Canadian Journal of Microbiology

Morphological changes and bioaccumulation in response to cadmium exposure in *Morchella spongiosa*, a fungus with potential for detoxification

Journal:	<i>Canadian Journal of Microbiology</i>
Manuscript ID	cjm-2020-0571.R1
Manuscript Type:	Article
Date Submitted by the Author:	03-Apr-2021
Complete List of Authors:	Xu, Hongyan; Qinghai University Guo, Jing; Qinghai University Meng, Qing; Qinghai University Xie, Zhanling; Qinghai University
Keyword:	<i>Morchella spongiosa</i> , Micro-morphology, Cd precipitates, Intracellular accumulation, Extracellular adsorption
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

SCHOLARONE™
Manuscripts

**Morphological changes and bioaccumulation in response to cadmium exposure
in *Morchella spongiola*, a fungus with potential for detoxification**

Hongyan Xu¹², Zhanling Xie^{1*}, Jing Guo¹, Qing Men¹

¹*College of Eco-Environmental Engineering, Qinghai University, Xining 810016, Qinghai, China*

²*Academy of Agriculture and Forestry Sciences, Qinghai University, Xining 810016, Qinghai, China*

Draft

*** Corresponding author:**

Address: Qinghai University, Ningda Road 251, Xining 810016, Qinghai Province, China

E-mail: xiezhanling2012@126.com

Tel: 0086-13997286692

Abstract

Morchella is a genus of edible fungi with strong resistance to Cd and the ability to accumulate it in the mycelia. However, the mechanisms conferring Cd resistance in *Morchella* are unknown. In the present study, morphological and physiological responses to Cd were evaluated in the mycelia of *Morchella spongiosa*. Variations in hyphal micro-morphology including twisting, folding and kinking in mycelia exposed to different Cd concentrations (0.15, 0.9, 1.5, 2.4, 5.0 mg/L) were observed using scanning electron microscopy. Deposition of Cd precipitates on cell surfaces (at Cd concentrations > 2.4 mg/L) was shown by SEM-EDS. Transmission electron microscopy analysis of cells exposed to different concentrations of Cd revealed the loss of intracellular structures and the localization of Cd depositions inside/outside the cell. FTIR analysis showed that functional groups such as C=O, -OH, -NH and -CH could be responsible for Cd binding on the cell surface of *M. spongiosa*. In addition, intracellular accumulation was observed in cultures at low Cd concentrations (< 0.9 mg/L), while extracellular adsorption occurred at higher concentrations. These results provide valuable information on the Cd tolerance mechanism in *M. spongiosa* and constitute a robust foundation for further studies on fungal bioremediation strategies.

Keywords

Morchella spongiosa; Cd; Micro-morphology; Cd precipitates; Intracellular accumulation; Extracellular adsorption

Introduction

Heavy metal pollution is of critical concern for ecologists. Cadmium(Cd), one of most common toxic heavy metals, can cause serious effects to the environment, and it has a major role in disturbing the earth's ecological balance due to its toxic effects in freshwater, surface water, marine water and groundwater environments (Abatenh et al. 2017). In the past three decades, China has been rapidly growing its industrial sector, leading to deterioration in environmental quality along with a serious threat to agricultural soils, especially heavy metal pollution; the standard rate of Cd contamination in Chinese soils has reached 7% (Wang et al. 2015). Cd affects not only the health of the soil but also the soil microbiota, thereby causing a reduction in biodiversity, changes in enzymatic activities in the microbes and alterations to normal metabolism (Tang et al. 2019).

To address this persistent problem, efficient low-cost methods are urgently needed. Several chemical methods, such as precipitation, oxidation or reduction, ion exchange, and electrochemical treatment, and physical methods including sludge separation, reverse osmosis, filtration, evaporative recovery, and activated charcoal adsorption are being used to remove heavy metals from polluted sites (Zhang et al. 2016). However, these physio-chemical procedures are very expensive and consume much time and energy, and they can also cause secondary environmental pollution (Price et al. 2001). More environmentally friendly methods for heavy metal removal are therefore required. Methods of bio-remediation employing microbiological strategies have potential as alternative approaches to the problem (Zafar et al. 2007). Many microbes are able to tolerate heavy metals at high concentrations due to mutations or adaptation (Wang et al. 2019). Microorganisms such as bacteria and fungi have proven their worth in ameliorating polluted environments by methods such as bio-stimulation,

bio-augmentation, bio-venting, bio-piles and bio-attenuation (Abatenh et al. 2017). Several studies have shown that fruiting bodies of mushrooms such as *Lentinus edodes* (Ma et al. 2019), *Agaricus subrufescens* (Stoknes et al. 2019), *Galerina vittiformis* (Damodaran et al. 2013), and *Agaricus bisporus* (Ali et al. 2017) can absorb Cd from polluted soil. There are also some reports on the application of Cd adsorption by fungi. Stoknes et al. pointed that *Agaricus subrufescens* culturing in waste-to-crop system could be a cost-efficient way to remediate Cd from the food chain (Stoknes et al. 2021). Mathialagan et al. tried to absorb Cd from aqueous solution by *L. edodes* and *A. bisporus* (Mathialagan et al. 2003). In order to make better use of these fungi, it is necessary for them to have a high tolerance to Cd and be able to remove Cd effectively. However, although there are some reports about the ability of macrofungi to remove Cd, the response of fungi to Cd stress was not clear.

In the last few decades, many investigations have focused on the strategies that fungi use to remove heavy metals from aqueous solutions. It has been reported that microorganisms have two mechanisms for achieving this: extracellular bio-absorption, which consists of metabolism-independent binding between negatively charged free groups and the fungal cell wall, and intracellular bioaccumulation resulting from energy-dependent influx of metals (Mishra 2017; Muhamma et al. 1992). *Pleurotus ostreatus* can remove lead from wastewater by mechanisms such as extracellular cation exchange, chelation that is dependent on extracellular organic acids, and intracellular bioaccumulation (Huang et al. 2012). *Agrocybe aegerita* can tolerate 20 mg/kg Cd owing to various strategies such as regulating the subcellular distribution of Cd, and increasing the production of antioxidant enzymes, glutathione and low-molecular-weight organic acids (LWMOAs) (Li et al. 2019).

