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Original Research

Decomposition of Plant Litter under Chromium Pollution and Associated Characteristics of Chromium Release

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Abstract

In vegetated constructed wetlands, plants used for phytoremediation may release pollutants back into the aquatic system as a result of decomposition after senescence, lessening wastewater disposal efficiency. After treatment of wastewater containing chromium with *Alternanthera philoxeroides* in constructed wetland, plant litter was used to study the release characteristics of chromium with the biomass decomposition under different levels of Cr pollution. Results indicate that decomposition rates of plant litter under zero and low-level Cr pollution were larger than those under high pollution concentration. Under low Cr intensity, the total Cr concentration in the residual increased in the first 40 days, and then decreased to 67.72% of the initial concentration. In the end, the residual ratios of plant litter in different pollution intensities were 57.91%, 48.16% and 71.79% of the initial mass on average separately, and about 57.45%, 67.14% and 38.32% of Cr had been released into the aquatic environment. The changes in percentages of chemical forms in residual should be correlated with the decomposition process reflected by the interactive effects. The release of Cr could be divided into two stages, i.e. immobilization and discharge. These results were possible coming from the moderating effect of different Cr intensities on microbial decomposers.

Keywords: vegetated constructed wetland, chemical forms, chromium, decomposition, Cr release

Introduction

Industrial development has caused serious environmental pollution with heavy metals, especially in developing countries [1]. Therefore, affordable and effective treatment technologies are highly desirable [2, 3]. Wetlands have been considered as structural best management practices for the treatment of municipal sewage, industrial effluents, agricultural wastewater, landfill leachate, and employing a serial of complex natural processes (e.g., substrates, vegetation and microbial activity) [3-5].

The mechanisms of pollutant removal in wetlands have been known to involve precipitation, adsorption, microbial assimilation and plant purification [6]. As one of the primary components, plants can stabilize

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hydraulic conductivity and provide huge available growth area for microorganisms, which enhances their abundance and diversity [7-9]. Furthermore, the roots of plants can directly absorb pollutants into their tissues, e.g., nutrient elements, micro-pollutants, and heavy metals [8, 10, 11].

Phytoremediation effects of wetlands on heavy metals removal from wastewater have drawn a lot of attention [1, 12-16]. Plant purification processes for wastewater containing heavy metals are influenced by several factors [17]. Plant decomposition plays a crucial role in the elements circulations in the ecosystems [18], which may drive the heavy metals return from plant residuals to water and directly determine the chemical forms of metals for plant uptake [17, 18]. With the growth and heavy metal absorption, the role of the plants in the wetland ecosystem function might change. Generally in constructed wetlands, plants for remediation germinate and grow in the spring and summer, start decay in the autumn, and are decomposed during the rest of the seasons [19]. In the other hand, plants in wetland are capable of retaining refractory pollutants in high concentrations. These accumulators can tolerate, absorb and transfer high concentrations of certain heavy metals that would be toxic to most organisms [14]. When the concentrations of heavy metals accumulated in plants exceed certain thresholds, the toxicity can lead to the organs of plants withering up or even dying [20, 21]. After natural or unnatural senescence, the retained heavy metals will be released and become another source of pollution to the water column or other parts of the ecosystem. Hence, it is necessary to take into consideration the amount of those heavy metal pollutants released back to the system while assessing pollutant retention capacities and the actual effectiveness of a vegetated constructed wetland [6, 19].

Chromium (Cr) has been recognized as one of the most toxic heavy metals to the ecological system and human health, especially Cr (VI). Chromium is widely used in alloy smelting, electroplating, tanning, dyeing, medicine, fuels and so on [22, 23]. Many studies have proven that vegetated constructed wetlands are used for the treatment of wastewater containing chromium pollutants [22-24]. As previously stated, the lack of counting the release rate of heavy metals might lead to the failure of the ecosystem to mitigate chromium efficiently. The previous studies rarely directly considered the effect of plant decomposition during the decay phase on chromium release to the environment and associated changes in pollutant forms [22-24]. In this study, we explore the release rate of chromium by plants after remediation, and examine the changes in chemical forms of chromium. These results are imperative for characterizing the chromium dynamics of a constructed wetland system during plant dormancy, and providing a better understand of the role of vegetation in wastewater treatment in vegetated constructed wetlands.

