen for 3-4 h, and then transferred to a refrigerator at -80 °C for storage and testing. DNA extraction, PCR (primer V3-V4), and high-throughput 16S rRNA pyrosequencing of each sample were performed following the methods described by Tabugo et al. [28].

Data Analysis

All statistical analyses were performed using SPSS IBM Statistics version 23, Illinois, USA. The four treatments' TN, TP, NH_3-N, and COD, differed ($P<0.05$) using one-way ANOVA, followed by the Tukey post hoc test to identify homogenous groups. Chlorophyll, protein content, MDA, and AsaA of *V. natans* in CV1 and CV2 were compared using independent samples t-test, respectively. The Shannon index was determined based on the calculated operational taxonomic units (OTUs) using Mothur ver. 1.30.1 for the sequence analysis of the sediment microbiota. The sequences were phylogenetically classified to the phylum and genus levels at 97% similarity for the community composition analysis. MEGA 6.0 was used to depict a phylogenetic tree using the neighbor-joining algorithm. Representative sequences from each OTU were subjected to the RDP-II Classifier of the Ribosomal Database Project (RDP) and the National Center for Biotechnology Information (NCBI) BLAST for taxonomic analysis.

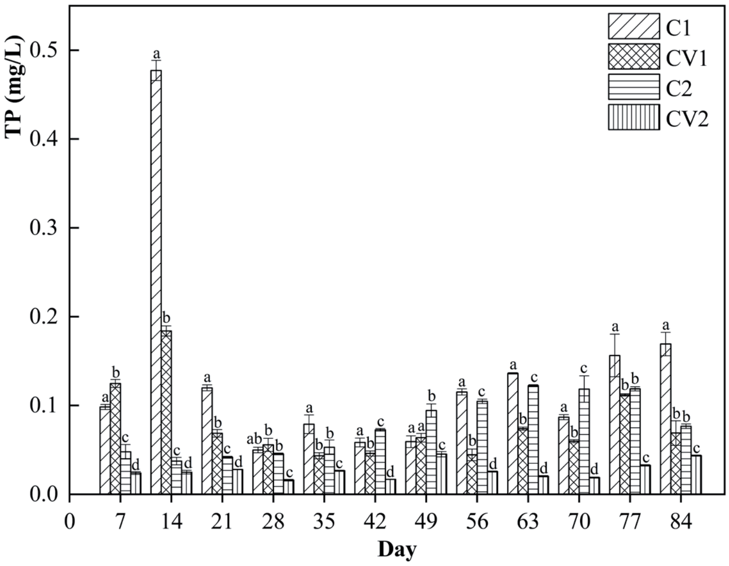

Fig. 2. The purification effect of *V. natans* and ZSC on TP (The different letters (a,b) indicate significant differences ($P < 0.05$) between different experimental groups at the same time, n = 3).

Results and Discussion

Water Quality

Total Phosphorous (TP)

During the experiment, TP in CV2 ranged from 0.02 mg/L to 0.06 mg/L. TP in C1, CV1, and C2 were 0.05~0.48 mg/L, 0.04~0.18 mg/L, and 0.04~0.12 mg/L. On day 7, TP in CV1 was 0.12 mg/L, higher than the others. On days 42, 49, and 70, TP in C2 was 0.08 mg/L, 0.09 mg/L, and 0.12 mg/L, respectively, which were higher than TP in C1. However, TP in CV2 was significantly lower than in others from day 7 to day 84 (P<0.05) (Fig. 2).

Total Nitrogen (TN)

TN in C1 ranged from 1.19 mg/L to 6.64 mg/L and was the highest from day 7 to day 49 and day 84. When ZSC was covered in sediments, TN in C2 (0.43 ~4.95 mg/L) was higher than in C1, especially from day 63 to day 77. Only planted *V. natans* obviously reduced the concentration of TN in CV1 (0.8 to 2.63 mg/L) compared to C1. TN in CV2 was significantly lower than others from day 7 to day 84 (P<0.05) (Fig. 3).