Morchella species have the ability to bioaccumulate cadmium. Literatures shows that Cd bioconcentrations for the *M. conica* ranged from 0.20 mg/kg to 0.89 mg/kg (Liu et al. 2015; Sarikurkc

et al. 2011; Ozturk et al. 2010; Gursay et al. 2009) and 0.72-1.1 mg/kg for *M. esculenta* and 0.3-1.89 mg/kg for other *Morchella* species (Gursay et al. 2009; Isildak et al. 2004). These implied that *Morchella* species have the potential of Cd-bioaccumulation. Zhang et al. studied the maximum tolerance concentration of Cd were in the range of 20-50 mg/kg for *M. importuna* (Zhang et al. 2017). An et al pointed that *M. esculenta* could growing in the culture media containing 32 mg/kg Cd (An et al. 2009). However, except for the bioconcentration factor, the information about the Cd-*Morchella* interaction and Cd accumulation strategy of *Morchella* is lacking. The present study was aimed at analyzing the capacity of the macro-fungus *M. spongiosa* for Cd removing from the aqueous solution, and exploring the influence of different concentrations of Cd on its cellular morphology and physiology. The cadmium removal potential of *Morchella* was also analyzed to lay a foundation for the bioremediation of polluted sites with the help of this fungus.

Materials and Methods

Microorganism and growth conditions

The fungal strain *M. spongiosa* M12-10 was obtained from the Laboratory of Microbiology, college of eco-environmental engineering, Qinghai University, China. *M. spongiosa* was cultured in Potato Dextrose Agar (PDA) medium (glucose 20.0 g/L; potato extract 200.0 g/L and agar 17.0 g/L, pH 7.0) at 20°C and 50% humidity.

Effect of initial Cd concentrations on mycelial growth

In order to study the effect of different concentrations of Cd on mycelial growth, CdCl₂ gradients covering low (0.03-0.15 mg/L), medium (0.3-2.7 mg/L) and high (3-5 mg/L) concentration ranges were prepared and mixed with PDA medium. Distilled water (dH₂O) was added into the PDA culture

plates as the control. For each treatment, an agar plug (5 mm diameter) cut from the margin of an *M. spongiola* culture (5 d incubation, 20°C) was inoculated into the center of a PDA petri dish containing 0-5 mg/L CdCl₂, which was placed in an incubator at a constant temperature of 20°C and 50% humidity. During incubation, the hypha density and color were observed, the diameter of the colony was measured daily and the growth difference ratio (GDR) was calculated as following formula (1) (Zhang et al. 2017). We defined Cd as having a promoting effect on mycelial growth when GDR > 0, while GDR < 0 represents an inhibitory effect. In addition, 0.5 mg wet mycelia of *M. spongiola* filtered from liquid PD medium was inoculated into 50 mL of growth medium at various initial CdCl₂ concentrations (0.15, 0.9, 1.5, 2.4, 5.0 mg/L) and harvested (150 rpm, 20°C) after 5 d culture to measure the biomass (Xu et al. 2015).

$$GDR = \frac{D - D_{CK}}{D_{CK}} \quad (1)$$

Where, D is the final diameter of test colony in each treatment group (mm); D_{CK} is the final diameter of the control group (without Cd) (mm).

Bioaccumulation mechanism studies

LM, SEM, and TEM observations

Light microscopy was carried out to study the mycelial morphology of *M. spongiola* at different initial Cd concentrations. Cells unexposed to Cd (control) or exposed to 0.15, 0.9, 1.5, 2.4, 5.0 mg/L of CdCl₂ for defined intervals were viewed using a Nikon stereo microscope (Nikon SMZ18, Japan). The average cell length and width (number of cells measured per treatment: 100) from bright images was determined using the Image J software package. SEM (ZEISS MERLIN Compact, Zeiss, Germany) coupled with electron dispersive spectroscopy (EDS) (Zeiss, Germany) was used to examine cell

surface properties in *M. spongiola*. Cells exposed to different initial Cd concentrations were harvested and fixed with 2.5% glutaraldehyde at 4°C overnight, then washed three times with phosphate buffered saline (PBS) (0.1 M, pH 7.0), dehydrated in an ethanol gradient series and sputter coated with gold following standard methods. For transmission electron microscopy (TEM) analysis, samples were fixed in 2.5% glutaraldehyde overnight and washed three times with 1% osmium acid, then dehydrated and sectioned, stained and observed under a TEM (JEM-1400, Jeol, Japan).

FTIR analysis

Control cells or cells exposed to different initial Cd concentrations were prepared, dried, made into KBr pellets and analyzed using FTIR (WQF-410, Beijing, China) in the range 400–4000 cm⁻¹.

Cd biosorption analysis

Control cells or cells incubated under different initial Cd concentrations were removed from flasks and washed twice with ddH₂O. They were then treated three times with HNO₃ (0.2 M) to remove heavy metal from the cell surface, and the supernatants from the three acid treatments were combined after centrifugation, digested with HNO₃ (0.2 M) at 180°C and analyzed by Atomic Absorption Spectroscopy (AAS) (ZEEnit 700, Jena, Germany) to determine the extracellular accumulation of Cd. The cell pellets after acid washing were digested and analyzed by AAS to determine the intercellular accumulation of Cd. The liquid media remaining after removal of cultured mycelia were digested and analyzed by AAS to determine the Cd removal capacity of each culture (Zhang et al. 2016).

Statistical Analysis

Every independent experiment was carried out at least three times. Average values (mean ± SD) were calculated during data interpretation. Two-way ANOVA statistics were used to analyze the difference between the means of Cd-treatment groups. All statistical analysis was performed using

SPSS (IBM SPSS Statistics version 22).

