



# Suppression of cadmium uptake in rice using fermented bark as a soil amendment



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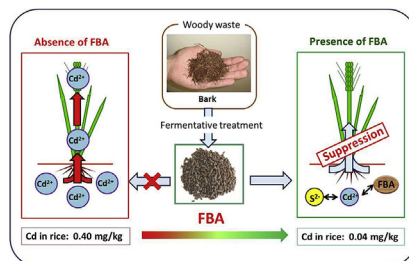
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## HIGHLIGHTS

- A fermented bark amendment (FBA) was used as an organic amendment in rice paddies.
- The application of FBA resulted in the fixation of cadmium (Cd) in the soil.
- Soil with FBA largely suppressed Cd uptake in rice under water-filling conditions.
- Soluble Cd<sup>2+</sup> ion interacted with S<sup>2-</sup> ion to form insoluble CdS species.
- FBA did not affect the uptake of Ca or Fe necessary for rice growth in paddies.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The contamination of rice paddies with heavy metals has become a serious concern due to their high toxicity to human health. In this study, we developed a chemical-free, fermented bark amendment (FBA) and used it for organic rice cultivation. The application of FBA resulted in the fixation of heavy metals, especially cadmium (Cd), in the soil and suppressed their uptake in brown rice. The suppression of Cd uptake was most effective, since its uptake in rice from FBA-supplemented soil was 10 times lower than that from untreated soil under ordinary water-filling conditions. These results could be explained by the rapid conversion of sulfate ions to sulfide ions, which subsequently react with Cd producing insoluble sulfide species, as well as Cd adsorption to the decomposed bark in soil. The FBA did not affect the uptake of metals, such as calcium and iron, which are necessary for the growth of rice. Thus, the FBA may suppress Cd uptake in rice, and its effectiveness is related to application time and water regime.

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## 1. Introduction

Cadmium (Cd) is a common pollutant in rice paddy fields and has been identified in many Asian countries such as India, Thailand, and China. Rice is contaminated with Cd when cultivated with the use of phosphorous fertilizers that contain Cd or irrigated with

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wastewater released from mines (Quezada-Hinojosa et al., 2015; Sandalio et al., 2001; Sebastian and Prasad, 2014). Heavy metal environmental pollution has negatively affected human health. The Cd-induced Itai–Itai disease in Toyama Prefecture (1910s–1970s) is one of the many documented examples that occurred due to the accumulation of Cd in the human body after the ingestion of rice and other plants.

Currently, the countermeasures against soil pollution by heavy metals include soil dressing (Arao et al., 2010), which replaces the contaminated soil, and chemical cleaning (Makino et al., 2008; Contin et al., 2008), which involves the use of chemicals to absorb heavy metals. However, the transport and operation of heavy machinery for soil dressing and the procurement of chelating agents for chemical cleaning are costly procedures.

In order to reduce the environmental impact of Cd at a lower cost, soil environment restoration, using incineration ash, wood, livestock waste materials, and plants, has been actively investigated. For example, phytoremediation, which involves the elimination of Cd in the soil using plants with high absorption capacity (e.g., plants belonging to the Poaceae family), has attracted much attention (Murakami et al., 2007; Chehregani et al., 2009; Murakami et al., 2009). However, the phytoremediation process takes at least 1–3 years and within this period, agricultural land cannot be used until Cd content is reduced to acceptable levels.

It is possible that Cd concentration in food exceeds the recommended levels, even if the levels in the soil are within the standard acceptable values (Arao et al., 2010). Thus, for soils with Cd content below the standard value, it is useful to develop methods that suppress Cd uptake by plants. This approach can be used in regions where rice and vegetables are Cd-contaminated, despite the low levels of Cd, as well as other heavy metals, in the soil. This would be effective for reducing the Cd content in rice below  $0.4 \text{ mg kg}^{-1}$ , even when the reference value of Cd content of the soil is  $150 \text{ mg kg}^{-1}$  (Ministry of Agriculture Forestry and Fisheries of Japan (2011)).

Water-filling of soil is an approach that has been used to suppress Cd uptake in rice. When the contaminated soil is filled with water, anaerobic bacteria convert sulfate ions ( $\text{SO}_4^{2-}$ ) into sulfide ions ( $\text{S}^{2-}$ ) that subsequently react with Cd producing insoluble sulfide species (Fan et al., 2010; Livera et al., 2011). However, long-term water-filling reduces the capacity of plant roots to absorb nutrients and oxygen, resulting in poor yields.

