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Enrichment of heavy metals in coal gangue by puff balls and mechanism research

CHEN Miao[1], ZHU Lijun[2], WU Yonggui[1], FU Tianling[1], and RAN Yan[3]
[1] *College of Resource and Environmental Engineering, Guizhou University, Guiyang 550025, China*
[2] *Department of Land and Resources of Guizhou Province, Guiyang 550001, China*
[3] *Guizhou Academy of Instrumental Analysis, Guiyang 550001, China*
[*] *Corresponding author, E-mail: chenmaio7879@126.com*

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Abstract Coal gangue with/without wild puff balls growing on or covering up were analyzed by means of inductively coupled plasma atomic emission spectrometry (ICP-AES) and atomic fluorescence spectrometry (AFS) to detect the contents of heavy metal elements in the plants. Seven heavy metals were chosen to do the experiments, which showed that the puff balls have strong enrichment capacity for heavy metals (As, Pb, Mn and Ni). The highest enrichment level was Mn, 220.3 mg/kg (dry weight) for puffball fruiting bodies, and the highest As, Cr and Pb enrichment levels for puffball mycelium were 111, 265.3 and 86 mg/kg, respectively. Cd, Mn and Ni could transfer from puffball mycelium to its fruiting bodies, while As, Cr, Pb and Co mainly accumulated in the mycelium. The contents of five organic acids (tartaric acid, glycolic acid, lactic acid, citric acid and malic acid) in puffballs were determined with high performance liquid chromatography. With water, ethanol and ethyl acetate as solvents, the puffball extracts performed dissolution reactions with coal gangue, in which the water extract (containing a large amount of organic acids) was most effective in releasing heavy metals, especially As, Pb, Cr, Mn, Co and Ni from coal gangue. As a pioneer species, puff balls absorbed the hazardous trace elements in coal gangue matrix to reduce its eco-toxicity, on the other hand, it raised the pH value of coal gangue, improving the vegetation growing conditions for coal gangue matrix to a certain extent and laying the foundation for ecological restoration of coal gangue dumps.

Key words Puff balls; coal gangue; heavy metal; absorption; enrichment

1 Introduction

Coal gangue is the solid waste in coal production and preparation. The coal gangue yield is 10%–30% of the raw coal. According to incomplete statistics, the annual gangue discharge is 1.5×10^8–2.0×10^8 tons in China (Su et al., 1998). Though part of the coal gangue is recycled as building materials and fuel, its industrial utilization rate is less than 5% (Wang and Lin, 2000). Therefore the disposal leads to various environmental problems, such as land occupation, decline of soil productivity, landscape devastation, and pollution of atmosphere, water and soil.

The treatment of coal gangue dumps has become a priority issue for the environmental improvement in coal mining area (Guo et al., 1998). The high sulfur contents in coal gangue are prone to produce acid after weathering (Chen and Zhang, 2000), while low contents of nutrient elements inhibit the growth of vegetation (Wu et al., 1998). The pot experiments conducted by the research group led by Bi et al. (2005) showed very low germination rate of the crops in coal gangue matrix. The crops were not able to survive even if they germinated. A new thought of biological comprehensive treatment was proposed by Bi et al. (2005), namely, the optimal treatment for coal gangue was land reclamation and planting, to make it resources available and improve the ecological environment of mining area.

Zhang (2001) put forward that the priority of ecological rehabilitation of coal gangue dumps lies in the improvement of coal gangue to be special growth substrate. Coal gangue is characterized by extremes of the pH scale, poor water and fertilizer reservation, high erodibility and poor soil microbial activity, making it not suitable for crop growth. Thus, the application of microbial technology for gangue matrix amelioration and vegetation restoration, has become an important approach to improve the ecological environment of coal mining area as well as the matrix fertility. To adopt artificial microbial inoculation is to take advantage of microbe activity to develop the potential fertility of coal gangue dumps and to help its ecological reconstruction.