Results

Effects of initial Cd concentrations on growth of *M. spongiola*

As shown in Figure 1, the growth of mycelia after 72 h of culturing depended on Cd concentration when the initial concentration of Cd was above 0.15 mg/L. Cd concentrations up to approximately 1 mg/L do not alter growth, whereas higher concentrations inhibit it relative to the control (Figure 1A). The same difference in mycelial growth can also be seen in Table S1. When the concentration of Cd was lower than that of 0.15 mg/L, $GDR > 0$ indicating that the addition of Cd might promote the mycelial growth of *M. spongiola* compared with the control group. With the increase of Cd concentration, there are different negative changes in the value of GDR, which means that higher concentration of Cd will inhibit the growth of mycelia (Table S1). In addition, we found that the biomass of *M. spongiola* was much more than that of the control group when the Cd concentration was lower than 0.9 mg/L, while it was significantly reduced with the concentration increased to 2.4 mg/L (Figure 1B). Therefore, we chose 0.15 mg/L and 0.9 mg/L with promoting effects and 1.5 mg/L, 2.4 mg/L and 5.0 mg/L with inhibitory effects to subsequent follow-up experiment.

Cellular morphological characteristics of *M. spongiola*

The mycelium could be seen in each Cd-treatments and control group after 24 h inoculation. The hypha density and colour in the medium for 5 days culturation were shown Figure 1C. It was indicated that the treatment of $CdCl_2$ with the contents from 0.15 to 0.90 mg/L could significantly promote the mycelia growth for *M. spongiola*. Moreover, obvious transparent aerial hyphae were observed in Cd-treatment groups (Figure 1C). Light microscopic observations of mycelia treated with 0.15 mg/L

Cd revealed normal hyphal branches and cell sizes consistent with the control group (Figure 2A). Swelling and lysis of some hyphal tips were observed when the Cd concentration was increased to 2.4 mg/L and 5.0 mg/L (Figure 2A), as was found in *Trichoderma virens* growing towards a metal-containing tile with 1 g/L sucrose and 2 mM Cu (Fomina et al. 2003). Furthermore, cells exposed to 0.9 mg/L showed an increase in cell length, while those exposed to 5.0 mg/L exhibited an increase in cell width (Figure 2B). Such a decrease in mycelial length was also observed in *Aspergillus lentulus* under Ni stress (Mishra and Malik 2012).

In the SEM micrographs (Figure 3), the cell surface was seen to be smooth with rope-like structures before bioaccumulation, and after bioaccumulation there were almost no obvious modifications in the case of cells from the culture with 0.15 mg/L, which suggested that there was very less effect on the hyphal structure of *M. spongiola* at this concentration. With increasing initial Cd concentration, some mycelia became twisted, folded or kinked, and this was especially evident when *M. spongiola* was exposed to 2.4 mg/L, which was similar to the results obtained by Mishra and Malik (Mishra and Malik 2012), who reported that in response to Cr and Cu stress *A. lentulus* underwent twisting and looping of individual hyphae. In addition, SEM macrographs of cells exposed to 2.4 mg/L and 5.0 mg/L exhibited irregular deposits on the cell surface/cell wall (Figure 3). The EDS spectra of these deposits confirmed that Cd was bound to the cells (Figure S1). Similar Cd crystal particles were found on the surface of *P. chrysosporium* mycelium exposed to 0.1 mM Cd(NO₃)₂ (Chen et al. 2014).

TEM analysis revealed short crystalline precipitates within cells of *M. spongiola* exposed to 1.5-5.0 mg/L Cd (Figure 4). Many more crystalline precipitates were observed both inside and outside the cells challenged with 2.4 mg/L and 5.0 mg/L Cd than for cells at lower concentrations (Figure 4E, 4F). Clear vacuolization of the entire cytoplasm region were observed in some cells treated with 1.5,

2.4 and 5.0 mg/L Cd (Figure 4D, 4E, 4F). Furthermore, there were clear plasma membrane separation and dark electron dense deposits between cell wall and cytoplasm at 2.4 and 5.0 mg/L Cd (Figure 4E, 4F). A clear boundary between cell wall and cytoplasm, and electron dense particles were also observed in the cell wall of *Bacillus cereus* under 100 mg/L Cd stress (Li et al. 2018).

Cd binding

Cd binding experiments were conducted in PD medium supplemented with 0.15-5.0 mg/L Cd. As shown in Figure 5A, intracellular accumulation was greater than extracellular adsorption when the Cd concentration was less than 0.9 mg/L. Extracellular adsorption increased as the initial Cd concentration increased, becoming much greater than intracellular accumulation at high exposure levels (>1.5 mg/L). This suggested that growing cells could take up Cd more effectively when its concentration was less than 0.9 mg/L. Interestingly, there was little obvious change in the intracellular accumulation even when the initial Cd concentration was much higher than 2.4 mg/L. We calculated that the maximum extent of Cd removal was 61.80% in the group treated with 0.90 mg/L Cd. On the basis of these results, we conjecture that growing cells of *M. spongiola* are efficient bioaccumulators of Cd from aqueous solutions.

FTIR analysis of *M. spongiola*

Table 1 shows the assignment of FTIR bands and details of wave number shifts. A marked shift in the adsorption peak was observed at 3420 cm^{-1} , representing –OH and –NH stretching of proteins, which is consistent with the peak at 3359 cm^{-1} assigned to surface-bonded hydroxyl or amine groups in *Pleurotus platypus* under conditions of Cd adsorption (Vimala and Das 2011). The peaks located at 2926, 2856, 1551, 1407 and 861 cm^{-1} were assigned to –CH and –NH groups (Huang et al. 2014). Those near 1745 cm^{-1} and 1643 cm^{-1} were attributed to the stretching of C=O in carboxyl groups (amide I

band) (Du et al. 2012). The peaks located at 1244, 1203 and 1154 cm^{-1} were assigned to phosphate groups (Chen et al. 2019). These peak shifts results indicated that functional groups such as C=O, -OH, -NH, -CH and P=O may be responsible for Cd binding on the cell surface in *M. spongiola*.