Materials and Methods

Focal Species and Study Sites

As one of the most detrimental invaders, the amphibious perennial herb *Alternanthera philoxeroides* (Mart.) Griseb (Amaranthaceae) is widely distributed in aquatic (e.g., rivers, lakes, canals) and terrestrial habitats (e.g., crop lands, lawn) and junctions (e.g., river banks) in tropic and subtropical areas [15, 16]. *A. philoxeroides* has developed fibrous roots, high tolerance and accumulation ability to many environmental stress factors, including the heavy metal chromium [25]. Thus, it can be used as a purifying plant in a constructed wetland.

On 21 June 2014, clonal fragments of *A. philoxeroides* were collected along the banks of the Moshui River in Qingdao, Shandong Province, China. The plants were transplanted and propagated in a greenhouse located at Qingdao Agricultural University, China. In the greenhouse, the mean diurnal temperature during the experiment was 25ºC and the average humidity was 40%.

Experimental Design

From May to August 2016 we conducted a series of greenhouse experiments on the artificial wastewater treatment containing chromium with *A. philoxeroides.* Original sewage was collected from Chengyang sewage treatment plant in Qingdao, China. Cr was added as a dichromate solution ($K_2Cr_2O_7$, AR, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) into the original sewage at different doses to simulate a chromium-polluted water environment.

A follow-up decomposition experiment was carried out from August to December. The cleaned fragments were separated into aboveground (leaves and stems) and underground (roots) parts, and cut into pieces about 3~5 cm. Some separated plant litter was dried to a constant weight. The water on the surface of the other fresh litter was dried with tissues.

For determining the decomposition rate, a batch of dried litter of 5 g was weighed and placed into 15×10 cm nylon bags of 5-mm mesh size. For the measurement of total Cr concentration and the forms of Cr, a batch of fresh litter of 5 g was disposed of in the same way. One bag of dried litter and one bag of fresh litter were put into a one-liter beaker at the sediment-water interface for the decomposition experiment. The beaker was filled with wastewater mixed with original sewage from a treatment plant and water from constructed wetland without Cr pollution in accordance with the proportion of one-to-one. Different doses of Cr were added as the dichromate into the wastewater and mixed thoroughly to simulate the polluted decomposition environments. A previous observation found that 1 mg L^{-1} Cr pollution had no obvious damage on plant, but 10 mg L^{-1} Cr pollution induced moderate to heavy damage to plants over a few days. The three Cr concentrations were 0 for the control, 1 mg L^{-1} for the low-level pollution, and 10 mg L^{-1} for the high-level pollution, respectively. Each treatment was repeated three times. During the experiment, the water in the beaker was maintained at one liter. The pH values were measured three times at the beginning, in the middle and at the end of the experiment.

Every 10 or 20 days, three bags of dried litter and three bags of fresh litter in each treatment were collected for measuring mass decomposition, total Cr concentration in the residual tissues and forms of Cr. The whole experiment lasted for 100 days. All the litter was carefully washed after collection. All the dried litter and half of the fresh litter were dried in a stove at 70ºC for 48. The mass of remaining dried litter was weighted. Dry samples of the originally fresh litter were ground with a ball mill (DECO-PBM-V-4L, Changsha, Hunan Province, China) and dried to a constant weight. The homogenized samples were put into Teflon crucibles (effective volume 25 ml) and mixed with 8 ml H_2O_2 (30%, GR, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) / HNO_3 (65%, GR, Nanjing Chemical Reagent Co., Ltd, Nanjing, China) at a ratio of 1:4 (*v/v*). After eight hours, the crucibles were sealed with steel cans and heated at 80°C for one hour. After the steel cans cooled, the crucibles were taken out and heated on an electrical heating panel (MWJ-3020, Wuxi, Jiangsu Province, China) at 120ºC for two hours to remove excess acid from the solution. Cr concentrations in the extracts were tested using inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 8000, Perkin Elmer, Massachusetts, the USA). The operational parameters of ICP-OES were as follows: the plasma gas flow was 15 L/min, the auxiliary gas flow was 0.2 L/min, the flow of atomizer was 0.6 L/min, the radio-frequency power was 1300 W, the injected quantity of sample was 1.5 ml/min, and the analyzing spectrum line was selected as 267.716 nm. The standard solution of Cr (1000 mg/L, National analysis and testing center of nonferrous metals and electronic materials, Beijing, China) was diluted stepwise by 2% HNO_3 into 0.0, 0.5, 1.0, 2.0, 5.0 and 10.0 mg/L. The blank solution was measured a score of times continuously, and the triple standard deviation of corresponding measurement result was used as detection limit (0.36 μg/L).