Bi et al. (2006) conducted biotic reclamation of coal gangue abandoned matrix by inoculation of mycorrhizal fungi and rhizobium. The experimental results showed that the plant roots could improve the physical and chemical properties of the matrix and increase its pH value, which makes plant growth at the suitable pH scale. Mycorrhizal fungi and rhizobium can form a good symbiotic relationship with plants and promote the plants' uptake and utilization of insoluble P in the solid waste of coal mining area. Hu et al. (2009) found that sulfate-reducing bacteria can make effective control on sulfuric contamination caused by coal gangue. The sulfate is reduced in this process and the rate of sulfate reduction reaches 95.5%. The sulfate-reducing bacteria can effectively remove sulfate of acid mine drainage from coal gangue, increase pH value, decrease redox potential, acid producing production potential and salt concentration, so as to inhibit the production of acid mine drainage from coal gangue and remediate the acid leaching caused by sulfur-containing coal gangue on the condition of rainfall and relevant environmental pollutions. It was found by recent study that thiobacilli has good desulfurization effect, though it causes the acidification of coal gangue dumps. $Fe(OH)_3$ precipitates out from water, deposits on river bed and forms a layer of orange sludge, which can almost kill all the aquatic organisms growing in river bottom.

Since the 1970s, the bioaccumulation of heavy metals in macrofungi has been gradually appearing in the spotlight. The research showed that compared with green plants, macrofungi can accumulate high concentrations of heavy metals such as Cd, Pb and Hg (An and Zhou, 2007). The international studies focused on the heavy metal contents of fruiting bodies of some species of wild macrofungi and the bioaccumulation characteristics of heavy metals in fruiting bodies. For example, the Cd content was 100–300 mg/kg (dry weight) in Agaricus macrofungi, Pb 100–300 mg/kg (dry weight) in various species of macrofungi growing around Pb smelters, and Cu 100–300 mg/kg (dry weight) in some species of macrofungi (Zhou et al., 2008). In China in recent years, the studies put emphasis on the bioaccumulation characteristics of heavy metals in macrofungi under artificial conditions. For example, Lei and Yang (1990) and Shi et al. (1991) studied the enrichment of Cu, Zn, Pb and Cd added in composts during the cultivation process of *Lentinus edodes*, *Agaricus bisporus*, *Auricularia auricular*, *Pleurotus sajor-caju* and *Flammulina velutipes*. Huang et al. (2007) studied the enrichment of Hg, As, Pb and Cd in fruiting bodies of Agaricus blazei Murrill during cultivation. Although the heavy metal enrichment of macrofungi in artificial cultivation was studied by most reports, there is seldom research on wild macrofungi growing in coal gangue matrix with heavy metal enrichment capacity.

A large amount of wild puff balls has been found growing in a large coal gangue dump in Huaxi District, Guiyang City, central Guizhou Province. Puff balls with a wide variety are commonly known as Niushigu, mainly include members of genera Lycoperdon, Bovista, Bovistella, Calvatia, Mycenastrum and Disiciseda, belonging to the class Agaricomycetidae, the order Agaricales and the family Lycoperdaceae. Puff balls are usually found after rain in late summer or autumn, appearing in solitary, scattered and tufted types. To further explore the adversity-adapting/resisting potential of biological gene resources, and to improve the ecological environment of coal mining area, puff ball and coal gangue samples were collected and related indexes tests and laboratory simulation were conducted. It was supposed that the research results can provide basic data contributing to the ecological restoration of coal gangue dumps.

2 Materials and methods

2.1 Sample collection

Puff ball fruiting bodies and mycelium growing on coal gangue, puffball-covered coal gangue (biotic coal gangue) and surrounding coal gangue without puff balls (control coal gangue) were collected in a large coal gangue dump of Maiping mining area in Huaxi District of Guiyang City in November, 2012 (Fig. 1). The diameters of puffball fruiting bodies range from 2–6 cm.

2.2 Experimental methods

2.2.1 Heavy metal tests

In accordance with trade standards JY/T015-1996, Cd, Cr, Mn, Ni, Pb and Co were determined by using an inductively coupled plasma atomic emission spectrometry (iCAP6300 Radial, ICP-AES, Thermo Fisher Scientific). As was determined by the atomic fluorescence spectrometry (AFS-3100, Beijing Kechuang Haiguang Instrument Co., Ltd) according to national standard test methods GB/3058-2008. The results were mean values of the tests.