Discussion

Fungal growth at different initial Cd concentrations

Mycelial growth in *M. spongiola* showed promote effects up to 0.3 mg/L Cd, in terms of either colony diameter or dry biomass (Figure 1A, 1B). This concentration is lower than those reported for *Morchella importuna* (5 mg/L) (Zhang et al. 2017) and *Morchella esculenta* (2 mg/L) (An et al. 2008), which could be explained by the existence of different tolerance mechanisms in different species. The concentration of Cd in polluted water is usually less than 1 mg/kg, but *M. spongiola* M12-10 can grow at a concentration of 5 mg/L (Figure 1B), imply that *M. spongiola* M12-10 has a certain ability to deal with the cadmium, and this Cd-accumulation ability of *M.spongiola* could be used in bioremediation. However, most of the yellow *Morchella* (including *M. spongiola*) can not be cultivated artificially, while the hyphae of *M. spongiola* could be easily collected. We also tested that the maximum Cd removal rate of *M. spongiola* was 61.80% (Figure 5), which was much higher than 45% of *Lentinus edodes* (Mathialagan et al. 2003), 12% of *Agaricus macrosporus* (García et al. 2005). These years, fungal hyphae have received extensive attention in heavy metal bioremediation because of their large cell growth span, tough texture and easy loading of micro-units in cells (Rozman et al. 2020; Lei et al. 2019). Therefore, *M. spongiola* might be adapted to remove the Cd by a promising modification method just as *Saccharomyces cerevisiae* and *Phanerochaete chrysosporium* do in the future (Lu et al. 2020; Ma et al. 2015).

SEM and TEM observations with different initial Cd concentrations

The results of SEM and LM revealed that the mycelial morphology and cell surface morphology changed to different degrees depending on the initial Cd concentration in the culture medium. We think that the main reason for this may be that different cell surface morphologies respond to different concentrations of Cd. At lower concentrations (0.3 mg/L), extracellular adsorption did not play a major role in the removal of Cd and there was no effect on cell surface morphology, while extracellular adsorption (such as surface chelation) may change the structure of the cell surface at higher concentrations (2.4 and 5.0 mg/L), resulting in an increase in cell surface roughness and the formation of cadmium precipitates (Huang et al. 2014). The TEM observations also supported this explanation. We found that the greatest differences in the effects of lower and higher Cd concentrations on cells were the formation of large vacuoles and cytoplasmic contraction (Figure 4), suggesting that vacuoles may play an important role in limiting the toxicity of high concentrations of cadmium (Kulakovskaya 2018; Poirier et al. 2013). The cell profile was regular at lower concentrations, while the cell width under higher concentrations was increased due to extracellular adsorption, vacuole formation and cytoplasmic contraction (Figure 4). These phenomenon also been proofed in the SEM observation that cell width remained unchanged at lower concentrations but increased sharply at higher concentrations (Figure 2B). Similar morphological changes have been reported by other researchers as adaptive responses to abiotic stress (Grąz et al. 2011, 2015; Kolhe et al. 2020).

FTIR analysis

In FTIR analysis, the wavelength of each group changed little at low concentration (0.3 mg/L), but at higher concentrations (2.4 and 5.0 mg/L), the peaks representing C-N, N-H and phosphate groups changed more markedly than those representing C=O, -OH and -CH groups. These results

indicate that under high Cd concentrations, the metal binding sites of *Morchella* are mainly associated with C-N, N-H and phosphate groups, which are likely to be components of cell wall proteins and cell membranes. Similar results were reported by Boyanov who identified the functional groups of *Bacillus subtilis* responsible for Cd adsorption at six pH values and reported that carboxyl groups became increasingly important at higher pH values (Boyanov et al. 2003).

The bioaccumulation mechanism in *M. spongiola*

In order to understand the difference in intracellular accumulation and extracellular adsorption by *Morchella* exposed to different initial Cd concentrations, we studied the levels of Cd removal, extracellular adsorption and intracellular accumulation. The results showed that the extracellular adsorption capacity increased with an increase in the initial concentration of Cd (Figure 5). This may be due to the fact that higher Cd concentrations may damage cells and expose potential metal binding sites, resulting in an increase in total uptake, while another possible reason might be that at high Cd concentrations a Cd efflux mechanism released more Cd ions to the medium for extracellular chelation. Similar results were obtained by Huang et al. (2014), who studied the characteristics of Cd biosorption in *Bacillus cereus* RC-1 cultured in medium with different initial Cd concentrations and found that removal of Cd increased with increasing concentration owing to the much larger negative surface charge. We also found that intracellular Cd accumulation contributed more to total Cd removal at lower concentrations than at higher concentrations, consistent with reports on *Tetraselmis suecica* exposed to 0.6-6 mg/L Cd for 72 h (Pérez-Rama et al. 2010), *P. ostreatus* at 10 mg/L Cd for 10 d (Li et al. 2017), and *Saccharomyces cerevisiae* exposed to 1 mg/L Cd for 96 h (Wang et al. 2012). Intracellular accumulation remained at a constant level once the concentration reached or exceeded 1.5 mg/L (Figure 5), which could be explained by the existence of a Cd-efflux system that removed Cd from the

cytoplasm (Huang et al. 2018). Furthermore, proteins might take part in Cd bioaccumulation at higher concentrations. It has been reported that some proteins on the surface layer of bacteria were responsible for the efflux of Cd to the exterior of the cell, which was considered to be an energy-dependent process (Nies 2003; Huang et al. 2014).

Conclusions

In this study, we evaluated the bioaccumulation strategy in *M. spongiola* exposed to different concentrations of Cd. Morphological and physiological changes in *M. spongiola* cells appeared to be associated with energy-independent extracellular adsorption and energy-dependent intracellular bioaccumulation, and an efflux mechanism may be the key at higher concentrations, reducing the intracellular accumulation of Cd. The results of this study should be useful in understanding the physiological responses of fungi exposed to environmental toxins. Further studies combining genomic and proteomic analysis are required in order to understand the genetic basis of these physiological responses and the signal transduction mechanism associated with them.