For determining chemical forms of Cr, the method of sequential extraction was applied with following solutions in turn: 80% ethanol, deionized water, 1 mol L^{-1} sodium chloride solution, 2% acetic acid solution and 0.6 mol L^{-1} hydrochloric acid solution. The ethanol extracted Cr in the forms of inorganic salt (mainly composed of nitrate and chlorate) and amino acid salt (represented by F1 hereafter). The deionized water extracted Cr in forms of water-soluble acylate and dihydric phosphate (represented by F2 hereafter). The sodium chloride solution extracted Cr in forms of pectate, and protein binding or adsorption (represented by F3 hereafter). The acetic acid solution extracted Cr in forms of water-insoluble phosphate, including hydrophosphate and orthophosphate (represented by F4 hereafter). The hydrochloric acid solution extracted Cr in forms of oxalate (represented by F5 hereafter). The remaining Cr was in residual form (represented by F6 hereafter) [26]. For each extracting step, one type of the extracting solutions mentioned above was used. The remaining fresh litter was put into a 100-ml centrifuge tube and submerged by 50 ml extracting solution. Then the centrifuge tube was placed in a constant temperature incubator shaker (SHA-B, Tianjin, China) and shaken for 12 hours under 30ºC. Next, the litter was extracted for two hours using the same 30 ml solution twice. All the extracting solutions were collected together and boiled on the electrical heating panel (MWJ-3020, Wuxi, Jiangsu Province, China) to nearly dry. Finally, the remaining solution was transferred to a 25-ml volumetric flask, diluted with deionized water to volume, and mixed [27]. Cr concentrations were tested using the same test instrument and method.

Data Processing and Analysis

The residual mass of dry litter and associated decomposition coefficient were used to describe the decomposition rate. Mass decomposition variation was fitted to the simple exponential model [29]:

$$X_t = X_0 e^{-kt}$$

…where X_t is the residual mass of dried plant litter (g), X_0 is the initial mass of dried plant litter (5 g), *e* is the natural log constant, *k* is the decomposition rate constants (day^{-1}, d^{-1} in short hereafter) and *t* is the decomposition time (day, d in short hereafter).

The percentage of Cr released from plant litter to water was calculated as follows:

$$\text{Percentage} = (X_0 C_0 - X_t C_t) / X_0 C_0$$

…where C_t is the total Cr concentration retained in plant litter of each period (mg kg^{-1}), and C_0 is the initial total Cr concentration before treatment (mg kg^{-1}).

Two-way ANOVA was used to test for the effects of different Cr concentrations and decomposition time on the mass decomposition rate, total Cr concentration and chemical forms composition of Cr. One-way ANOVA with the Tukey test method was used to test the initial variations in different traits under different intensities of Cr pollution, separately. SPSS 21 (IBM Inc., Armonk, New York, USA) was used for all of the statistical analyses. $P<0.05$ was used as the significance level. The regression equations in the figures were generated using Sigmaplot 12.5 (Systat Software Inc., Erkrath, Germany).

Table 1. Results of two-way analyses of variance (ANOVA) for effects of different Cr concentrations (C), decomposition times (T), and their interactions (C×T) on residual mass, total Cr concentration, released Cr amount and different chemical forms of Cr in plant litter

Effect	Residual mass	Total Cr	Released Cr amount	F1	F2	F3	F4	F5	F6
C	238.13***	0.06^{ns}	25.01***	69.51***	14.44***	51.51***	1087.82***	118.15***	518.68***
T	197.48***	7.19***	72.86***	86.10***	18.61***	20.45***	16.43***	20.26***	16.58***
C×T	5.20***	0.50^{ns}	1.98*	1.84^{ns}	0.37^{ns}	4.24***	32.47***	1.33^{ns}	4.88***

F values and significance levels are given ($^{***}P<0.001$, $^{*}P<0.05$, $^{ns}P>0.05$).