In this study, we considered the potential of bark, an environmentally friendly material produced in abundance from the thinning of cedar in Gunma Prefecture, Japan, for soil restoration. Bark consists of natural polymers, such as lignin and cellulose, and has been primarily used for fuel production or as a soil substitute (Yoshioka et al., 2005) in horticulture. Lignin, which represents about 20%–35% of the cell wall of conifer wood, is particularly known for absorbing heavy metals (Guo et al., 2008; Sciban et al., 2011), since it is not easily decomposed by bacteria or hydrolyzed as happens with cellulose. However, white-rot fungus found in humic soil is known to decompose bark (Tuomela et al., 2002; Vane et al., 2006). The assimilation of bark in the soil can be accelerated by applying it in a decomposed state, instead of using it directly. Bark composts consist of decomposing bark that is rapidly assimilated into the soil to improve plant growth and soil quality (Fukushima et al., 2009; Starzyk et al., 2013). However, the preparation of bark compost is time consuming and requires approximately 6–12 months for the complete decomposition.

Our group has developed a soil amendment that could be used to reduce Cd uptake in brown rice. The fermented bark amendment (FBA) is produced by mixing recycled bark, white-rot fungus, and bean curd to accelerate the fermentation process and can be obtained in 2–10 d using a rapid fermentation procedure as described

in Fig. S1.

The present study aimed to investigate the ability of FBA to stabilize Cd in the soil and identify the amount of FBA and application time that reduce Cd uptake in brown rice. Changes in the oxidation–reduction potential (ORP), soil pH, and the chemical forms of Cd in the soil after the application of FBA were also analyzed, and the results were compared with those of other heavy metals such as copper (Cu) and zinc (Zn).

## 2. Materials and methods

### 2.1. Preparation of FBA

Cedar bark was provided by the Forestry Association of Gunma Prefecture, while rice bran (RB), white-rot fungus, and bean curd were provided by Sasutera Ltd. (Niigata, Japan). The FBA, with a moisture content of 60%, was prepared by mixing equal quantities of bark, white-rot fungus, and bean curd using fertilizer production equipment (Sasutera Ltd., Niigata, Japan). The mixture was fermented using white-rot fungus at room temperature for 2–10 days, and materials were mixed until the FBA reached a moisture content of 15%. A schematic illustration of this procedure is shown in Fig. S1. The component analysis of FBA (total-carbon [C], total-nitrogen [N], C/N ratio, and phosphorous [P] content) was performed by an organic element analyzer (Micro Corder JM10 Element Analyzer, J-Science Labo., Ltd., Kyoto, Japan), and inductively couple plasma-atomic emission spectrometer (SPETROBULE ICP-AES, SPECTRO Analytical Instruments, Kleve, Germany).

### 2.2. Cation exchange capacity (CEC)

The CEC of FBA was determined based on the Standard for Soil Quality Testing (ISO 11260) as recommended by the Japanese Geotechnical Society. The details are described in the Section 2 of Supplementary Materials. The CEC of FBA was compared to that of bark (<2 mm) and bark-compost (BC; Aglitech, Miyagi, Japan).

### 2.3. Rice cultivation

Rice was cultivated for approximately 120 d between July and November in 2009–2014 on a rooftop at Gunma University, Gunma, Japan. The soil was collected from a site close to a Cu–Cd mine located close to an upper stream, with a Cd content of  $2.2 \pm 0.7 \text{ mg kg}^{-1}$ , which was lower than the reference value of  $150 \text{ mg kg}^{-1}$  (Table S1). The nursery plants used in this experiment were produced by a rice farmer in Gunma Prefecture, Japan.

FBA was mixed with 5 kg of soil at weight ratios ranging from 0.1% to 2.0%. At the same time, a high-grade compound fertilizer (N:P:K, 14:14:14) was added to all test soil samples at a rate of 0.5 g per pot ( $530 \text{ cm}^2$ ). Fertilizing was repeated at the same rate of 0.5 g per pot 45 d after planting. Three different water regimes were applied: (a) ordinary water-filling (approximately 5 cm water depth), (b) water-filling with midterm drying, and (c) no water-filling. Water-filling with midterm drying is a common method for rice cultivation in Japan, which strengthens the roots of plants by temporarily allowing the soil to dry-out during paddy cultivation. The ORP and pH of the soil were measured every 2 d throughout the cultivation period using pH/ORP meter electrodes (Horiba, Kyoto, Japan) inserted into the soil at a depth of 3 cm. Fermented rice bran (RB), which is commercialized from Toshuka, Ltd. (Tokyo, Japan), was also supplemented to tested cultivation soil as a compared amendment to FBA, as well as BC.

Gases generated from the soil surface during rice cultivation were collected by water replacement method using 20-mL glass vials. The  $\text{CO}_2$ ,  $\text{O}_2$ ,  $\text{N}_2$ , and methane ( $\text{CH}_4$ ) fractions were then

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