Acknowledgements

The authors thank Dr. Shuo Sheng and Dr. Benyin Zhang for discussions on designing of experiment.

Conflicts of interest

The authors declare that there is no conflict of interest.

Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Hongyan Xu, Zhanling Xie, Qing Meng, and Jing Guo. The first draft of

the manuscript was written by Hongyan Xu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Information This work was supported by Qinghai Science and Technology Department project (2021-HZ-802) and Science & Technology innovation project of Qinghai University (2019-QH-16).

Data availability statement

All data and materials as well as software application support their published claims and comply with field standards.

References

- Abatenh, E., Gizaw, B., Tsegaye, Z., and Wassie, M. 2017. The role of microorganisms in bioremediation- a review. *Open Journal of Environmental Biology*, **2**:038–046. doi:10.17352/ojeb.000007.
- Ali, A., Guo, D., Mahar, A., Wan,g P., Shen, F., Li, R., and Zhang, Z. 2017. Mycoremediation of Potentially Toxic Trace Elements—a Biological Tool for Soil Cleanup: A Review. *Pedosphere*, **27**: 205-222. doi:10.1016/S1002-0160(17)60311-4.
- An, X.L., Zhou, Q., and Li, T. 2008. Growth and enrichment response of *Morchella esculenta* hyphae to single and combined pollution of Cd and Pb. *Journal of Basic Science and Engineering*, **16**:35-42 (in Chinese).
- Boyanov, M.I., Kelly, S.D., Kemner, K.M., Bunker, B. A., Fein, J. B., and Fowle, D. A. 2003. Adsorption of cadmium to *Bacillus subtilis* bacterial cell walls: a pH-dependent X-ray

- absorption fine structure spectroscopy study. *Geochimica Cosmochimica Acta*, **67**:3299-3311.
doi:10.1016/s0016-7037(02)01343-1.
- Chen, A.W., Zeng, G.M., Chen, G.Q., Liu, L., Shang C., Hu, X.J., Lu, L.H., Chen, M., Zhou, Y., and Zhang, Q.H. 2014. Plasma membrane behavior, oxidative damage, and defense mechanism in *Phanerochaete chrysosporium* under cadmium stress. *Process Biochemistry*, **49**:589-598.
doi:10.1016/j.procbio.2014.01.014.
- Chen, S.H., Cheow, Y.L., Ng, S.L., and Ting, A.S.Y. 2019. Mechanisms for metal removal established via electron microscopy and spectroscopy: a case study on metal tolerant fungi *Penicillium simplicissimum*. *Journal of Hazardous Materials*, **362**: 394-402.
doi:10.1016/j.jhazmat.2018.08.077.
- Damodaran, D., Shetty, K.V., and Mohan, B.R. 2013. Effect of chelaters on bioaccumulation of Cd (II), Cu (II), Cr (VI), Pb (II) and Zn (II) in *Galerina vittiformis* from soil. *International Biodeterioration and Biodegradation*, **85**: 182-188. doi:10.1016/j.ibiod.2013.05.031.
- Du, L.N., Wang, B., Li, G., Wang, S., Crowley, D.E., and Zhao, Y.H. 2012. Biosorption of the metal-complex dye Acid Black 172 by live and heat-treated biomass of *Pseudomonas sp.* strain DY1: Kinetics and sorption mechanisms. *Journal of Hazardous Materials*, **205-206**:47-54. doi:10.1016/j.jhazmat.2011.12.001.
- Fomina, M., Ritz, K., and Gadd, G.M. 2003. Nutritional influence on the ability of fungal mycelia to penetrate toxic metal-containing domains. *Mycological Research*, **107**:861-871.
doi:10.1017/s095375620300786x.
- Fomina, M., and Gadd, G.M. 2014. Biosorption: current perspectives on concept, definition and application. *Bioresource Technology*, **160**:3-14. doi:10.1016/j.biortech.2013.12.102.

- García, M.A., Alonso, J., and Melgar, M.J. 2005. *Agaricus macrosporus* as a potential bioremediation agent for substrates contaminated with heavy metals. *Journal of Chemical Technology and Biotechnology*, **80**: 325-330. doi:10.1002/jctb.1203.
- Grąz, M., Pawlikowska-Pawłęga, B., and Jarosz-Wilkolazka, A. 2011. Growth inhibition and intracellular distribution of Pb ions by the white-rot fungus *Abortiporus biennis*. *International Biodeterioration and Biodegradation*, **65**:124-129. doi:10.1016/j.ibiod.2010.08.010.
- Grąz, M., Pawlikowska-Pawłęga, B., and Jarosz-Wilkolazkaa, A. 2015. Intracellular distribution of Cd during the growth of *Abortiporus biennis* on cadmium amended media. *Canadian Journal of Microbiology*, **61**:545–554. doi:10.1139/cjm-2015-0102.
- Guo, J., and Xie, Z.L. 2015. Determination and analysis of the content of mineral elements of *Morchella* in Qinghai province. *China Fungul*, **3**:58-59, 68 (in Chinese).
- Gursoy, N., Sarikurkcu, C., Cengiz, M., and Solak, M.H. 2009. Antioxidant activities, metal contents, total phenolics and flavonoids of seven *Morchella* species. *Food Chemical Toxicology*, **47**:2381-2388. doi:10.1016/j.fct.2009.06.032.
- Huang, F., Guo, C.L., Lu, G.N., Yi, X.Y., Zhu, L.D., and Dang, Z. 2014. Bioaccumulation characterization of cadmium by growing *Bacillus cereus* RC-1 and its mechanism. *Chemosphere*, **109**:134-142. doi:10.1016/j.chemosphere.2014.01.066.
- Huang, F., Wang, Z.H., Cai, Y.X., Chen, S.H., Tian, J.H., and Cai, K.Z. 2018. Heavy metal bioaccumulation and cation release by growing *Bacillus cereus* RC-1 under culture conditions. *Ecotoxicology Environmental Safety*, **157**:216-226. doi:10.1016/j.ecoenv.2018.03.077.

