Graduate School of Science and Technology, Gunma University

Environment Engineering Science Program

Doctor's thesis

Study on Manipulations for Uptake of As, Cd and Cs by Plant Using Magnetic Nanoscale Powder and Fermented Bark Amendment

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Chapter 1

Introduction

1.1 Toxicity to living organisms

Elements make up living and non-living organisms on Earth, and human exposure comes from natural or anthropogenic sources. Several elements are known as nutrients and are necessary for living organisms, namely basic elements such as calcium, magnesium, potassium, sodium and zinc, as well as trace elements including selenium, iodine and molybdenum.

At the same time, there are many elements that cause toxicity to living organisms, which interfere with cell function and destroy health, such as mercury, lead, cadmium and arsenic. Furthermore, in Tohoku as well as Kanto in Japan, radioactive cesium is added as a toxic element due to the leakage of radioactive substances due to the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident in 2012. Therefore, studies on environmental, biological and physiological functions by such elements are continued using various research knowledge.

In this thesis, I focused on uptake of As, Cd and radioactive Cs (radio Cs) by plant. As and Cd are well-known to be toxic elements that affect human body by people ingesting foods containing them in large quantities. Radio Cs is strongly required for its removal from farmland soil to prevent ingestion of foods containing radio Cs, especially from victims by FDNPP accident.

As the countermeasures mentioned above, I used magnetic nanoparticle powder (MNP) and fermented bark amendment (FBA) to manipulate the uptake of As, Cd and radio Cs by plants. The results in these studies are detailed in this thesis.

I believe that the paper can provide important information for many researchers who are studying on the countermeasures of their elements and general persons who hope to overcome the problems by the contaminations of soils.

1.2 Radioactive Cesium (radio Cs)

1.2.1. Laws and regulations of the radioactive substance

A mass of radioactive material was reported to spread to the surrounding areas due to the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident caused by the Great East Japan Great Earthquake that occurred on March 11, 2011[1].

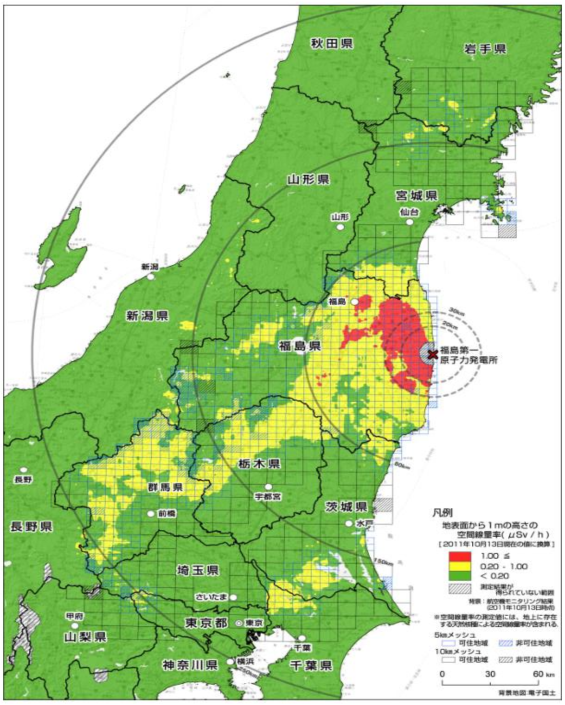

Fig. 1-1 Radioactive Cs concentration map in Fukushima and its surrounding areas

Since the FDNPP accident, many researchers in worldwide are conducting the monitoring surveys of contaminated areas to ascertain the distribution and concentration of radioactive materials diffused to the environment mainly in Tohoku and Kanto, Japan regions [2]. The main radionuclides that were generated by the fission reaction uranium-235, which is mainly used as fuel in nuclear power plants, are summarized in Table 1-1 [3]. Especially, iodine and Cs were likely to volatilize widely. Human should pay attention to the atomic volatilization after the FDNPP accident. While iodine-131 own brevity half-life for 8 days, radioisotope Cs (radio-Cs; ^{134}Cs and ^{137}Cs) will particularly continue to emit radiation over the few decades [4–6]. In addition, as many of radio Cs are water-soluble like sodium and potassium, which belong to alkali metals, it is expected to be transferred into plants such as agricultural crops through environmental waters (ground water, river water, or lake water). Therefore, the concentrations of radio Cs and the radioisotopes in foods are continuously monitored according to provisional regulation value of radioactive Cs and new reference values (Table 1-2) [7].

Table 1-1 Half-life and stable isotope of each radionuclide

Radionuclide	Half-life	Stable isotope
Cobalt-60 (^{60}Co)	5.3 years	Cobalt-59 (^{59}Co)
Strontium-90 (^{90}Sr)	28.8 years	Strontium-88 (^{88}Sr)
Iodine-131 (^{131}I)	8.0 days	Iodine-127 (^{127}I)
Cesium-134 (^{134}Cs)	2.1 years	Cesium-133 (^{133}Cs)
Cesium-137 (^{137}Cs)	30.2 years	Cesium-133 (^{133}Cs)

Table 1-2 Provisional regulation values and new reference values of radioisotopes [7]

Food variety	Regulation value（Bq/kg）	Reference value（Bq/kg）
Drinking water	200	10
Milk/Dairy products	200	50 (Milk)
Vegetables	500	100 (common food)
Shell	500	50 (for baby)
Meat/fish/egg/other	500	

1.2.2. Radio Cs contamination to agricultural land and its countermeasure

The FDNPP accident caused a large region of radiation contaminations in groundwaters, soils and plants over east area of Japan. Particularly, in agricultural land, increasing of radiation substance in crop was serious evolution [8]. As summarized in the Table 1-2, a new reference value for goods were set up by Ministry of Health, Labour and Welfare Japan since April 2012. The approaches of radioactive substances accumulation in crops are considered to be two pathways, mainly. One is direct depositions of radioactive compounds onto crops via the atmosphere, and the other one is absorption from the cultivation soil contaminated by the radioactive compounds [9]. The radioactive depositions from atmosphere can be removed by collection, while to remove the radioactive in contaminated-soil or to suppress absorption into agricultural crops are both tanglesome [8].

Current countermeasures for agricultural land decontamination of radioactive substances is a topsoil scraping, removing the topsoil, using heavy equipment. As shown in Fig.1-1, the method can apparently reduce the radioactive substances on surface layer of the soil. This is a simple and reliable method for removing the pollutant, and it has widely been applied to against the Chernobyl accident [10].

However, soil inversion method, burial of the topsoil in a layer typically of 15 to 35 cm, is an advantage method which the radioactive substances-contaminated soil will not move to any other areas. However, the method cannot basically suppress uptake of radioactive substances by crops.

As an alternative method mentioned above, applications of clay mineral materials such as zeolite and bentonite were effective method to increase Cs retention in soil and to suppress Cs absorption into crops [11-13]. However, the effect of clay mineral materials greatly varies resting with the genre of soil.

Fig.1-2 Photos of topsoil scraping procedures [14].