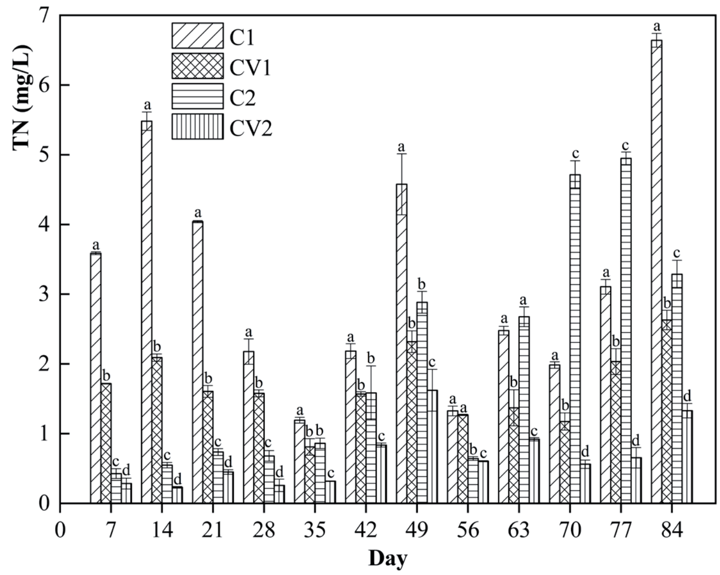

Fig. 3. The purification effect of *V. natans* and ZSC on TN (The different letters (a,b) indicate significant differences ($P < 0.05$) between different experimental groups at the same time, n = 3).

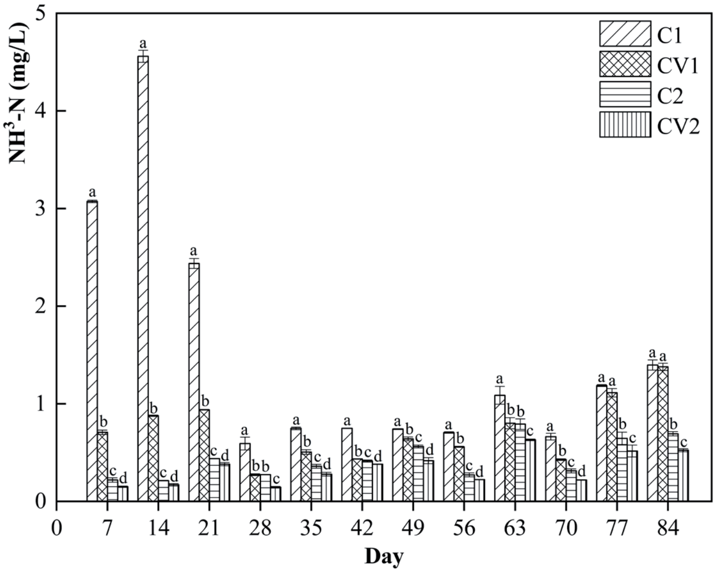

Fig. 4. The purification effect of *V. natans* and ZSC on NH_3-N (The different letters (a,b) indicate significant differences ($P < 0.05$) between different experimental groups at the same time, n = 3).

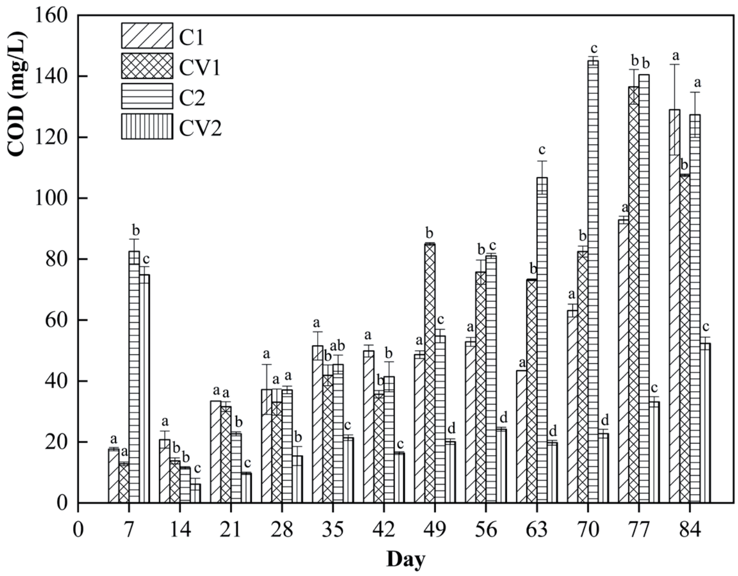

Fig. 5. The purification effect of *V. natans* and ZSC on COD (The different letters (a,b) indicate significant differences ($P < 0.05$) between different experimental groups at the same time, n = 3).