- Huang, H., Cao, L., Wan, Y., Zhang, R., and Wang, W. 2012. Biosorption behavior and mechanism of heavy metals by the fruiting body of jelly fungus (*Auricularia polytricha*) from aqueous solutions. *Applied Microbiology and Biotechnology*, **96**:829-840.
doi:10.1007/s00253-011-3846-6.
- Isildak, Ö., Turkecul, I., Elmastas, M., and Tuzen, M. 2004. Analysis of heavy metals in some wild-grown edible mushrooms from the middle black sea region, Turkey. *Food Chemistry*, **86**:547-552. doi:10.1016/j.foodchem.2003.09.007.
- Iskandar, N.L., Zainudin, N.A.I.M., and Tan, S.G. 2011. Tolerance and biosorption of copper (Cu) and lead (Pb) by filamentous fungi isolated from a freshwater ecosystem. *Journal of Environmental Sciences*, **23**:824-830. doi:10.1016/s1001-0742(10)60475-5.
- Kolhe, N., Zinjarde, S., and Acharya, C. 2020. Impact of uranium exposure on marine yeast, *Yarrowia lipolytica*: Insights into the yeast strategies to withstand uranium stress. *Journal of Hazardous Materials*, **381**:121226. doi:10.1016/j.jhazmat.2019.121226.
- Li, F., Wang, W., Li, C., Zhu, R., Ge, F., Zheng, Y., and Tang, Y. 2018. Self-mediated pH changes in culture medium affecting biosorption and biomineralization of Cd²⁺ by *Bacillus cereus* Cd01. *Journal of Hazardous Materials*, **358**:178-186. doi:10.1016/j.jhazmat.2018.06.066.
- Li, X., Ma, H., Li, L., Gao, Y., Li, Y., Xu, H. 2019. Subcellular distribution, chemical forms and physiological responses involved in cadmium tolerance and detoxification in *Agrocybe Aegerita*. *Ecotoxicology and Environmental Safety*, **171**:66-74.
doi:10.1016/j.ecoenv.2018.12.063.
- Li, X., Wang, Y., Pan, Y., Yu, H., Zhang, X., Shen, Y., Jiao, S., Wu, K., La, G., Yuan, Y., and Zhang S. 2017. Mechanisms of Cd and Cr removal and tolerance by macrofungus *Pleurotus*

ostreatus HAU-2. Journal of Hazardous Materials, **330**:1-8.

doi:10.1016/j.jhazmat.2017.01.047

Liu, H., Xu, J., Li, X., Zhang, Y., Yin, A., Wang, J., and Long Z. 2015. Effects of microelemental fertilizers on yields, mineral element levels and nutritional compositions of the artificially cultivated *Morchella conica*. Scientia Horticulturae, **189**: 86-93.

doi:10.1016/j.scienta.2015.03.047.

Lu, N., Hu, T., Zhai, Y., Qin, H., Aliyeva, J., and Zhang, H. 2019. Fungal cell with artificial metal container for heavy metals biosorption: equilibrium, kinetics study and mechanisms analysis.

Environmental Research, **182**:109061. doi:10.1016/j.envres.2019.109061.

Ma, H, Li, X, Hou, S, Peng, D, Wang, Y, Xu, F., and Xu, H. 2019. The activation and extraction systems using organic acids and *Lentinus edodes* to remediate cadmium contaminated soil.

Environmental Pollution, **255**:113252. doi:10.1016/j.envpol.2019.113252.

Mathialagan, T., Viraraghavan, T., and Cullimore, D.R. 2003. Adsorption of cadmium from aqueous solutions by edible mushrooms (*Agaricus bisporus* and *Lentinus edodes*). Water Quality

Research Journal of Canada, **38**(3), 499-514. doi:10.2175/106143003X140872.

Mishra, A., and Malik, A. 2012. Simultaneous bioaccumulation of multiple metals from electroplating effluent using *Aspergillus lentulus*. Water Research, **46**:4991-4998.

doi:10.1016/j.watres.2012.06.035.

Mishra, G.K. 2017. Microbes in Heavy Metal Remediation: A Review on current trends and patents.

Recent Patents on Biotechnology, **11**:188-196. doi:10.2174/1872208311666170120121025.

Muhamma, W., Abdus, S., and Affab, I. 1992. Nutrient and heavy metal contents compared among five mushrooms from Pakistan. Karstenia, **32**: 1-5. doi:10.29203/ka.1992.286.

- Nies, D.H. 2003. Efflux-mediated heavy metal resistance in prokaryotes. *FEMS Microbiology Reviews*, **27**:313-339. doi:10.1016/s0168-6445(03)00048-2.
- Ozturk, I., Sahan, S., Sahin, U., Ekici, L., and Sagdic, O. 2010. Bioactivity and mineral contents of wild-grown edible *Morchella conica* in the Mediterranean Region. *Journal of Consumer Protection and Food Safety*, **5**:453-457. doi:10.1007/s00003-010-0625-8.
- Pérez-Rama, M., Torres, E., Suárez, C., Herrero, C., and Abalde, J. 2010. Sorption isotherm studies of Cd (II) ions using living cells of the marine microalga *Tetraselmis suecica* (Kylin) Butch. *Journal of Environmental Management*, **91**:2045-2050. doi:10.1016/j.jenvman.2010.05.014.
- Poirier, I., Hammann, P., Kuhn, L., and Bertrand, M. 2013. Strategies developed by the marine bacterium *Pseudomonas fluorescens* BA3SM1 to resist metals: A proteome analysis. *Aquatic Toxicology*, **128-129**:215-232. doi:10.1016/j.aquatox.2012.12.006.
- Price, M.S., Classen, J.J., and Payne, G.A. 2001. *Aspergillus niger* absorbs copper and zinc from swine wastewater. *Bioresource Technology*, **77**:41-9. doi:10.1016/S0960-8524(00)00135-8.
- Rozman, U., Kalčíková, G., Marolt, G., Skalar, T., and Žgajnar, G. A. 2020. Potential of waste fungal biomass for lead and cadmium removal: Characterization, biosorption kinetic and isotherm studies. *Environmental Technology and Innovation*, **18**:100742. doi:10.1016/j.eti.2020.100742.
- Sarikurkcü, C., Copur, M., Yildiz, D., and Akata, I. 2011. Metal concentration of wild edible mushrooms in Soguksu National Park in Turkey. *Food Chemistry*, **128**: 731-734. doi:10.1016/j.foodchem.2011.03.097.
- Stoknes, K., Scholwin, F., Jasinska, A., Wojciechowska, E., Mleczek, M., Hanc, A., and Niedzielski, P. 2019. Cadmium mobility in a circular food-to-waste-to-food system and the use of a