2.2.2 Organic acid tests

According to the high performance liquid chromatography method of national standards (Determination of Organic Acid in Foods, GB/T 5009.157-2003), the test procedures involved in sample homogenate extraction and centrifugation, 0.3 μm membrane pore filtration of sample solution, $(NH_4)_2HPO_4$-H_3PO_4 butter solution at pH 2.7 as mobile phase, separation on C18 chromatographic column with a high performance liquid chromatography (Agilent1100, USA), 210 nm ultraviolet detection, and quantitative determination of organic acid by calibration curve.

3 Results

3.1 Heavy metal contents in puff ball fruiting bodies and mycelium

The contents of heavy metal elements in puffball fruiting bodies and mycelium (wet weight) were shown in Table 1. The contents of Cd, Mn and Ni in fruiting bodies were higher than those in mycelium. The contents of As, Pb, Cr and Co in fruiting bodies were lower than those in mycelium. There were high contents of Mn, Cr and As in fruiting bodies with the mass percent of 0.0048%, 0.0015%, and 0.0011% respectively. There were high contents of Cr, As, Pb and Mn in mycelium with the mass percent of 0.0058%, 0.0024%, 0.0019%, and 0.0011% respectively. Based on the tests, the moisture content of puff balls was 78.1%. Converting the heavy metal contents in wet weight into the contents in dry weight, the enrichment content of Mn in puffball fruiting bodies reached 220.3 mg/kg (dry weight), the enrichment contents of As, Cr and Pb in puffball mycelium were 111, 265.3 and 86 mg/kg respectively. Compared with the enrichment content of heavy metals in wild macrofungi ranging from 100–300 mg/kg by overseas studies (Zhou et al., 2008), puff balls showed a high capacity of heavy metal enrichment.

3.2 pH value and heavy metal contents in coal gangue

The heavy metal contents in two types of coal gangue, as well as the decreased percent of heavy metal contents in the biotic coal gangue compared with those in the control coal gangue were listed in Table 2. Based on the data in Table 2, it can be indicated that the percent of As and Ni contents in the biotic coal gangue decreased obviously. It can be seen from Table 3 that one of the reasons for the largely decreased percent is the intensive absorption and enrichment of As and Ni by puff balls.

Fig. 1. Puff balls and puff ball-covered coal gangue.

Table 1 Heavy metal contents in puff ball fruiting bodies and mycelium ($\mu g \cdot g^{-1}$)

Sample	Statistic	As	Pb	Cr	Cd	Mn	Co	Ni
Puff ball fruiting body	Mean	11.330	7.100	15.342	0.356	48.236	1.451	7.514
	Standard deviation	0.282	0.068	0.340	0.011	0.241	0.037	0.045
	Mass percent (%)	0.0011	0.0007	0.0015	0.00004	0.0048	0.0002	0.0008
Puffball mycelium	Mean	24.302	18.842	58.096	0.354	10.548	5.701	3.792
	Standard deviation	0.142	0.153	0.616	0.011	0.148	0.050	0.075
	Mass percent (%)	0.0024	0.0019	0.0058	0.00004	0.0011	0.0006	0.0004

Table 2 pH value and heavy metal contents in coal gangue (μg·g^{-1})

Sample	Statistic	pH	As	Pb	Cr	Cd	Mn	Co	Ni
Control coal gangue	Mean	3.190	7.710	5.087	102.840	1.097	25.610	35.722	5.131
	Standard deviation	0.044	0.040	0.018	0.594	0.010	0.081	0.110	0.026
	Mass percent (%)		0.0008	0.0005	0.0103	0.0001	0.0026	0.0036	0.0005
Biotic coal gangue	Mean	3.830	6.121	5.040	102.400	1.041	25.224	35.490	3.724
	Standard deviation	0.040	0.029	0.068	0.718	0.002	0.080	0.099	0.038
	Mass percent (%)		0.0006	0.0005	0.0102	0.0001	0.0025	0.0035	0.0004
Decreased percent of heavy metal contents in biotic coal gangue (%)			20.610	0.921	0.430	5.105	1.510	0.650	27.420