1.3. Cadmium (Cd) pollution

1.3.1. Cd distribution and its existence in nature

Application of Cd is extremely wide such as, pigments for paints and plastics. And in electronic devices industry, Cd is generally used as Ni-Cd batteries [15]. Most of production on Cd is on mining, smelting, and refining sulfide ores of zinc. From 1950 to 1990, the global Cd production increased for four times, and has slightly decreased in the recent decade (Fig. 1-3).

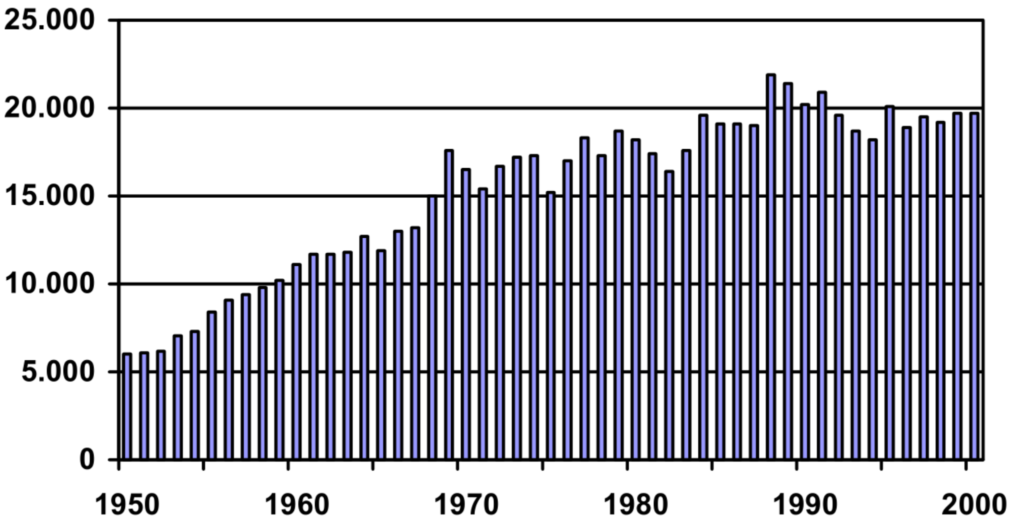

Fig. 1-3 Global Cd refinery production 1950-2000 [16]

Distribution of Cd element in the oceans is similarity property with the macro-nutrients such as zinc (Zn), implying that for marine life cadmium element is regarded as nutrient-type. Although Cd is regarded as toxic materials at high concentrations [17,18], several researchers reported that cadmium could play a role of micronutrient and instead of Zn in the enzyme carbonic anhydrase [19,20]. However, Cd intake of human, particularly, is known to be affected even in the trace level [21-23].

1.3.2. Cd toxicity to plant and human

In 1858, from Nordberg's report on acute gastrointestinal symptoms and respiratory symptoms causing by Cd carbonate powder, toxic of Cd exposure was firstly found from clinical medicine [24]. In 1920, incident of workers' Cd poisoning was reported by Stephens [24]. At the same time, Alsberg and Schwartze gave an account of a variety of clinical sign and morphological transformation in organs of vertebrates via animal experiments and made a contribution to toxicological [25,26]. In Prodan's report about cat exposure observation, after exposure in Cd oxide fume for one day, serious damages in lung, liver and kidneys were observed [27]. In the 1940's, it was reported that consuming food and drink with Cd contaminated lead to acute gastrointestinal and accompanying with vomiting and diarrhea [28]. In Nicaud's report, osteomalacia, which is a systemic consequent, was observed in Cd exposure worker [29], and this contributed to the later research for Itai-itai disease. In general, osteomalacia has characteristic of increasing serum levels of alkaline phosphatase as well as decreasing in calcium and phosphate, and biochemical of this disease also represent the same symptoms. The slight symptoms of anemia, gastrointestinal and renal dysfunction were also observed [30], and Cd content in urine was observed to increase [31].

In 1968, the Japanese government declared the source of the disease with osteomalacia was Cd dissolved from the mines and transported to downstream by the Jinzu River. The Cd polluted water was used to irrigate the farmland cultivating for rice. Rice accumulated lots of Cd in the farmland soil, and was consumed by livestock or human. The United States Department of Agriculture reported that rice is one of the most vigorous cereal for Cd absorption [32]. In a decade ago, Chinese Ministry of Agriculture launched an investigation about rice in the national market, and reported that in 10.3% of rice exceeded the national standard for Cd [33].

Considering China's agricultural trait, it is not easy to prevent Cd pollution at this stage. In China, rice cultivation is main by field owner, the farmers, and most fields are free for quality requirements. In the case of field pollution caused by industrial or mining enterprises, farmers are unwilling or unable to establish repair system.

1.3.3. Cd agricultural land treatment

Previously, several modified fertilizers could make an effectively contribution to Cd suppression during pot experiments in laboratory, such as phosphate fertilizers, clay minerals and alkaline substances [34-36].

Otherwise, red mud works efficaciously remove either mobility or bioavailability Cd in the contaminated soil via activating biological activity of microbial [37]. Lime could reduce the invalid transformation of Cd via changing of soil pH, as the solubility of Cd significantly reduced by conversion of insoluble form ($Cd(OH)_2$) with addition of lime [38,39]. Diatomite is also an effective material available to decrease this mobility of Cd from cultivation field to rice because of efficient porosity, roomy surface area and evident adsorption capacity.

However, most studies on the soil amendments, even though they are effective for reductions either mobility or bioavailability of Cd, were merely inside laboratory for limited cycle. Therefore, such applications to real agricultural production are still unknown.

1.4. Arsenic (As) pollution

1.4.1. As distribution and its existence in nature

Chemicals containing As are more than 245 [40]. Compare with volcanic activity or other natural processes, human activities enhanced accumulation of As in environmental groundwater or soil [41]. The enhancements of As concentrations are caused by 1) industrial chemicals disposal, 2) smelting of As containing minerals, 3) fossil fuel combustion and 4) use of As compounds in various products, passing over the last hundred years [42].

In earth’s crust, As mainly combined with sulfide minerals [43], for example, orpiment (As_2S_3), realgar (AsS), mispickel (FeAsS), loellingite ($FeAs_2$), niccolite

(NiAs), cobaltite (CoAsS), tennantite ($Cu_{12}As_4S_{13}$) and enargite (Cu_3AsS_4) [44,45]. According to reports by Bissen and Frimmel in 2003 [41], As content in soil of Germany ranged 2.5~15 mg kg^{-1}, Italy was 1.8 to 60 mg kg^{-1}, the United States were 1.0 to 20 mg kg^{-1}. The As content in the gold deposit reached 20 g kg^{-1} in Zimbabwe, [46]. Xiao in 2008 [47] reported that, 70% among cosmopolitan explored As stores located in China and most Chinese As mine distribute in southwest. A higher As content as much as 35,000 mg kg^{-1} was discovered inside coals in As enrichment areas in south-western Province Guizhou, China [48]. Basing on another report in western Canada, the As average contents in milled coal were 1.6 mg kg^{-1}, in bottom ash were 1.6 mg kg^{-1} and in flying ash from a coal fired power plant were 17.5 mg kg^{-1} [49].