Ammonia (NH_3-N)

NH_3-N in C1 was 0.66~4.56 mg/L from day 7 to day 84. When covering ZSC or planting *V. natans*, the concentration of NH_3-N decreased. NH_3-N in CV1 was 0.27~1.38 mg/L, much less than in C1 except on day 77 and day 84. NH_3-N in C2 and CV2 remained at 0.22~0.79 mg/L and 0.14~0.63 mg/L, respectively. NH_3-N in CV2 was observably lowest during the experiment (P<0.05) (Fig. 4).

Chemical Oxygen Demand (COD)

COD in C1 went up gradually and was 17.65~129 mg/L from day 7 to day 84. After covering ZSC and planting *V. natans*, COD in CV1 and C2 were not effectively reduced and were higher than that in C1 from day 49 to day 84. COD in CV2 remained at 6.15~74.83 mg/L and was significantly the lowest during the experiment except on day 7 (P<0.05) (Fig. 5).

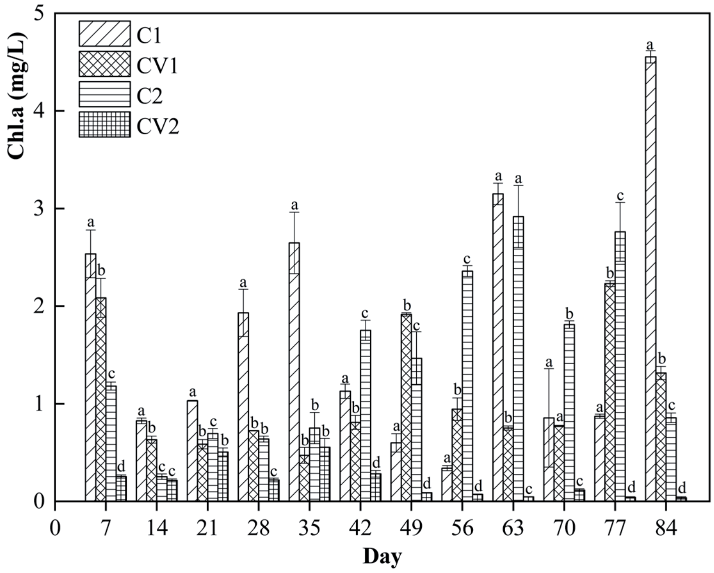

Fig. 6. The purification effect of V. natans and ZSC on Chl.a (The different letters (a,b) indicate significant differences (P < 0.05) between different experimental groups at the same time, n = 3).

Chlorophyll a (Chl.a)

Chl.a in C1 remained between 0.34 mg/L and 4.55 mg/L during the experiment. After only covering ZSC or only planting *V. natans*, Chl.a in CV1 and C2 was higher than in C1 from day 42 to day 77. However, the concentration of Chl.a in CV2 showed a decreasing trend and remained the lowest compared to other treatments (P<0.05) (Fig. 6).

In conclusion, covering ZSC with *V. natans* can effectively keep the TP, TN, NH_3-N, COD, and Chl.a low content in the overlying water. This is because ZSC adsorbs nutrients from sediments to slow their release to water, promoting the utilization of nutrients by *V. natans*.

Morphological and Physiological Parameters of *V. natans*

Biomass and Height

At the end of the experiment, the weights of *V. natans* in CV1 and CV2 were 414.67 g and 606 g. The heights of *V. natans* in CV1 and CV2 were 51.33 cm and 73.33 cm. Significant differences existed in terms of weight and height between CV1 and CV2 (weight: P<0.05; height: P<0.05) (Fig. 7). This suggested that covering ZSC promoted the growth of *V. natans*. The possible reason for this phenomenon was that ZSC can provide excellent growth conditions [16].