Results

Plant *A. philoxeroides* is good for the purification of sewage containing Cr in the constructed wetland. However, the fallen leaves or the senescent ramets may return the retained Cr back into the wetland, which would create endogenous pollution conditions in receiving waters. On the whole, the residual mass decreased over time (Table 1; Fig. 1). From the beginning to 60 days, the plant litter decayed at a relatively rapid speed. After 60 days, the decomposition process entered a relatively slow phase (Table 1; Fig. 1). The decomposition rate constants (d^{-1}) of plant litter under 0, 1 and 10 mg L^{-1} Cr pollution were 0.0056, 0.0074 and 0.0033 d^{-1}, respectively, suggesting that different intensities of Cr pollution had various effects on the decomposition process (Table 1; Fig. 1). High levels of Cr pollution inhibited the decomposition of plant litter, but the low concentration acted conversely (Table 1; Fig. 1). Twenty days later, the residual mass of plant litter under low-level pollution (4.03±0.04 g) was significantly smaller than that under high-level pollution (4.58±0.10 g; P = 0.001). For decomposition without pollution, the significant difference in residual mass with high-level pollution appeared after 30 days (3.88±0.02 g *v.s.*4.35±0.09 g, P = 0.011). At the end of the experiment, the residual ratios of plant litter in different pollution intensities were 57.91%, 48.16% and 71.79% of the initial mass on average separately (Fig. 1), which showed significant differences (F = 141.26, $P<0.001$). Theoretically, the decomposition periods of 50% litter under different Cr levels were approximately 124, 94 and 210 days, and the decomposition periods of 99% litter were 822, 622 and 1396 days. The pH of original wastewater under 0, 1 and 10 mg L^{-1} Cr were 7.84, 7.71 and 7.78, separately. With time, the pH of different treatments decreased to 7.45, 7.47 and 7.41 in the middle of the experiment. In the end, the pH stabilized around 7.52, 7.56 and 7.48.

The total Cr concentration and related changes in percentages of chemical forms in plant litter should be correlated with the decomposition process reflected by the interactive effects (Table 1; Figs 2-4). There was no significant difference in total Cr concentrations between decay phases of plant litter suffering from zero and high-level Cr pollution (Fig. 2). The concentration of Cr retained in plant litter under low-level Cr pollution increased in the first 40 days, and then decreased to be 67.72% of the initial values (423.43±27.08 mg/kg *v.s.* 625.24±58.33 mg/kg), which was distinctly lower than the increasing phases (Fig. 2). In the end, the total Cr concentrations showed no significant difference between

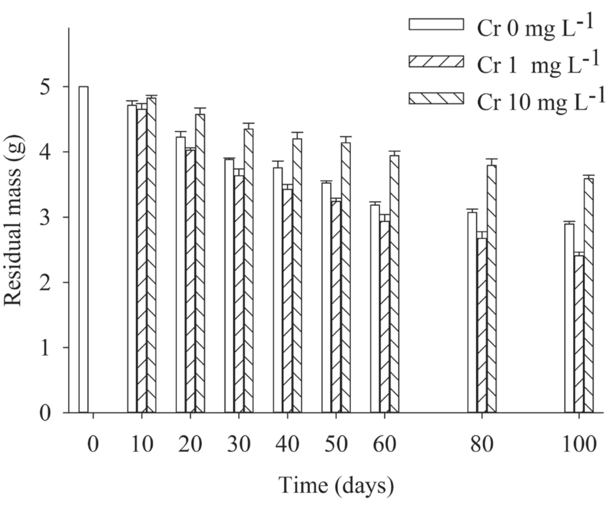

Fig. 1 Variations in residual mass of plant litter under different levels of Cr pollution over decomposition time. Data are mean± SE without transformation.