In the report by Matschullat [45], the As source in the atmosphere is associated with volcanoes, oceans, wood and oil burning. The volcano eruption 17,150 tons of As entrances atmosphere, the ocean discharged 27 tons, and burning of wood and petroleum also natural occurring forest fires resulted to the emissions of 125 to 3345 tons. Without the influenced by human activities, some underdevelopment areas own cleaner air with only a few amounts of As.

In seawater, As concentrations ranged from 0.09 μg L^{-1} to 24 μg L^{-1} (average 1.5 μg L^{-1}), and those in fresh water were from 0.15 μg L^{-1} to 0.45 μg L^{-1} [50]. In organisms, As metabolism occurs in plants from inorganic to organic [51], transit biomethylation to form monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), tetramethylarsonium ions (TETRA) and trimethylarsonium oxide (TMAO) (Fig. 1-4 and 1-5) [53,54].

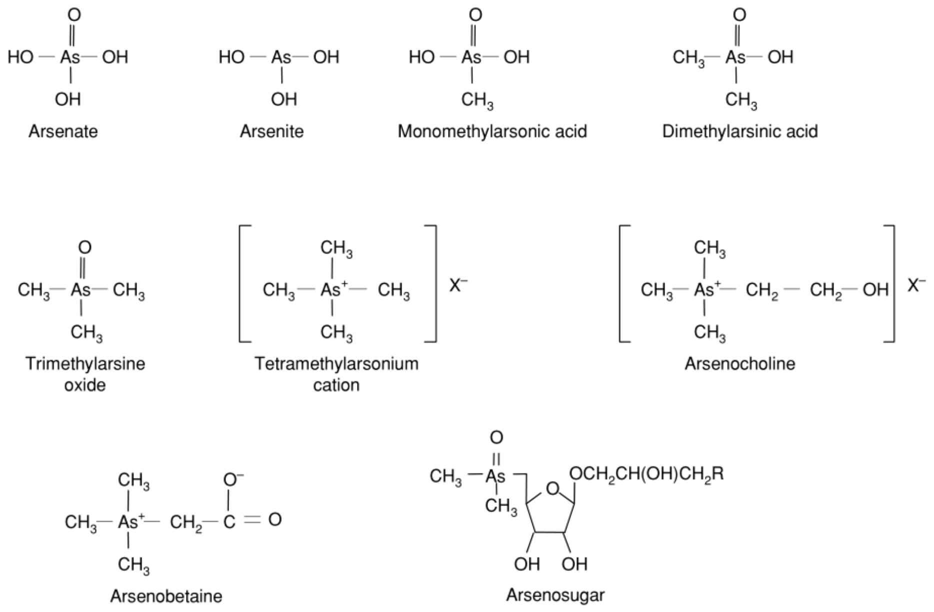

Fig. 1-4 Diagrammatic representation of As species detected in terrestrial plants [53].

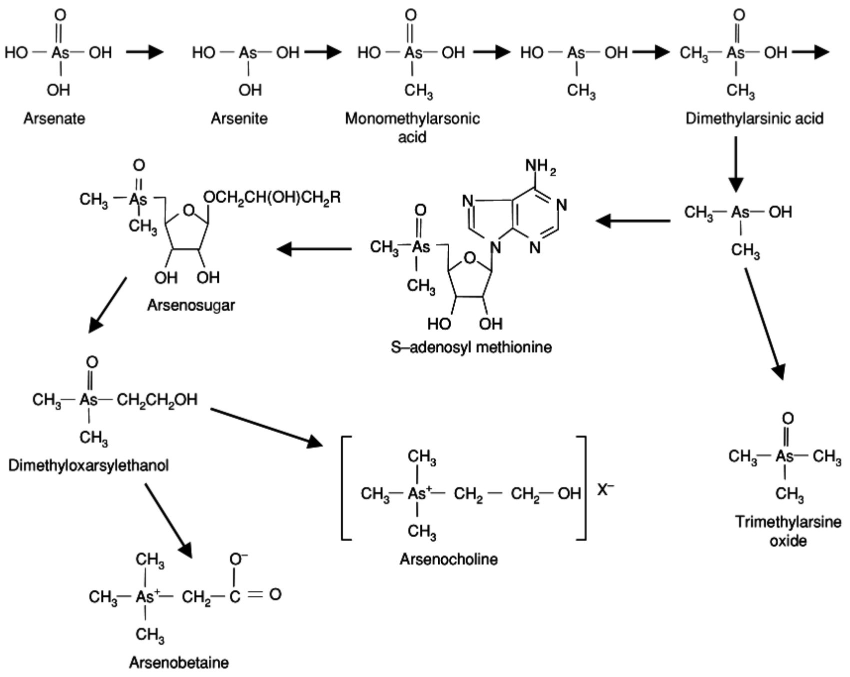

Fig. 1-5 Potential pathways for the reduction and methylation of As by terrestrial plants based on pathways for aquatic organisms and fungi [53].

1.4.2. As toxicity to plant and human

The As compounds that have toxicity for human and organisms is classified and determined by its morphology [54]. Arsine gas (AsH_3) is the highest virulence compound of As composition and fatal dose is 250 mg m^{-3} after exposing for 0.5 hour [54]. In a similar way, inorganic As is found to be much higher toxicity than organic As.

The uptake as well as accumulation of As insides crops vary on types either many factors [55]. The effects on arsenical pesticide residues in rice were studied as the earliest project for the toxicity of As species [56]. In aerobic soils, the dominant form of phytoavailable of As was arsenate (As(V)), which express suppression of root growth or cause some variety of plants (some of plant types are not resistive for As sustain) to die [47]. Many significant evidences demonstrate, exposure with inorganic As relates to a production of reactive oxygen species (ROS) [61]. Such a relationship would because the transition of As(V) to arsenite (As(III)) occur in crops' internal with enzymatic antioxidants synthesis [62-64]. Inorganic As species are high poisonous to crops under normal conditions. Rely on the similar structures, As(V) disguises to a phosphate analogue then transports thread the well brane using phosphate transport system [65]. As a highly toxic matter, As(III) general perform a reaction with sulfhydryl groups (–SH) in enzymes and tissue proteins [66]. When As is absorbed into the human body via air, water or food, it destroys the redox ability of the cells and affects the metabolism of cells [67].

The accumulation processes were illustrated in Fig. 1-5. Once As acts to a part of the human body, the symptoms are initially stimulated and tissue necrosis lasts for a long time. As has a stimulatory effect on the mucous membrane and could directly damage the capillaries [68]. As and its compounds were absorbed through the mucous membranes (including the vagina) or the skin and mainly deposited on the hair, nails, bones, liver and kidneys [69]. Whether it is chronic As poisoning or acute As poisoning, as long as one survives for more than one week after poisoning, more As can be found in the hair.