Ascorbic Acid (AsaA)

AsaA plays an important role in plant cell resistance to oxidative stress, cell division and elongation, and regulation of enzyme activity [29]. On day 21, the AsaA of *V. natans* in CV1 and CV2 were 8.47 μg/g FW and 7.62 μg/g FW. On day 84, the AsaA of *V. natans* in CV1 and CV2 were 6.24 μg/g FW and 4.67 μg/g FW. There were significant differences between CV1 and CV2 on day 21 (P<0.05) and day 84 (P<0.05), respectively, which indicated that covering ZSC can effectively reduce the content of AsaA in *V. natans* (Fig. 8). This is because AsaA is generally positively correlated with the stress tolerance of plants [30]. It provided a good growth environment for *V. natans* by covering ZSC, leading to the decrease of AsaA in *V. natans.*

Malondialdehyde (MDA)

MDA is an important physiological indicator of the severity of plants under stress, mainly causing membrane lipid peroxidation and destroying the structure and permeability of the cell membrane, leading to a series of physiological and biochemical reactions [31]. On day 21, the MDA of *V. natan*s in CV1 and CV2 were 2.69 μmol/g FW and 1.88 μmol/g FW. There was a significant difference between CV1 and CV2 (P<0.05). On day 84, the MDA of *V. natans* in CV1 and CV2 were 1.62 μmol/g FW and 1.01 μmol/g FW, which showed a significant difference (P<0.05) (Fig. 9). This suggested that covering ZSC could effectively reduce MDA in *V. natans*, which is similar to the research showing that the content of MDA in plants was positively correlated to the degree of plants subjected to stress [32, 33]. These results indicated that covering ZSC could relieve the stress of *V. natans* caused by sediments.

Protein

Under stress conditions, plants usually accumulate large amounts of intracellular active proteins to enhance tolerance [34, 35]. On day 21, the protein of *V. natans* in CV1 and CV2 was 0.18 mg/g FW and 0.17 mg/g FW. There was no significant difference between CV1 and CV2 (P>0.05). On day 84, the protein of *V. natans* in CV1 and CV2 was 0.09 mg/g FW and 0.04 mg/g FW, which showed a significant difference (P<0.05) (Fig. 10). The results indicated that sediments without ZSC accelerated the synthesis of proteins in *V. natans.* This may be a response to environmental stress in order to reduce cells' osmotic potential through protein production.

Chlorophyll

Photosynthesis is considered one of the most sensitive physiological processes for plants under environmental changes, and chlorophyll is the main pigment for light absorption in photosynthesis [26, 35]. The effects of covering ZSC on the chlorophyll of *V. natans* were mainly analyzed from chl.a, chl.b, and carotenoids. On day 21, chl.a, chl.b, and carotenoids in CV2 were 25.20 mg/g FW, 9.38 mg/g FW, and 6.80 mg/g FW, respectively. The chl.b and carotenoids in CV2 were much less than those in CV1 (chl.b: 11.85 mg/g FW, P<0.05; carotenoids: 8.10 mg/g FW, P<0.05). On day 84, chl.a, chl.b, and carotenoids in CV2 were 33.86 mg/g FW, 13.74 mg/g FW, and 8.05 mg/g FW, which were

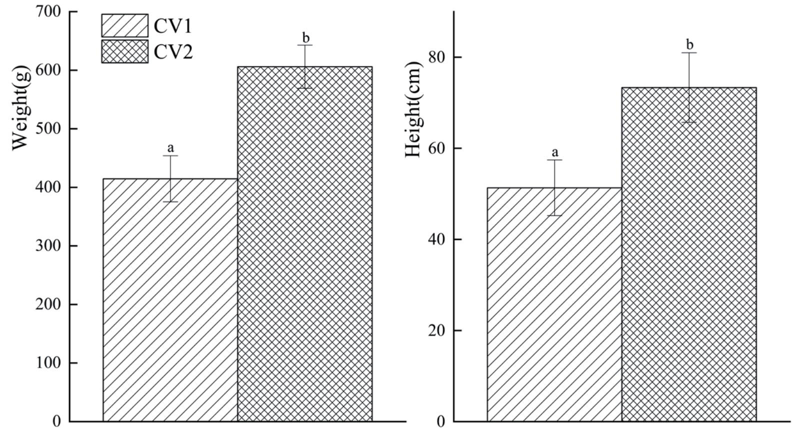

Fig. 7. Biomass and height of *V. natans* at the end of the experiment (The different letters (a,b) indicate significant differences (P < 0.05) between different experimental groups at the same time, n = 3).