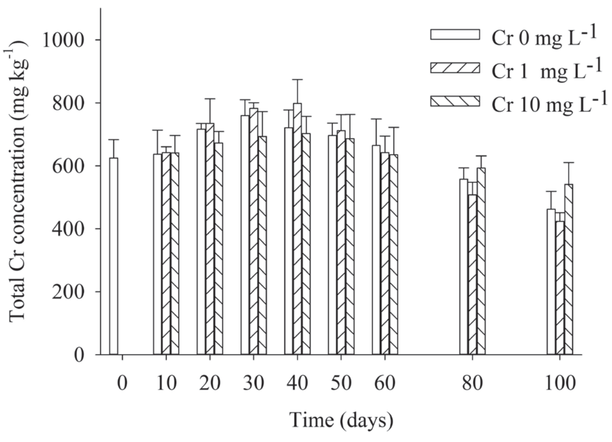

Fig. 2 Variations in total Cr concentration of plant litter under different levels of Cr pollution over decomposition time. Data are mean± SE without transformation.

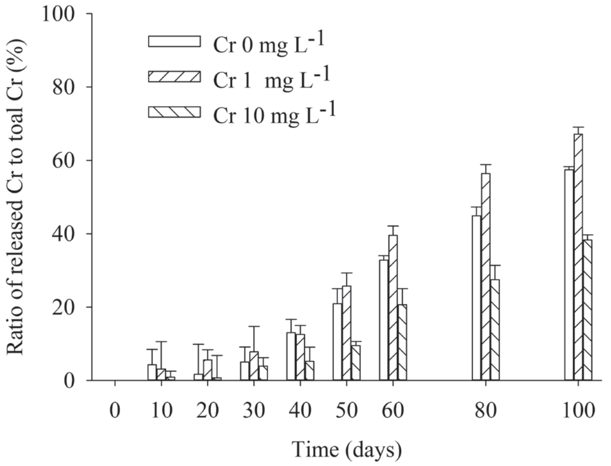

Fig. 3 Variations in ratio of Cr released into surroundings to the initial values of plant litter under different levels of Cr pollution over decomposition time. Data are mean± SE without transformation.

different Cr pollution treatments ($P = 0.355$; Fig. 2). The final Cr concentrations of all the treatments also had no difference with the initial values ($P = 0.122$; Fig. 2).

With the decomposition of plant litter, Cr fixed by plants was released to the water (Fig. 3). Though the decomposition process of litter was at a relatively rapid speed at the beginning, the release of Cr to water was relatively few (Figs 1 and 3). After 50 days, the release rate significantly accelerated (Fig. 3). In the 100 days, about 57.45%, 67.14% and 38.32% of Cr in 0, low-level and high-level pollution were liberated, which showed obvious significance ($P<0.001$; Fig. 3), suggesting that more than most of the Cr in 0 and low-level treatment was returned to the environment.

Before the decomposition treatment started, the chemical forms 6, 1 and 2 accounted for the major proportions (33.93%, 24.49% and 18.41%), while the other three chemical forms took up relatively small proportions (Fig. 4). With the decomposition process of plant litter, the proportions of each chemical form also changed gradually (Table 1; Fig. 4). The directions of chemical form variations differed between Cr pollution intensities (Table 1; Fig. 4). Furthermore, significant interactive effects of Cr pollution intensity and decomposition time were found for F3, F4 and F6 (Table 1; Fig. 4). In all three pollution treatments, F1 and F2, representing the easily soluble reactive forms, reduced rapidly as the decomposition process (Fig. 4). For F1, the percentages decreased by 88.27%, 80.81% and 65.28% in 40 days and by 94.51%, 85.84% and 82.98% in 100 days (Fig. 4). For F2, the percentages decreased by 53.49%, 51.23% and 31.34% in 40 days and by 62.61%, 61.06% and 43.99% in 100 days (Fig. 4). Overall, the F3 of plant litter in zero pollution decreased after 50 days ($P = 0.001$), litter in low-level pollution showing reduction after 40 days ($P = 0.002$), and the reduction of litter in high-level pollution happened in 80 days ($P = 0.004$). There were no differences in F4 values of plant litter in zero and low-level pollution, but the values of high-level increased by large scale (Fig. 4). F5 of the three pollution treatments increased from 7.17% to 15.63%, 13.88% and 25.87% separately (Fig. 4). The concentration of F6, the residual form, was determined by the concentrations of the other chemical forms. The F6 values of zero and low-level pollution were nearly double that of the initial proportion, but the values of high-level pollution were reduced by nearly 60% (Fig. 4).