1.4.3 As mobility in nature

In complicated nature environment, As species exist as complexes via creaturely mediation, organic matter, and crucial inorganic matters [70]. Additionally, the chemical form abundance ratios of As species are strongly affected by the pH in environmental water and soil, as an important key. As shown in Fig. 1-6, dominant chemical form in neutral environment is $As(OH)_3$ while a small fraction of $As(OH)_2O^{-}$ represents less than 1.0%. At pH 7, existence of $AsO_3(OH)^{2-}$ is almost same much as $AsO_2(OH)^{2-}$. In the case of MMA, at neutral pH, $CH_3AsO_2(OH)^{-}$ is major species of MMA, while $CH_3AsO_3^{2-}$ will also be present as minor species. The major species of DMA at pH 7 are $(CH_3)_2AsO(OH)$ and $(CH_3)_2AsO^{2-}$. In the soil, inorganic As compounds exist as higher abundant than organic compounds. The pH of soil is in relation to possibility of inorganic As species uptake into plants base on mentioned above [71].

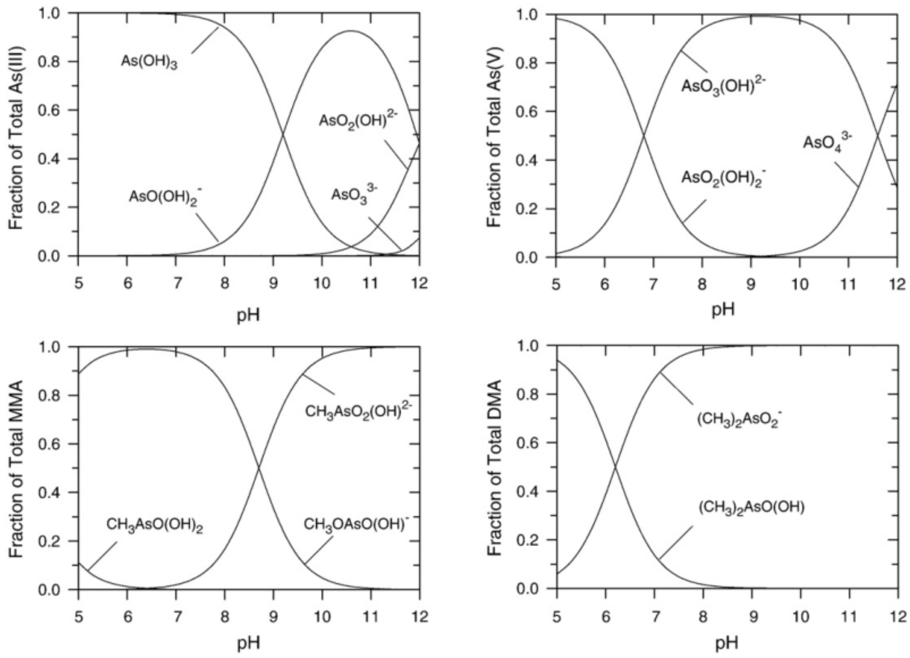

Fig. 1-6 Distribution of As(III), As(V), MMA, and DMA hydroxide species as a function of pH at 25 ℃ [71]

Therefore, there are numerous studies on the remediation tools for purification of As contaminated water [72]. Iron oxides and hydroxides, which exist extensively in natural aqueous systems, have diversity of composition and degree of crystallinity, and either seriously affect the As mobilization. Precipitation reaction of Fe(III) in aqueous solution is rapid and results in production of Ferrihydrite (approximately $Fe_2O_3 \cdot 2H_2O$). On account of large amorphous with a roomy surface, ferrihydrite could be applied as simulate solid-state adsorption surfaces in aqueous phase [73]. Otherwise, As adsorption of iron and aluminum oxyhydroxides [74,75] and iron hydroxide [76] were aggressively studied in the past.

1.5. Wood-based waste

1.5.1 Generation amount of wood-based waste

Recently, the effective consumption of fossil fuels by biomass or wood chips are recommended in terms of the recycling of biomass material [77]. For example, ash generated in the process of wood burning has a disadvantage because of the high disposal cost [78,79], but it is usable to compensate biomass as a useful fertilizer [80]. Every year, a mass of wood waste is generated during manufacturing activity of wood products. Only 2015, Germany has generated 401 million tons of wastes for one year, and 11.9 million tons of those was waste wood [81,82]. In Japan, about 2.2 million tons of wood-waste were generated by constructions and 4 million tons produced by lumbering in forest in 2015. In 2000, to promote recycling, reducing the generation of waste, and the reuse of parts and components (so called “3R policy”), Japan enforced a basic law for the promotion of a recycling-oriented society. According to 3R policy, material recycle of wood-based waste is focused on as mild recycle. Also, material recycle of woody biomass will reduce amount of carbon dioxide derived from burning of woody biomasses. However, most wood wastes would be burned or unused as in Fig. 1-7 [83].

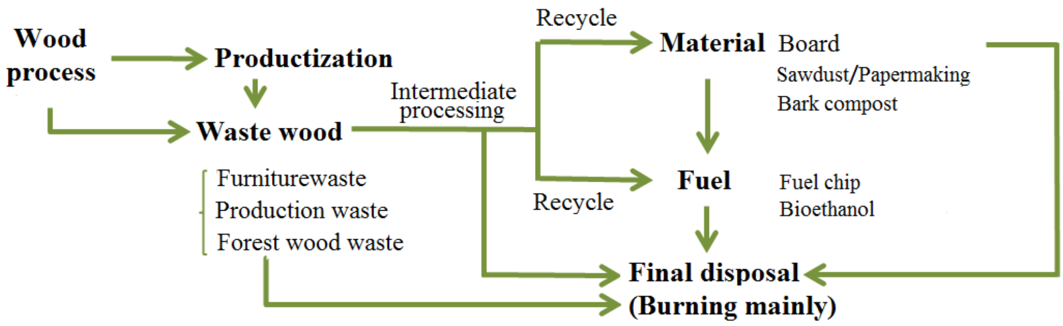

Fig. 1-7 Generation amount of wood-based waste and treatment [84]

1.5.2. Previous study on recycling of wood-based waste

Cellulosic fiber in the wood waste was reported as converting to a sorbent in removing heavy metals, e.g., mercury [85]. Moringa oleifera bark is efficient in removal of Ni(II) from water phase [86]. However, ion-exchange or adsorption capacities of most original wood materials were lower than those of other sorbents chemically produced. Therefore, chemically modifications of various original wood waste materials were required to improve the exchange and adsorption capacities. The treated juniper fiber was available as a cheap and efficient adsorbent for dissolved heavy metals removal caused by rain-water runoff, in the report by Min et al. [87]. However, bark-soluble components such as flavonides, tannins and terpenes led to increasing the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) in aqueous medium. Some appropriate treatments of wood wastes may be required to suppress the increase of COD and BOD that threatens the aquatic organisms.

1.6. Application of magnetic nanoscale powder (MNP)

Magnetic nanoparticles (MNPs) would be deemed as a new subject of special interest in medical applications [88]. Main fabrication methods for MNPs are electric explosion of wire (EEW) [89], either laser target evaporation (LTE) [90,91].

Nanoparticles of iron oxides or iron hydroxide are also comprehensive used during the specific elimination of As in environments [92-94]. Iron compounds like hematite, goethite, iron oxide-coated materials and granular ferric hydroxide have been an excellent group for As adsorption for decreasing As leaching from exhausted adsorbent [95]. Qiao et al., had employed iron to immobilize As species in soil, and indicated that could suppress the As uptake into paddy [96]. Also, rice cultivation with the zero-valent iron biochar significantly reduced uptake of Cd either As in rice, comparing with that of the blank experiment [96]. Very recently, the modifications of adsorbents such as nickel binary oxide or zirconium binary oxide onto iron oxide particle have aggressively been reported to enhance the adsorption capabilities for As in water or soil [97,98].