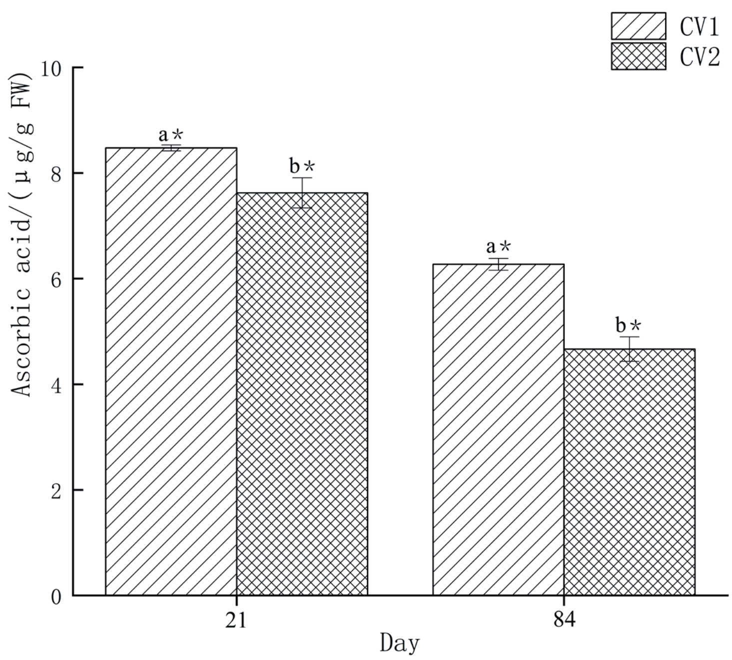

Fig. 8. AsaA content of *V. natans* (The different letters (a,b) indicate significant differences (P < 0.05) between different experimental groups at the same time. * represents significant differences (P < 0.05) between the same experimental group at different times, n = 3).

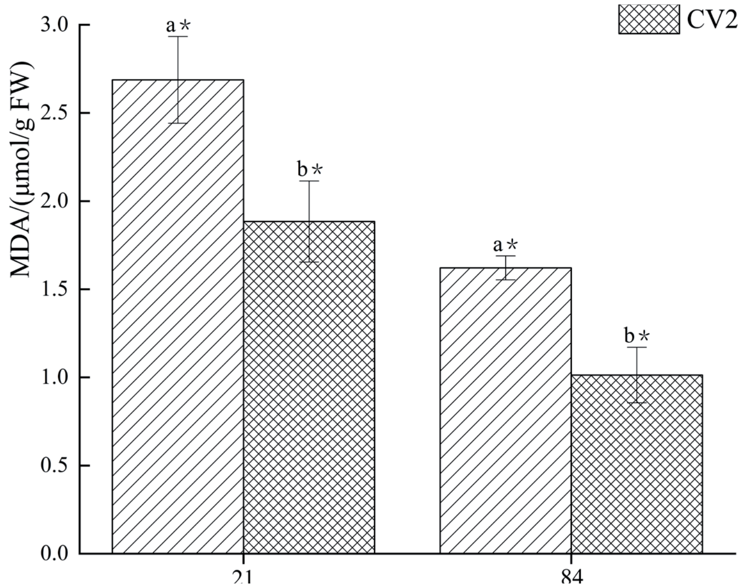

Fig. 9. MDA content of *V. natans* (The different letters (a,b) indicate significant differences ($P < 0.05$) between different experimental groups at the same time. * represents significant differences ($P < 0.05$) between the same experimental group at different times, n = 3).