- cultivated mushroom (*Agaricus subrufescens*) as a remediation agent. Journal of Environmental Management, **245**: 48-54. doi:10.1016/j.jenvman.2019.03.134.
- Tang, J., Zhang, J., Ren, L., Zhou, Y., Gao, J., Luo, L., Yang Y., Peng, Q., Huang, H., and Chen, A. 2019. Diagnosis of soil contamination using microbiological indices: A review on heavy metal pollution. Journal of Environmental Management, **242**:121-130. doi:10.1016/j.jenvman.2019.04.061.
- Vimala, R., and Das, N. 2011. Mechanism of Cd (II) adsorption by macro-fungus *Pleurotus platypus*. Journal of Environmental Sciences, **23**:288-293. doi:10.1016/s1001-0742(10)60405-6.
- Wang, H., McCarthney, A., Qiu, X., and Zhao, R. 2012. Cd²⁺ impact on metabolic cells of *Saccharomyces cerevisiae* over an extended period and implications for bioremediation. Geomicrobiology Journal, **29**:199-205. doi:10.1080/01490451.2011.558558.
- Wang, L., Cui, X., Cheng, H., Chen, F., Wang, J., Zhao, X., Lin, C., and Pu, X. 2015. A review of soil cadmium contamination in China including a health risk assessment. Environmental Science and Pollution Research, **22**:16441-16452. doi:10.1007/s11356-015-5273-1.
- Wang, P., Chen, H., Kopittke, P.M., and Zhao, F. 2019. Cadmium contamination in agricultural soils of China and the impact on food safety. Environmental Pollution, **249**:1038-1048. doi:10.1016/j.envpol.2019.03.063.
- Xu, P., Zeng, G., Huang, D., Dong, H., Lai, C., Chen, M., Tang, W., Li, F., Leng, Y., Cheng, M., He, X., and He, Y. 2015. Cadmium induced hydrogen peroxide accumulation and responses of enzymatic antioxidants in *Phanerochaete chrysosporium*. Ecological Engineering, **75**:110-115. doi:10.1016/j.ecoleng.2014.11.060.

- Zafar, S., Aqil, F., and Ahmad, I. 2007. Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. *Bioresource Technology*, **98**:2557-2561. doi:10.1016/j.biortech.2006.09.051.
- Zhang, N., Zhao, M., Xie, J., Wang, Y., Wen, X., and He, X. 2017. Tolerance of *Morchella importuna* towards heavy metals. *Mycosystema*, **36**:367-375. doi:10.13346/j.mycosystema.160146.
- Zhang, S., Zhang, X., Chang, C., Yuan, Z., Ting, W., Yong, Z., Yang, X., Zhang, Y., La, G., Wu, K., Zhang, Z., and Li, X. 2016. Improvement of tolerance to lead by filamentous fungus *Pleurotus ostreatus* HAU-2 and its oxidative responses. *Chemosphere*, **150**:33-39. doi:10.1016/j.chemosphere.2016.02.003.

Draft

Figure captions

Figure 1 Effect of initial Cd concentration on the diameter (A), biomass (B) and colony morphology (C) of *M. spongiola*. For the solid culture, an agar plug (5 mm diameter) cut out from the margin of a culture (7 d incubation, 20°C) was inoculated onto solid PDA media (20 mL) mixed with CdCl₂ of different concentrations; for the liquid culture, 0.5 mg of wet mycelia of *M. spongiola* was inoculated into liquid PD media containing CdCl₂ of different concentrations (0, 0.15, 0.9, 1.5, 2.4, 5.0 mg/L) and collected after 5 d culture to measure the fresh biomass. “*” represents the significant difference between the Cd-treatment group and the control group ($P < 0.01$)

Figure 2 Effect of initial Cd concentration on the mycelial morphology of *M. spongiola* (A) Light microscopy of control and Cd treated mycelia (magnified to 400x). The mycelium was inoculated onto solid PDA media (20 mL) mixed with CdCl₂ of different concentrations (a-0.00 mg/L; b-0.15 mg/L; c-0.90 mg/L; d-1.50 mg/L; e-2.40 mg/L; f-5.00 mg/L) and observed using a stereo microscope. (B) Determination of average sizes of mycelia exposed to different concentrations of Cd. Length and width of cells (number of cells measured: 100) were determined using the Image J software package. Results are shown as the means $\pm$ SD from three repetition experiments. “*” represents the significant difference between the Cd-treatment group and the control group ($P < 0.01$)

Figure 3 Scanning electron microscopy (SEM) of *M. spongiola* at different initial Cd concentrations. Representative scanning electron micrographs of mycelia exposed to CdCl₂ of different concentrations (A-0.00 mg/L; B-0.15 mg/L; C-0.90 mg/L; D-1.50 mg/L; E-2.40 mg/L; F-5.00 mg/L) for 5 d. Red arrows and green arrows show, respectively, irregular cell surfaces and partially fractured mycelia due to the presence of Cd deposits in mycelia treated with 2.4 mg/L and 5.0 mg/L of Cd