1.7. Purpose of this study

As mentioned above, bark (bark) used in this experiment is unused wood waste occurs in the course of wood processing. I focused on property of bark containing many sites that can bind to heavy metals [99], and converted bark into material with added-value to improve the reusability of bark. On the other hand, I employed magnetic nanoparticles (MNPs) as a sorbent that effectively immobilize As.

As a countermeasure to heavy metals and Cs pollution problems of crops, my group developed a soil reforming material (fermented bark amendment: FBA) prepared from waste bark, and applied FBA only or a mixture of FBA and MNP to establishment of cultivation method to suppress uptake of As and radio-Cs by plants.

First, the composition and properties of FBA or MNP was characterized via X-ray diffraction, X-ray fluorescence, Zeta potential analyzer, etc.

Next, the adsorption capacities of FBA and MNP for radio-Cs, As and heavy metals were evaluated in aqueous solution.

Finally, FBA and MNP were individually or simultaneously applied to cultivation media (soil and agar), and the effects of radio Cd, As, and heavy metals uptakes by plants were evaluated. In addition, the uptake mechanisms of analytes by plants when applying the amendments were discussed.

This thesis summarizes the above research results and the discussions whether the developed cultivation methods are applicable to real agricultural production, as follows.

Chapter 1 is an introduction. It describes properties of radioactive materials, heavy metals and As species as analytes in this study, applications of woody wastes and magnetic nanoparticles to treatment of contaminated groundwater or soil, besides the current problems on the environmental remediation by the waste materials and subject as well as the purpose of this research.

Chapter 2 describes the composition and properties of original bark and FBA.

Chapter 3 describes the effect on stable- and radio-Cs uptakes by rice into a Cs-contaminated soil with supplementation of FBA in pot experiment, comparing with that with supplementation of ammonium salt.

Chapter 4 describes the effect of investigating the influence for uptakes of As or heavy metals into radish with supplementation of FBA and MNP into agar media. Finally, FBA and MNP were simultaneously applied to radish cultivation.

Chapter 5 summarizes all results in chapters 2 – 4, besides discussion the uptake mechanisms of analytes by plants cultivated in soil or agar with supplementations of two amendments. This also provides issues to be examined in the future.

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Chapter 2

Characterization of fermented bark amendment

2.1 Introduction of fermented bark amendment

In Japan, reuse and recycle of bark largely produced in every year are required. Bark is composed of natural polymers such as lignin and cellulose, but it is more difficult to pulp or chip because of containing more fibrous than the tree trunk. Although fuel conversion is being considered as thermal recycling, the moisture content in bark is 30% higher than the tree trunk. In practice, most bark is hardly reused in the world [1]. On the other hand, 20% to 35% of lignin in coniferous bark contains carboxyl group besides phenolic hydroxyl group, and lignin is known to adsorb heavy metals [2-4]. My group focused on adsorption ability of this lignin to the heavy metal and tried to develop the cedar bark to a kind of environment friendly materials. Although bark itself could adsorb heavy metals, my group further modified bark as a soil amendment material of heavy metals-contaminated agricultural land, considering the effective potential of bark. However, lignin in original bark is so hard to decompose only using ordinary bacteria or hydrolysis [5,6]. Therefore, bark should be forbidden to spray directly on farmland. The most normal method to apply bark on agricultural land is to ferment it with water and make compost. However, it takes as long as 0.5~1 year to compost.

Our laboratory develops a production process which could ferment bark in a short period, and names production as fermented bark amendment (FBA) [7] (Fig. 2-1). In the process, bark, okara containing white rot fungus (hereinafter referred to as rot fungus) and rice bran were mixed added into bark in ratio of 1: 1: 1. If the FBA provides higher heavy metal adsorption capacity than original bark, it should be applied as a new utilization technique of bark.

In this chapter, the FBA obtained by above procedures were characterized from the chemical composition by organic elemental analysis and energy dispersion X-ray fluorescence (EDX), and cation-exchange capacity (CEC) by titration method. These obtained results were compared to those of original bark. Through this study, I discussed the adsorption capability of metal cations in soil.

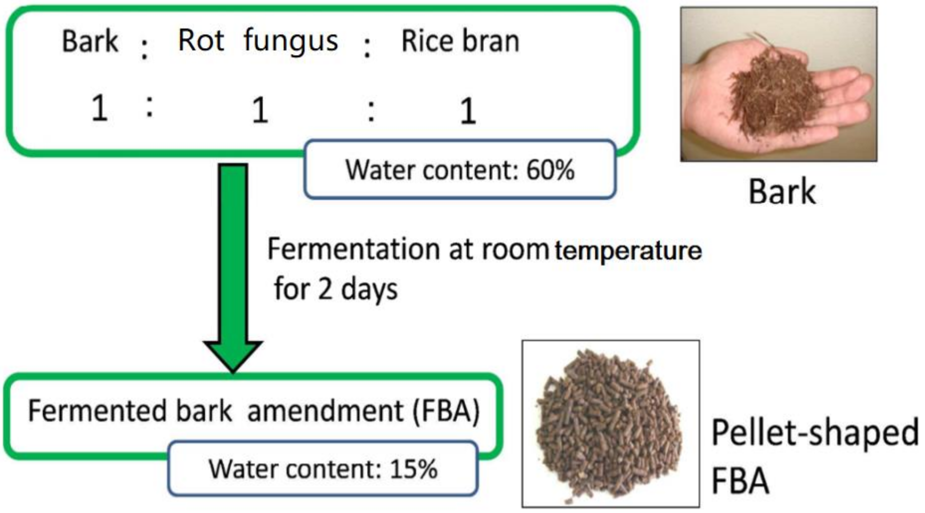

Fig. 2-1 Procedure of FBA production

2.2. Experimental

2.2.1. Preparation of FBA

The bark used in this experiment is from “Sinrin Kumiai Rengoukai” of Gunma Prefecture and FBA prepared by the process shown in Fig. 2-1. During the process of FBA production, white-rot fungus, while rice bran (RB) and bean curd were from Sasutera Ltd. (Niigata, Japan).

FBA preparation should employ mixture of equal volumes of bark, rice bran, and okara containing white rot fungus (hereinafter referred to as rot fungus), and tap water was added so that the initial water content was 60%, and all mixture were finally fermented in fertilizer manufacturing machine (Shirane Land Transport Co., Ltd.). The fermentation treatment was performed by the microbial activity in white rot fungus without an electric heating. They were mixed to be less than 15% of water content. The procedure was carried out from 2 to 30 days.

2.2.1. EDX analysis and organic elemental analysis

As a pretreatment, each of bark and FBA was lyophilized and then powdered (less than 2 mm) using a mortar before the experiments.