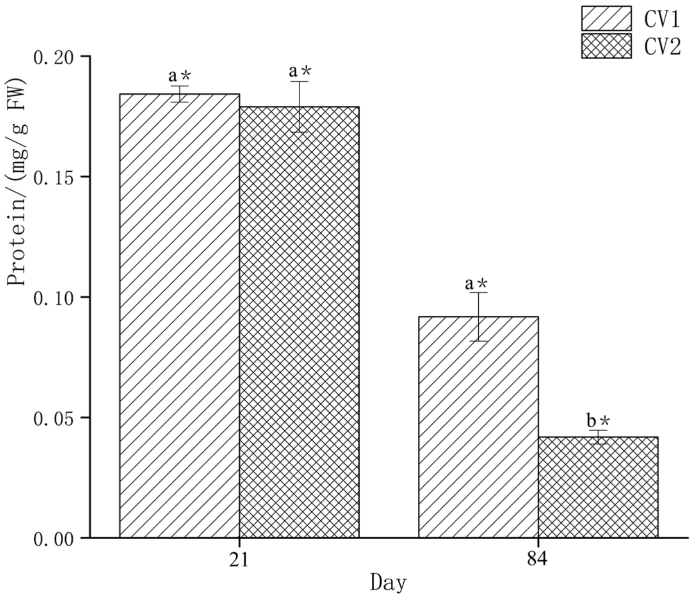

Fig. 10. Protein content of *V. natans* (The different letters (a,b) indicate significant differences ($P < 0.05$) between different experimental groups at the same time. * Represents significant differences ($P < 0.05$) between the same experimental group at different times, n = 3).

much higher than those in CV1 (chl.a: 24.51 mg/g FW, P<0.05; chl.b: 8.00 mg/g FW, P<0.05; carotenoids: 6.73 mg/g FW, P<0.05) (Fig. 11). These results indicated that the polluted sediment could hinder chlorophyll synthesis of plants, and it is beneficial to chlorophyll synthesis by covering ZSC, especially at the end of the experiment, which was consistent with other studies that the content of chlorophyll showed a downward trend when plants were under stress [36, 37].

Meanwhile, the content of AsaA, MDA, and protein in CV1 and CV2 decreased significantly on day 84 compared to that on day 21 (AsaA: Fig. 8, P<0.05; MDA: Fig. 9, P<0.05; protein: Fig. 10, P<0.05), which showed that *V. natans* can gradually adapt to the environment, leading to resistance to stress decreasing during the growth process. The content of chl.a, chl.b, and carotenoids in CV1 on day 21 was significantly more than those in CV1 on day 84, but the content of chl.a, chl.b, and carotenoids in CV2 on day 21 was significantly less than those in CV1 on day 84 (Fig. 11), indicating that covering ZSC promotes the synthesis of chlorophyll in *V. natans*.

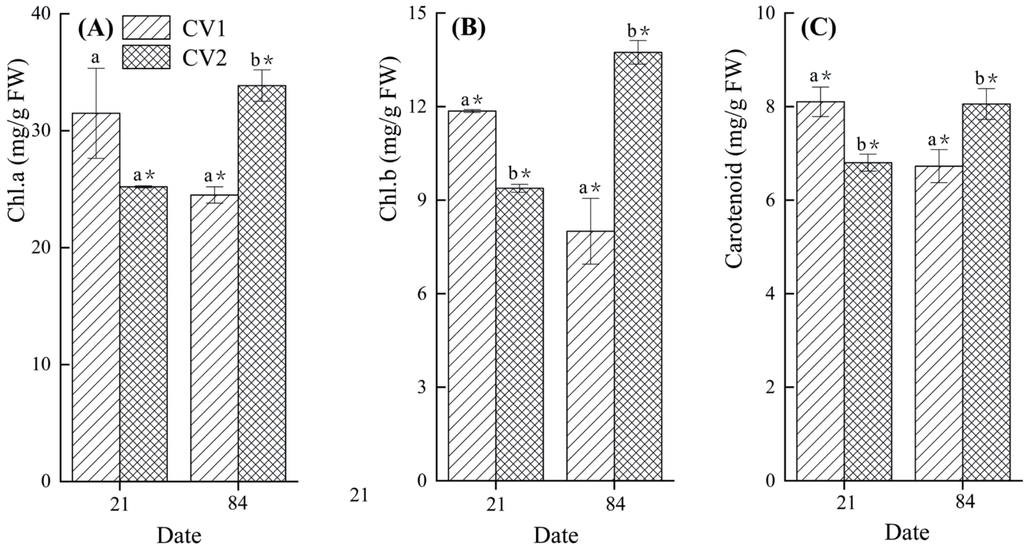

Fig. 11. Chlorophyll content of *V. natans* (The different letters (a,b) indicate significant differences (P < 0.05) between different experimental groups at the same time. * Represents significant differences (P < 0.05) between the same experimental group at different times, n = 3).