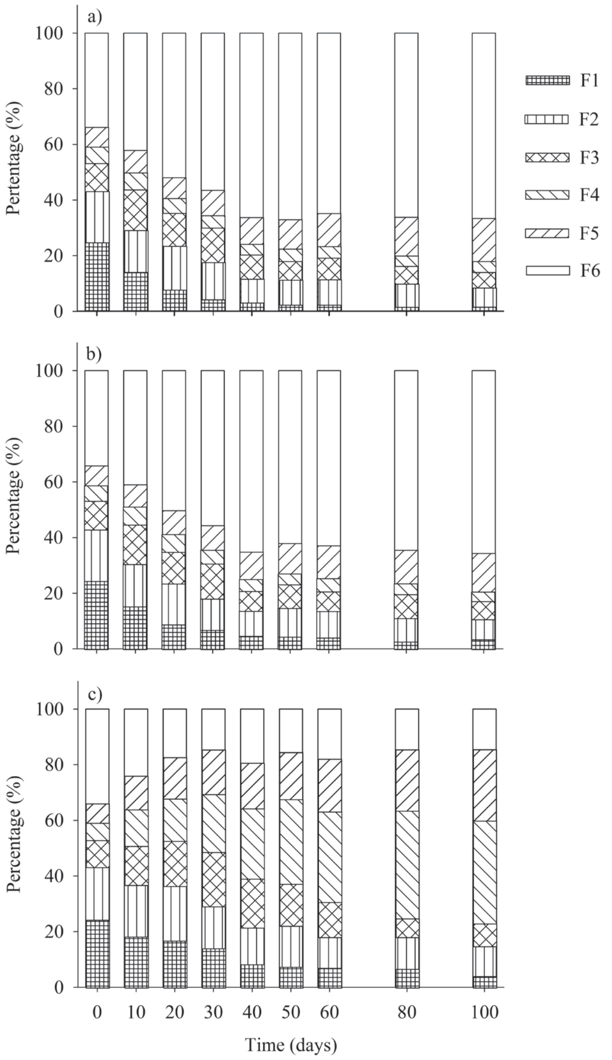

Fig. 4 Variations in the distribution of different Cr chemical forms of plant litter under 0 a), 1 mg/L b) and 10 mg/L c) of Cr pollution over decomposition time. The chemical forms of Cr were represented by different patterns of bars.

Discussion

As an integral section of vegetated constructed wetland, plants play an important role in the removal of various pollutants in the wetlands, e.g. organics, nitrogen, phosphorus, heavy metals, and so on [1, 19, 30-32]. Pollutant (such as nitrogen, phosphorus and heavy metals) storage in plant tissue is a saturable process, indicating that plants can return the retained nutrients or heavy metals back to the surrounding environment during decomposition [19, 30]. Plant litter decomposition plays a crucial role in ecosystem elements cycling. However, most research has focused on the leaching of nutrients after plant dieback, which would make eutrophic conditions in receiving an aquatic environment during the winter months (from November to January). There is little quantitative research available comparing plant growth characteristics, treatment performances on heavy metals and associated decomposition process in constructed wetland ecosystems [24]. By conducting a decomposition study on plants after remediation, we were able to investigate the differential release characteristics of Cr in plant litter.