In order to analyze the composition of bark and FBA, hydrogen(H), carbon(C), nitrogen(N) and C/N ratio were determined by elemental analysis. Nitrogen(N), phosphorus(P), Potassium(K) and sulfur(S) were also analyzed later and fluorescent X-ray analysis (EDX) (EDX-800 manufactured by Rayny) was used for further measurement.

2.2.2. CEC of original bark and FBA

Materials and reagents

Bark and FBA were was lyophilized and then powdered (less than 2 mm) using a mortar as a pretreatment. Cd contaminated soil in a certain area in Akita prefecture was used as soil sample.

Reagents used in the analysis were most manufactured by Wako Pure Chemical Industries, barium acetate (purity: 99%), including sodium hydroxide (purity: 97%), thymol blue sodium indicator, bromothymol, magnesium sulfate (95%), barium chloride dihydrate (99%). Amidosulfuric acid (99%) and lanthanum nitrate hexahydrate (99%) were manufactured by Kanto Kagaku Co., Ltd.

Ultra-pure water equipment (Simpli Lab) manufactured by Japan Millipore Co., Ltd. was used for ultra-pure water preparation. For the measurement of Mg concentration, inductively coupled plasma emission spectrometer (ICP - AES) (ICPS - 1000 III provided by Shimadzu) and magnetic stirrer (Pasolina Mini Stirrer CT - 1) were used.

Barium acetate method (fertilizer control law) [8].

Samples of bark and FBA was respectively taken by 0.2 g and added into 25 mL of 0.05 M hydrochloric acid, stirring for 20 minutes, filtrating mixture for twice. And during this procedure, the cation exchange group of the sample was replaced with hydrogen ion.

After that, excessive hydrogen ions were washed away by water, then adding 25 mL of barium acetate solution into the sample and stirring. After 1 hour standing, repeating this procedure again and hydrogen ions were replaced by barium ions.

Next, excessive barium ions were washed away by water, then thymol blue was added into the sample and the subsequent eluate was titrated with 0.05 M sodium hydroxide. At this time, CEC was obtained by the equation 1. Sulfuric acid amide was used for orientation of sodium hydroxide.

$$\text{CEC (cmol/kg)} = (w_1 - w_0) \times 5F / W \qquad (1)$$

Here, w_0 is the amount of sodium hydroxide (mL) (50 mL of barium acetate solution + 150 mL of water), w_1 is the amount of dropwise addition of sodium hydroxide in the eluent (mL), W is the amount of sample (g) as dry matter, F denotes a correction coefficient.

Test method for soil cation exchange capacity (standard of Japanese Geotechnical Society) [9].

Adding 30 ml, 0.1 mol/L barium chloride liquor into 2.5 g of dry soil sample then shaking for 1 hour. Then centrifuge was taken at 3000 rpm for 10 mins, then supernatant should be removed. This operation should be repeated twice. 30 ml of a 0.0025 mol / L barium chloride liquor was added into sample, shaking the mixture for 10~14 hours, then the mixture should be centrifuged (3000 rpm, 10 minutes) then supernatant was removed. Next, 30 g of 0.02 mol/L magnesium sulfate was added into the sample, shaking the mixture for 1014 hours, then the mixture was centrifuged (3000 rpm, 10 minutes) and the supernatant was filtered with filter paper. Following, 0.3 mL of 0.1

mol/L barium chloride solution and 10 mL of 10 mg La/L acidic lanthanum solution should be added into 0.2 mL of the filtrate, after this mixture solution should be made up to 100 mL and became sample solution. Then, instead of the filtrate, 0.2 mL of 0.02 mol / L magnesium sulfate, barium chloride, acidic lanthanum was added into and this mixture should be made up to 100 mL and became blank liquor. The magnesium concentration of this sample solution and blank solution was measured using ICP-AES, and measurement result would be fitted into Equation 2 and Equation 3 to obtain CEC (cmol / kg).

$$C_2 = C_1 \times (a + m_2 - m_1) / a \qquad (2)$$

$$\mathrm{CEC}\,(\mathrm{cmol/kg}) = (C_b - C_2) \times 100 \times a / m \qquad (3)$$

Here, C_1 is magnesium concentration (mmol/l) in the sample, C_2 is magnesium concentration (mmol/l) in the corrected sample, m_1 is the mass (g) of the centrifuge tube containing dry soil, m_2 is the mass (g) of the centrifuge tube containing wet soil. The mass (g) of the centrifuge tube containing soil is as follows: “a” is the mass of the magnesium sulfate solution for cation exchange (30 g), C_b is magnesium concentration for blank (mmol/l), m is mass for dry soil (kg).

2.3. Results and discussion

2.3.1. EDX analysis and organic elemental analysis

First, FBA obtained in this study were characterized for chemical composition using elemental analysis and EDX as well as original bark. The results of organic element analysis and EDX analysis were summarized in Table 2-1 and Table 2-2.

In elemental analysis, organic carbon (C), nitrogen (N) in original bark was respective 51.8% and 0.10%, respectively, and the C/N ratio was 518. On the other hand, C and N of FBA were 35.9 ± 0.4% and 2.92 ± 0.10%, and C/N ratio was 12.3.

The C/N ratio is one of important indicator when evaluating the difficulty among decomposition of organic matters by microorganisms, the ease of manifestation of fertilizer effect, and the degree of compost maturity [11]. As summarized in Table 2-1, obviousness to understand, the C/N ratio of FBA is much smaller than that general bark, which makes it easier to assimilate into soil. Also, in general, compost with a C/N ratio over 12.3, against the average C/N ratio of commercial chemical fertilizers is approximately 0.1. The C/N ratio of FBA was significantly lower than that of bark, but the application of FBA in large quantities causes a reduction in the amount of N available to the crop.

Next, the N, P besides K rate of FBA was respective 2.9%, 1.5%, and 2.8%, and giving a total content of 7.2%. According to fertilizer control law in Japan, summation of N, P, K of chemical-fertilizer specified in domestic fertilizer should be 30%. For FBA was positioned as a low-fertilizer material, the addition of a small amount of chemical fertilizer was required in the rice cultivation as described in Chapter 3.

In the EDX analysis, the main component elements of original bark were Ca> Fe> K> Zn, and in FBA was Ca> K> Fe> S. Although the main components of original bark and FBA were almost similar, sulfur (S) in FBA was higher content than that in original bark. This might be related to the fact that soybean curd and white rot fungus contained sulfur.

Table 2-1 Elemental analysis results for bark and FBA

Sample	Content (wt%)			
	H (hydrogen)	C (Carbon)	N(Nitrogen)	C/N ratio
Original bark	5.62	51.8	0.10	518
FBA	6.19	35.9	2.92	12.3

Table 2-2 EDX analysis results of original bark and FBA

Original bark		FBA	
Ca (%)	50.4	Ca (%)	68.5
Fe (%)	36.0	K (%)	17.0
K (%)	5.57	Fe (%)	8.23
Zn (%)	4.02	S (%)	3.96
Other (%)	4.04	Other (%)	2.29

2.3.2. CEC of original bark and FBA

Generally, the surface of soil particle has negative charge, and electrostatically binds cations such as alkali metals (Na^+, K^+) also alkaline earth metals (Mg^{2+}, Ca^{2+}), in order to keep the electrical neutral [12]. When a fertilizer is added into a soil, the cations in fertilizer are retained into the soil particle based on the cation-exchange reaction [13]. On the contrary, the cations which retained previously in the soil particle are released [14]. Therefore, the cation exchange capacity (CECs) of soil or fertilizer is an important factor affecting the quantity of cations in fertilizers in agriculture [15].