Microbial Communities of Sediments

To identify the bacterial communities from sediments samples, more than 51200 sequences were obtained for each sample. After downstream analyses, 9891 OTUs were obtained at a 97% sequence identity threshold. In each sample, 3166 to 3551 OTUs were observed. The sequence information and diversity index (Shannon) of the samples are shown in Table 3. Relative bacterial community abundance of CV1 and CV2 was characterized at the phylum and genus levels (Fig. 12).

At the phylum level, there were 45 phyla classified for each, and the major phyla of the samples were uniform, but their relative abundances differed. For example, the relative abundances of Chloroflexi and Proteobacteria in CV1S were 30.97% and 15.93%, which were less abundant than in CV2S (Chloroflexi: 37.34%, P<0.05; Proteobacteria: 19.43%, P<0.05). However, Firmicutes (16.07% vs. 15.88%), Caldiserica (12.65% vs. 6.27%), Bacteroidetes (8.16% vs. 5.72%), and Actinobacteria (2.21% vs. 2.02%) were more abundant in CV1S as compared to CV2S. The relative abundances of Chloroflexi, Proteobacteria, Firmicutes, Bacteroidetes, and Planctomycetes were 7.70%, 25.50%, 6.43%, 19.51%, and 6.87%, respectively (Fig. 12a).

At the genus level, the prevalent species in CV1S were *Caldisericum* (12.65%), *Levilinea* (8.81%), and *Longilinea* (6.69%). The relative abundances of *Levilinea*, *Longilinea,* and *Caldisericum*, as the dominant species in CV2S, were 15.26%, 8.11%, and 6.27%, respectively. The relative abundances of *Caldisericum* in CV2S were much less than in CV1S (P<0.05), which was inverse to *Levilinea* and *Longilinea* in CV1S and CV2S (Fig. 12b). The results showed that covering ZSC could cause the change of relative abundance of microorganisms in sediments, such as the relative abundance of *Caldisericum* decreasing and the relative abundance of *Levilinea* and *Longilinea* increasing. *Caldisericum* is a kind of hydrolytic acidifying genus and also belongs to the thermophilic filamentous genus, which is a heterotrophic anaerobic high-temperature filamentous bacterium [38, 39]. Covering ZSC can lower sediment temperature, leading to a decrease in the relative abundance of *Caldisericum*. *Levilinea* and *Longilinea* belong to anaerobic genera. Covering ZSC can obstruct the transportation of dissolved oxygen from water to sediment, thus promoting the growth and reproduction

Table 3. Richness and diversity indices of the microbial communities in sediment.

Sample	Number of sequences	OTUs number	Shannon index
CV1S	111405	3551	5.13
CV2S	84402	3174	5.18
CV2Z	51277	3166	6.60

Note: CV1S, CV2S, and CV2Z represent the sediment of CV1, the sediment of CV2, and the ZSC of CV2, respectively. OTUs is abbreviated as operational taxonomic units.