3.3 Enrichment and translocation of heavy metals in coal gangue by puff balls

The enrichment of heavy metals in coal gangue by puff balls can be evaluated by the following two indexes: element content (mg/kg) and enrichment factor (ET, e.g. the ratio of the element content in microbe to the element content in matrix). The higher the EF value, the higher the enrichment capacity of the element (Liu et al., 2011; Zhang et al., 2010). The transport and migration of heavy metals in coal gangue by puff balls can be evaluated by translocation factor (TF). Translocation factor is the ratio of element content of overground part of plants to that of the underground part, mainly used to evaluate the pollutant transport capacity of plants from underground part to overground part (Huang et al., 2011). The enrichment coefficients and transfer coefficients were calculated based on the data in Tables 1 and 2, and the results can be seen in Table 3.

It can be seen from Table 3 that puff balls have a strong enrichment capacity of heavy metal As, Pb, Mn and Ni. The enrichment of heavy metals by fruiting bodies from strong to weak is in this order: Ni>Mn>As>Pb>Cd>Cr>Co. Meanwhile, the enrichment of heavy metals by mycelium from strong to weak: As>Pb>Ni>Cr>Mn>Cd>Co. The heavy metal Cd, Mn and Ni can transfer from puffball mycelium to its fruiting body, and As, Cr, Pb and Co mainly accumulate in mycelium. No matter where heavy metals exist in microbe, the remediation of matrix always begins when mycelium absorbing and releasing active molecules. Thus, the response of macrofungi mycelium to heavy metals is essential for the ecological restoration function of macrofungi to heavy metals.

3.4 Organic acid content in puff balls

It can be seen from Table 3 that puff balls can enrich and transfer heavy metals, meanwhile macrofungi can excrete organic acids and other metabolites to dissolve heavy metals and minerals containing heavy metals (Chanmugathas and Bollag, 1988). The organic acid contents in puff ball samples were analyzed and the results can be seen in Table 4, which indicates that the total organic acids in puffball fruiting bodies is higher than that in mycelium; expect for tartaric acid, the contents of other organic acids in fruiting bodies are higher than those in mycelium.

3.5 Dissolution of heavy metals from coal gangue

With solvents of different polarities, puffball extracts performed dissolution reactions with heavy metals in coal gangue collected from Xingren County, Guizhou Province. Water, ethanol and ethyl acetate were selected as solvents. 10.5 g puff balls was divided into three equal portions, and under the condition of 1:10 solid-liquid ratio suspended in 35 mL deionized water (No. 1), 35 mL ethanol (95% analytical reagent, No. 2) and ethyl acetate (99.5% analytical reagent, No. 3) respectively. At 26℃ and 130 r/min rotational speed of constant temperature oscillator, after 16 h oscillation and static centrifugation (3000 r/min, 10 min), supernatants were collected and added with 0.5 g coal gangue, then diluted to 250 mL. To make a control solution sample, 0.5 g coal gangue was directly added, dilution with deionized water to 250 mL (No. 4), with 4 h continuous oscillation and static centrifugation, then the supernatant was collected. The dissolution effects of heavy metals from coal gangue can be seen in Table 5.

It shows in Table 5 that the changes of pH value before and after experiments are not obvious. The water extract of puff balls was the most effective in the release of heavy metals from coal gangue, especially when releasing As, Pb, Cr, Mn, Co and Ni from coal gangue. The ethanol extract has a strong dissolution effect for As, Pb, Cr and Ni from coal gangue, while ethyl acetate extract has a strong dissolution effect for Pb and Ni. The dissolved concentrations of Cd by all the tested solvent extracts were extremely low. The highest dissolved concentration of Cd by water extract was merely 0.45 ng/mL. The water extract and ethanol extract have a strong effect of heavy metals dissolution from coal gangue, for the reason that the large amount of tartaric acid, lactic acid, citric acid and glycolic acid in puff balls is soluble in water and ethanol. The ethyl acetate extract has a weak effect of heavy metals dissolution from coal gangue, in that only glycolic acid in puff balls is soluble in ethyl acetate. It indicatess that the dissolution, absorption and enrichment of heavy metals in coal gangue by puff balls are associated with the large amount of organic acids in puff balls. It can be proved by the data in Table 3 and 5 that the poor Cd enrichment by puff balls is due to that Cd is hard to be dissolved by organic acids.