Figure 4 Transmission electron microscopy (TEM) of *M. spongiola* under different initial Cd concentrations Representative micrographs of mycelia exposed to CdCl₂ of different concentrations (A-0.00 mg/L; B-0.15 mg/L; C-0.90 mg/L; D-1.50 mg/L; E-2.40 mg/L; F-5.00 mg/L) for 5 d. White arrows represent intracellular structures in control cells which are not observed in Cd treated cells. Red arrows and black arrows show, respectively, intracellular and extracellular Cd deposits in Cd exposed cells

Figure 5 Effect of initial Cd concentration on the biomass and Cd bioaccumulation of *M. spongiola*. Extracellular adsorption and intracellular Cd accumulation by *M. spongiola* mycelia cultured in liquid PD medium mixed with CdCl₂ of different concentrations. The final Cd concentrations were quantified with an atomic absorption spectrophotometer. Results are shown as the means $\pm$ SD from three experiments. “*” represents the significant difference between the Cd-treatment group and the control group ($P < 0.01$)

Figure S1 EDS spectra of the surface of mycelia at different initial Cd concentrations Representative EDS spectrum of mycelia exposed to CdCl₂ of different concentrations (A-0.00 mg/L; B-0.15 mg/L; C-0.90 mg/L; D-1.50 mg/L; E-2.40 mg/L; F-5.00 mg/L)

Table captions

Table 1 Association between the bands observed in FTIR spectra and the corresponding functional groups

Table S1 Growth difference ratio at different initial Cd concentrations

Draft

Table 1 Association between the bands observed in FTIR spectra and the corresponding functional groups

Original wave number (cm ⁻¹)	Altered wave number (cm ⁻¹)					Possible assignment
	Cd0.15	Cd0.9	Cd1.5	Cd2.4	Cd5.0	
3420	3418	3418	3418	3422	3424	-OH and N-H stretching vibrations
2926	2927	2927	2927	2927	2926	C-H asymmetric stretching
2856	2857	2857	2857	2856	2856	C-H symmetric stretching
1745	1745	1744	1744	1746	1745	C=O stretching of ester
1643	1643	1641	1641	1641	1641	C=O stretching (amide I region)
1550	1551	-	1559	1552	1558	-NH bending in amide II
1407	1407	1410	1409	1411	1408	C-N stretching and N-H
1243	1244	1249	1243	1239	1240	Phosphate group
1203	1205	-	-	-	-	Phosphate group
1154	1153	1153	1153	1153	1154	Phosphate group
861	857	890	-	865	856	Aromatic C-H stretching
762	760	762	760	761	760	Aromatic C-H stretching
707	705	707	708	710	709	Aromatic C-H stretching

Figure 1 Effect of initial Cd concentration on the diameter (A), boimass (B) and colony morphology (C) of *M. spongiola*. For the solid culture, an agar plug (5 mm diameter) cut out from the margin of a culture (7 d incubation, 20°C) was inoculated onto solid PDA media (20 mL) mixed with CdCl₂ of different concentrations; for the liquid culture, 0.5 mg of wet mycelia of *M. spongiola* was inoculated into liquid PD media containing CdCl₂ of different concentrations (0, 0.15, 0.9, 1.5, 2.4, 5.0 mg/L) and collected after 5 d culture to measure the fresh biomass."*" represents the significant difference between the Cd-treatment group and the control group (P < 0.01).Lowercase letters show significant differences among all of treatments (P<0.01).

537x353mm (59 x 59 DPI)

Figure 2 Effect of initial Cd concentration on the mycelial morphology of *M. spongiola* (A) Light microscopy of control and Cd treated mycelia (magnified to 400x). The mycelium was inoculated onto solid PDA media (20 mL) mixed with CdCl₂ of different concentrations (a-0.00 mg/L; b-0.15 mg/L; c-0.90 mg/L; d-1.50 mg/L; e-2.40 mg/L; f-5.00 mg/L) and observed using a stereo microscope. (B) Determination of average sizes of mycelia exposed to different concentrations of Cd. Length and width of cells (number of cells measured: 100) were determined using the Image J software package. Results are shown as the means±SD from three repetition experiments. Lowercase letters represents the significant difference between the Cd-treatment groups (P < 0.01)

551x690mm (59 x 59 DPI)

Figure 3 Scanning electron microscopy (SEM) of *M. spongiola* at different initial Cd concentrations. Representative scanning electron micrographs of mycelia exposed to CdCl₂ of different concentrations (A- 0.00 mg/L; B-0.15 mg/L; C-0.90 mg/L; D-1.50 mg/L; E-2.40 mg/L; F-5.00 mg/L) for 5 d. Red arrows and green arrows show, respectively, irregular cell surfaces and partially fractured mycelia due to the presence of Cd deposits in mycelia treated with 2.4 mg/L and 5.0 mg/L of Cd

168x99mm (150 x 150 DPI)

Figure 4 Transmission electron microscopy (TEM) of *M. spongiola* under different initial Cd concentrations. Representative micrographs of mycelia exposed to CdCl_2 of different concentrations (A-0.00 mg/L; B-0.15 mg/L; C-0.90 mg/L; D-1.50 mg/L; E-2.40 mg/L; F-5.00 mg/L) for 5 d. White arrows represent intracellular structures in control cells which are not observed in Cd treated cells. Red arrows and black arrows show, respectively, intracellular and extracellular Cd deposits in Cd exposed cells.

170x100mm (150 x 150 DPI)

Figure 5 Effect of initial Cd concentration on Cd bioaccumulation of *M. spongiola*. Extracellular adsorption and intracellular Cd accumulation by *M. spongiola* mycelia cultured in liquid PD medium mixed with CdCl₂ of different concentrations. The final Cd concentrations were quantified with an atomic absorption spectrophotometer. Results are shown as the means±SD from three experiments. Lowercase letters represents the significant difference between the Cd-treatment groups (P < 0.01).

207x99mm (300 x 300 DPI)