Table 2-3 summarizes the CECs of original bark, FBA and soil sample used in Chapter 3. The CEC of FBA was time-dependence and much larger compared with original bark. It is easy to understand that increasing in FBA CEC is in connection with degree of decomposition of organic matter in bark.

On the other hand, for the CEC of the soil was lower than FBA and bark, retention of heavy metal ions and Cs ions in soil was expected to be weak [16]. Therefore, the supplementation of FBA may effectively enhance cation exchange in the soil and retain heavy metals, thereby suppress absorption of heavy metal into plant.

Table 2-3 Cation exchange capacities (CEC) of original bark, FBA and cultivation soil

	Fermentation period (days)	**CEC（cmol/kg）**
Original bark	--	20.5 ±3.6[a]
FBA	2	32.1 ±6.0
	3	48.1 ±2.0
	6	68.5 ±2.7
	10	72.0 ±3.9
	30	72.3 ±2.2
Cultivation soil	--	10.6 ±1.1

[a] The CEC indicates mean value and standard deviation estimated from triplicate measurements.

2.4. Conclusions

From the experimental results, the C/N rate of FBA was smaller compared with original bark. Low C/N of FBA means to easier decompose in soil than bark. Additionally, the N, P, K concentration of FBA was respective 2.9%, 1.5%, and 2.8%, and giving a total content of 7.2%, so that FBA is identified as a low-chemical fertilizer. Furthermore, as the CEC values were order of original bark < FBA, the supplementation of FBA may effectively enhance cation exchange in the soil.

It were expected that the decomposition of original bark by microorganisms would improve the bark adsorption abilities for heavy metals. That is, the supplementation of FBA might be expected to be effective to suppress mobilization of heavy metal in soil.

In the next chapter, I attempted the adsorptivity of FBA for Cs and the uptake of Cs by rice with supplementation of FBA, comparing that for heavy metals.

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Chapter 3

Radio-Cs uptake by rice during supplementation with a fermented bark amendment to radio-Cs-contaminated soil

3.1. Introduction

As mentioned in Chapter 1, radioactive materials that were released due to accidents at nuclear power plants gave severe contaminations in agricultural lands in eastern Japan, especially in Fukushima prefecture [1]. Cs-137 (^{137}Cs) with a longer half-life caused the extensive soil contamination, more serious than other radioactive substances [2]. The crops cultivated there have the possibility that adsorb radioactive substances from the contaminated soil. Therefore, the accumulation of radioactive substances in rice, which is a staple food in Japanese, might contribute to a serious problem for millions of people [3]. In Japan, there are strict reference values for radioactive material contamination in rice (see Table 1.2).

Current countermeasures are topsoil scraping and reverse cultivation using heavy equipment and those are implemented in some agricultural lands. However, there are still difficult problems about final treatment, either the contaminating soil during topsoil scraping or the accumulation radioactive materials in plants.

In this chapter, referring to the previous report by our research group [4], the effect of FBA supplementation was evaluated via rice cultivated in the contaminated soil, while ammonium salt was applied as same to compare. Finally, impact of FBA on radio Cs accumulation by rice was discussed.

3.2. Experimental

3.2.1. Reagents

A multi-element mixed standard solution WV (Cr, Mn, Fe, Ni, Cu, Zn, Cd, Pb) (ICP analysis) besides a Cs standard solution (1000 mg-Cs L^{-1}) used for creation of the calibration curve were from Wako Pure Chemical Corporation (Wako, Osaka, Japan). Preparation of an artificial Cs contaminated soil needed analytical grade reagent of Cs chloride (CsCl) (Wako). The ammonium salt supplied during cultivation was ammonium sulphate ($(NH_4)_2SO_4$). 2-Morpholinoethanesulfonic acid, monohydrate (MES), was purchased from Dojindo Laboratories, Ltd. (Mashiki-machi, Kumamoto, Japan). Ultrapure water (Simplicity® ultrapure water system; Millipore, Burlington, MA, US) was used during all preparations of standard solutions and dilutions of sample solutions.

3.2.2. Analysis Device

A freezing dry device (FDU-12AS type, AS ONE Corporation, Osaka, Japan) was employed to dry all harvested paddy. Pearl material microwave high temperature pressurized acid decomposition vessel (Model 4781) and Fujitsu General Microwave oven (BE-50C5-H) were used to decompose the paddy sample. The concentrations of heavy metals and stable isotope Cs (^{133}Cs) in sample solutions was quantified by inductively coupled plasma-mass spectrometer (ICP-MS) (HP 4500 manufactured by Agilent). Concentration of radio Cs (^{137}Cs) was quantified by gamma ray spectrometer (GEM20-70, Seiko EG & G, LTD, Tokyo, Japan).

During rice cultivation in the pot, variations of pH values in the soil were monitored with a portable pH meter (C-62, AS ONE Corporation). The electric conductivity (EC) and temperature of soil during the cultivation were measured using portable EC/℃ tester HI 98331 (HANNA, Hanna Instruments, Woonsocket, RI, USA). The oxidation-reduction potential (ORP) in soil surface during the cultivation was measured by an ORP electrode (9300-10D, Horiba Ltd., Kyoto, Japan) jointed in portable pH meter D-72 (Horiba).

3.2.3. Adsorption tests of Cs and heavy metals to original bark and FBA

To clarify the adsorption of FBA to Cs and heavy metals in aqueous solution, the adsorption test was performed as following;

Original bark and FBA were first smashed into powder of diameter within 2 mm. 0.5 g of mashed powder was putted to 50 mL of MES buffer liquor containing Cs and heavy metal ions solution (pH 6), and was stirred for 1 hour at room (ambient) temperature. After filtrating by syringe filter (0.45 μm), metal ions concentration in sample solutions were quantified by ICP-MS. Equations (3-1) and (3-2) were used to calculate the adsorption rate.

$$C_2\,(\mathrm{mol/L}) = C_0 - C_1 \qquad (3\text{-}1)$$

$$\text{Adsorption rate}\,(\%) = C_2 / C_0 \times 100 \qquad (3\text{-}2)$$

Here, C_0 means initial concentration (mol/L), C_1 means Cs and metal ion concentration (mol/L) after stirring, and C_2 is the adsorption amount (mol/L).

3.2.4. Rice cultivation

Three types of cultivation soil were prepared in this study; Soil #1 was collected from the south of Gunma Pref. on July 20, 2012 and was artificially contaminated with 100 mg/L CsCl solution; Soil #2 was a blend of Cs-contaminated soils collected from Chiba Pref., Fukushima Pref. and south of Gunma Pref. Soil samples in the Chiba Pref. and Fukushima Pref. were collected at the beginning of April 2013, and that in south of Gunma Pref. was on July 20, 2012. The blended weight ratio of the three soils was Gunma: Chiba: Fukushima = 1.5: 4: 3. The contents of radioCs and common heavy metal contents in each soil sample were summarized in Table 3-1.