4 Conclusions

(1) Puff balls have strong enrichment capacity for heavy metals (As, Pb, Mn and Ni). The highest Mn enrichment level was 220.3 mg/kg (dry weight) for puffball fruiting bodies, The highest As, Cr and Pb enrichment levels were 111, 265.3 and 86 mg/kg respectively for puffball mycelium. The enrichment of heavy metals by fruiting bodies from strong to weak is in this order: Ni>Mn>As>Pb>Cd>Cr>Co, meanwhile, the enrichment of heavy metals by mycelium from strong to weak: As>Pb>Ni>Cr>Mn>Cd>Co. The heavy metal Cd, Mn and Ni can transfer from puffball mycelium to its fruiting body, and As, Cr, Pb and Co mainly accumulate in mycelium.

(2) The total organic acids in puff ball fruiting bodies are higher than those in mycelium. Expect for tartaric acid, the contents of other organic acids in fruiting bodies are higher than those in mycelium.

(3) The water extract and ethanol extract have a strong effect of heavy metals dissolution from coal gangue, for the reason that the large amount of tartaric acid, lactic acid, citric acid and glycolic acid in puff balls is soluble in water and ethanol. The dissolution, absorption and enrichment of heavy metals in coal gangue by puff balls are associated with the large amount of organic acids in puff balls.

(4) The growth of puff balls can raise the pH value of coal gangue matrix and change the content of heavy metal elements in coal gangue. Puff balls can be planted in the prophase period of ecological restoration of coal gangue dumps to improve the growth substrate of coal gangue and to contribute to the development of ecological remediation patterns. Due to the unique environmental biological characteristics of macrofungi, mature artificial domestication and cultivation technology, high relative annual biomass of fruiting bodies, and easy aftertreatment of fruiting bodies after the ecological remediation of heavy metals, macrofungi has significant advantages in ecological remediation of heavy metals. The heavy metal contents of macrofungi depend on the heavy metal absorption from matrix, the selective absorption of heavy metals, and the precipitation of heavy metals in fungal tissues. It can be seen from Fig. 1 that puff ball mycelium crisscross in coal gangue matrix. Numerous mycelium long chains become interlaced and form a mycelium network with a vast occupation temporally and spatially, which is conducive to the absorption of heavy metals by mycelium.

Table 3 Enrichment coefficient and transfer coefficient of puff balls to heavy metals in coal gangue

	As	Pb	Cr	Cd	Mn	Co	Ni
Enrichment coefficient of fruiting body	1.851	1.409	0.150	0.342	1.912	0.041	2.018
Enrichment coefficient of fruiting body	3.970	3.738	0.567	0.340	0.418	0.161	1.018
Transfer coefficient	0.466	0.377	0.264	1.006	4.573	0.255	1.982

Table 4 Organic acid contents in puff ball fruiting bodies and mycelium (ug/g)

Sample	Tartaric acid	Glycolic acid	Lactic acid	Citric acid	Malic acid	Total
Puff ball fruiting body	1355.947	1446.505	4234.069	460.795	-	7497.317
Puff ball mycelium	4014.178	-	1632.136	237.513	-	5883.827

Note: "-" means undetected.

Table 5 Dissolution effects of heavy metals in coal gangue by different solvent extracts (ng/mL)

No.	Before experiment pH	After experiment pH	As	Pb	Cr	Cd	Mn	Co	Ni
1	3.560	3.490	45.030	3.927	4.822	0.450	155.200	7.438	22.960
2	3.610	3.520	78.270	20.336	3.595	0.443	3.487	0.263	14.950
3	3.690	3.570	1.795	3.877	2.730	0.413	3.435	0.243	14.590
4	3.730	3.680	1.837	-	2.268	-	150.600	7.421	14.520

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