Previous studies had proven that the decomposition process of litter and release of nutrients to an ecosystem were characterized by obvious stages [24, 30, 33]. In this study, the decomposition of plant litter exhibited relatively high speed in 60 days, and the whole decomposition period may last for a long time theoretically, even a few years. As is known to all, a number of environmental factors are crucial for controlling the litter decomposition process in contrasted wetlands, e.g. plant species, hydrological conditions, pH, temperature, nutrient content, pollutants, and microbial, fungal, invertebrate and other decomposer communities [33, 34]. The effects of heavy metals on the decomposition and release process were rarely reported. In practice, the microorganism in the wetland substrate has a great influence on the decomposition rate of plant litter, and different kinds of microorganisms play different roles in the decomposition process. Normally, bacteria mainly control the decomposition rate, which results in an approximate 90% loss of litter mass, and fungus chiefly breaks down the litter by changing their chemical and physical characteristics [35]. The intrinsic degradability could be modulated by external environmental factors [33]. For instance, in nutrient-rich ecosystems, high nutrient availability may increase the abundance of decomposers, change the composition of decomposer communities and promote their activity of decomposers, resulting in a high decay rate [33, 36, 37]. On the contrary, high concentrations or long-term stress of heavy metals has an adverse influence on the growth and metabolization of microorganisms, even making microorganisms lose the function of bioremediation [38, 39]. Compared with relatively lower concentrations of Cr such as 50, 100 and 150 μg/ml, the growth of bacterial isolates was slightly inhibited at Cr concentration of 200 μg/ml and highly inhibited at 500 μg/ml in 96 hours [38]. It was also suggested that different exposure times might result in different toxicities to bacteria [39]. Our results showed that the decay rate in low-level pollution was faster than those in control and high-level Cr concentration (Table 1; Fig. 1), implying that low Cr intensity may have a promotive effect on the growth of microorganic communities, but performances of communities including the activity, abundance or community composition were restrained under high-intensity stress. On the whole, the pH of decomposition environment decreased over time, possibly resulting from the accumulation of Cr in water and an increase in microorganisms.

The decomposition rate of plant litter varied with the Cr content of the wetlands, illustrating the importance of environmental conditions during the decomposition process. Accordingly, the total Cr concentrations retained in the plant litter changed in response to the mass loss and associated outside conditions. Faster reduction in residual mass of litter under low-level pollution caused a slight increase in total Cr concentration in the first 40 days. As the intensity of decomposition increased, the chromium content gradually decreased to be lower than the initial values. It is assumed that the increase in mass loss is related to the positive effects of low-level pollution on decomposers [24, 33]. According to the results, the release of elements could be divided into two stages, the chemical and/or physical immobilization at an early stage (i.e., Cr is accumulated in the residuals) and discharge at the next stage (i.e., mobilization to surrounding terrestrial and aquatic environments).

Presently, research on chemical forms of heavy metals primarily concern the sediment in constructed wetland, but few pay attention to the forms in plant residuals [1, 24]. The significance of chemical forms of heavy metals is the variation in biological effectiveness, which has potential risk for sewage treatment efficiency and ecosystem health. In original plant litter, the chemical forms 6, 1 and 2 accounted for the major proportions while forms 3, 5 and 4 took up a minor percentage (Fig. 4). Forms 1 and 2 are the most active parts in chromium rank, and percentages of forms 6 and 4 were smaller than the other forms [27]. In this study, we found that forms 1 and 2 were easily lost as the decomposition process. High activity made the transformations and chemical transport into water processes of F1 and F2 easily. Compared with F1 and F2, the biological activity and solubility of F4 and F6 were relatively low. Under high-level pollution, the percentage of F4 increased and F6 decreased, but the sum of F6 and F4 had no significant difference. Though high-level Cr stress restrained performances of microorganisms, it did not improve the activity of Cr in the residual. It was also possible that the plant residual can adsorb Cr from the surroundings and be stored in the tissues, which affected the significant performances between the chemical forms of plant litter suffering from different levels of Cr pollution.

Conclusions

Environmental quality had a strong effect on the decomposition process of plant litter in the constructed wetland. In the zero and low-level Cr pollution environment, decomposition of plant litter was faster compared with that under high Cr intensity. Furthermore, high-level Cr concentration can also lead to lower leaching speed of Cr into the surrounding aquatic environment, but it would not increase the activity of Cr retained in the residual. The release of Cr could be divided into two stages: the chemical and/or physical immobilization and discharge. These performances were possibly achieved by the moderating effect of different Cr intensities on microbial decomposers. The results obtained from this study are useful for the design and management of efficient constructed wetlands, and contribute effective information to the database on heavy metal release with litter decomposition.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

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