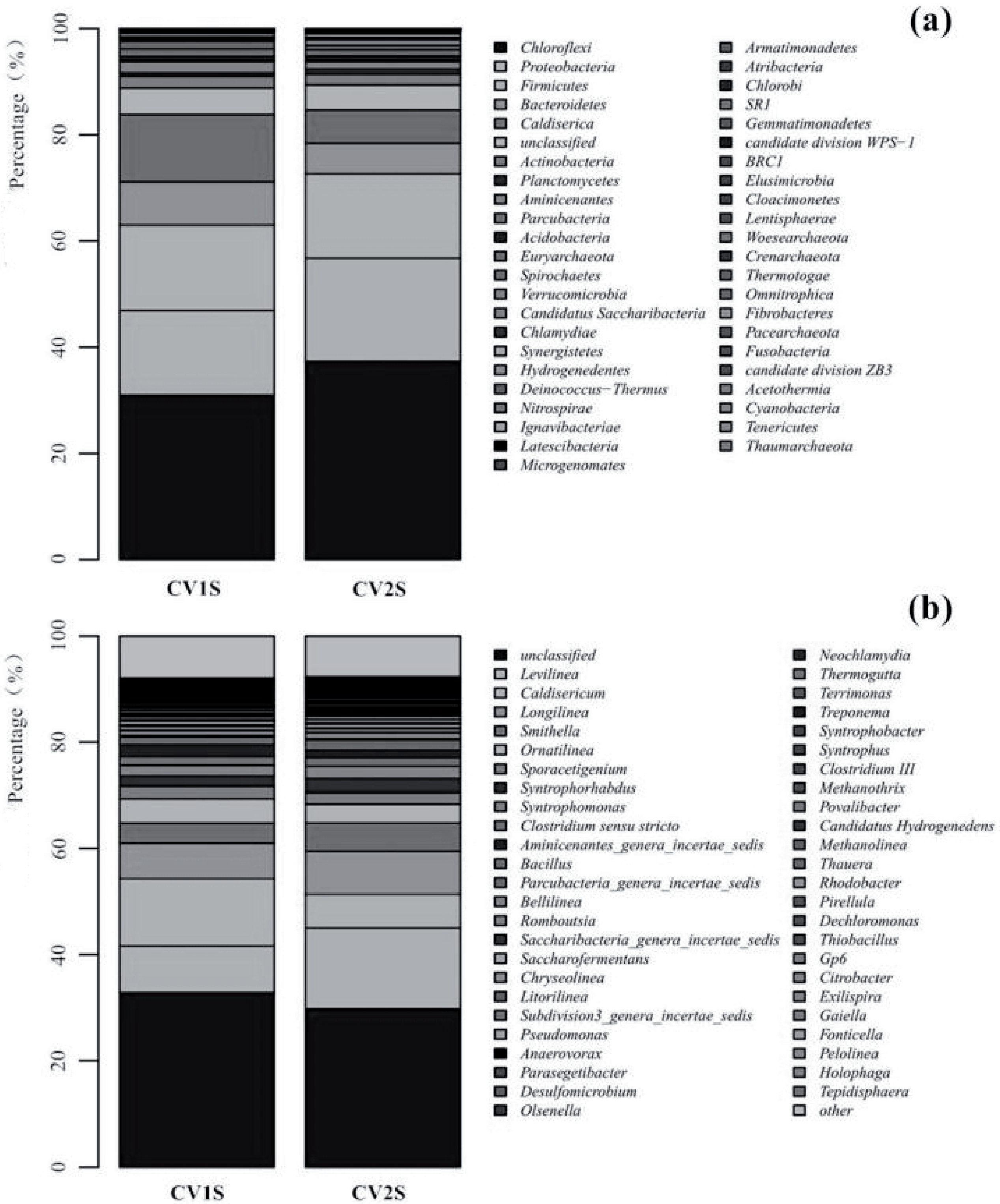

Fig. 12. Taxonomic composition of bacterial 16S rRNA gene reads at (a) phylum level and (b) genus level from CV1S, CV2S, and CV2Z samples.

of *Levilinea* and *Longilinea*. As a result, the relatively increasing abundance of *Levilinea* and *Longilinea* may accelerate sediment waste anaerobic digestion [40-43].

Conclusions

This study was undertaken to explore the ecological remediation of water bodies by ZSC and *V. natans*. The results showed that ZSC combined with *V. natans* could effectively maintain the low TP, TN, NH_3-N, COD, and Chl.a content in the overlying water. Covering ZSC promoted the growth and chlorophyll of *V. natans* and decreased the content of AsaA, MDA, and protein in *V. natans* by effectively alleviating the environmental stress of sediment. Covering ZSC could cause a relative decrease in the abundance of *Caldisericum* and the relative increasing abundance of *Levilinea* and *Longilinea*, which may accelerate waste anaerobic digestion of sediment. However, sediment capping with zeolite and shale ceramsite (ZSC) can reduce the water storage capacity, so it is unsuitable for waters with certain water depth requirements. Future research can focus on developing more efficient sediment covering materials and types of submerged macrophytes to improve the removal efficiency of pollutants such as nitrogen and phosphorus.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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