Table 3-1 Contents of radio-Cs (^{137}Cs) and common heavy metals in three types of soil samples

Soil#	^{137}Cs (Bq/kg)	Metal concentrations (mg/kg)					
		Cs [a]	Cu	Zn	As	Cd	Pb
1	ND [b]	74	33	31	7.4	0.12	39
2	6.8×10^3	0.024	36	25	0.085	0.042	147

[a] Stable Cs in soil sample was quantified as ^{133}Cs by ICP-MS.

[b] ND = not detected.

The paddy variety sown was “Goropikari”, which belongs to Japonica and is one of the main varieties produced in Gunma Pref. The nursery plants provision was a farmer of Gunma Pref., Japan.

Rice was grown in pots for approximately 90 days between July and September 2012–2015 on rooftop in Gunma University, Kiryu-city, Japan. Soil (4 kg) was added into 15-L plastic pots and tap water was poured to 15 cm-height from the soil surface. Meanwhile about 0.5 g high-grade fertilizer (N: P: K = 14:14:14) should be added into each sample (530 cm^2 of each bucket). FBA should be mixed into 4 kg of soil at weight rate of 0.1%, 0.5% and 1.0%, 20 days after planting of rice seedlings. The photo in the pot experiment is shown in Fig. 3-1.

Fig. 3-1 Cultivation of paddy in bucket

The cultivation process employed water filling besides midterm drying, as a common paddy cultivation method in Japan, is good for roots growth. Midterm drying was performed for one week on 50th days after planting. To estimate the variation of analytical results for each cultivation condition, three pots were prepared for the same cultivation condition. The EC, temperature, ORP and pH of the soil should be tested once for every 2 days during period by EC/temperature meter (Hanna) and pH/ORP meter electrodes (Horiba) inserted into the soil at a depth of 3 cm.

3.2.5. Influence of FBA and NH_4^+ salt application

Cultivation of rice lasted for approximately 90 days from July to September 2015 on rooftop of Gunma University, Gunma, Japan. Soil type used in this experiment was Soil C, which is a radio Cs contaminated soil. Here, 0.1wt% $(NH_4)_2SO_4$ was added just after planting of seedlings with fertilizer. Furthermore, I also prepared two different soil conditions; soil sprayed with 1% FBA on 50th days after planting and soil with 0.1wt% $(NH_4)_2SO_4$ sprayed 1% at same time. All the other conditions were the same as described in section 3.2.5.

3.2.6. Analyses of Cs contents in rice and soil

First, root and stem samples were cut in two parts at its top and bottom. Approximately 0.1 mg crushed, freeze-dried sample was dissolved into 2.5 mL of concentrated hydrofluoric acid (HF), 1 mL of 60% perchloric acid before being subjected to microwave wet decomposition. The HF in the solution was removed by concentrated nitric acid because HF corrodes the nebulizer or torch of the ICP-MS; the 50-mL treated solution was analyzed by ICP-MS.

In addition, approximately 6.0 g of dried soil was sieved through a 2-mm mesh screen, shaken, and churned in 200 mL of 1 M hydrochloric acid. The mixture was filtered and analyzed using ICP-MS for stable-Cs (^{133}Cs) and heavy metals. The concentration of radio Cs (^{137}Cs) was quantified by gamma ray spectrometer (GEM20-70, Seiko EG & G, LTD, Tokyo, Japan).

3.2.7. Leachability test of radio-Cs by several salt solutions

I performed the leachability test of radio-Cs by using several salt solutions during releasing Cs adsorbed on soil particles (NaCl, KCl, NH_4Cl and $MgCl_2$) to investigate the ion-exchange capability of metal salts. The operation of leachability test was according to the procedures of first fractionation (*i.e.*, ion-exchange form) sequential extraction proposed from Tessier et al. (1979) [5]. In this experiment only, I used the radio-Cs contaminated soil from the area surrounding Lake Onuma at Mt. Akagi

(N36°32'38.7", E139°11'20.9": Maebashi, Gunma Pref., Japan) on June 10, 2014. On the basis of extractant-to-soil sample ratio as 8 (mL g^{-1}), the fraction was made by stirring mixture of 40 g sample and 320 mL of 1 M metal chloride solution (pH 7) at ambient temperature for 1 h.

Extractant and the residue of the fractionation were separated by the suction filtration method. The extractants were later mixed with pure water using a 2-L volumetric flask, thus radio-Cs was quantified as described above (section 2.2). In addition, gamma-ray spectrometry was employed to quantify the radio-Cs in the solid residue obtained following the fractionation above.

3.3. Result and discussion

3.3.1. Adsorption capabilities of metal cations

In the solution of pH 6, the adsorption ratio of Cs^+ on original bark or on FBA was determined, and compared with adsorption rates of heavy metals ion (Cu^{2+}, Cd^{2+} and Pb^{2+}). In Table 3-2, the adsorption rate of Cs^+ on bark and FBA were extremely lower than those of Cu^{2+}, Cd^{2+} and Pb^{2+}. This might due to that the chemical form of Cs^+ is a monovalent cation with a large ionic radius and the surface charge density is small. The adsorption of Cs^+ on bark and on FBA would base on the cation-exchange interaction. The maximum adsorption capacities of Cs^+ on bark and on FBA were 1.1 μmol g^{-1} and 1.6 μmol g^{-1}, respectively. Besides, the maximum adsorption capacities of heavy metals on FBA were 17 μmol g^{-1} of Cu^{2+}, 11 μmol g^{-1} of Cd^{2+} and 15 μmol g^{-1} of Pb.

Table 3-2 Adsorption rates of Cs and heavy metal ions to original bark and FBA

	Adsorption rate (%)			
	Cs^+	Cu^{2+}	Cd	Pb
Original bark	33	97	89	98
FBA	15	85	93	90

3.3.2. Concentrations of Cs and heavy metal in cultivation soil

Prior to rice cultivation, the Cs contents in the soils were quantified after complete acid digestion. In Soil #1, as 100 mg L^{-1} CsCl was artificially added, the content of Cs quantified was high (74±15 mg L^{-1}, $n = 3$) and radio-Cs was lower than the limit of quantification (< 1.0 Bq kg^{-1})[6]. In Soil #2, the ^{137}Cs content was 6,800±301 Bq kg^{-1} (measured on May 13, 2013) because it was obtained blending with the contaminated-soils of 5,000–8,000 Bq kg^{-1} ^{137}Cs. The results are summarized in Table 3-3.

Table 3-3 Cs and heavy metal content in each cultivation soil (Unit: mg/kg; ^{137}Cs Unit: Bq / kg)

Sample #	Cu	Zn	As	Cd	Pb	^{133}Cs	^{137}Cs, Bq kg^{-1}
1	33	31	7.4	0.12	39	74	--
2	36	25	0.085	0.042	147	0.024	6800
Soil average value	34	86	--	0.1	29	--	208 (Gunma Pref.)
Soil environmental standard value	125 (Agricultural land)	--	150	150	150	--	5000

3.3.3. Changes in temperature, EC, pH and ORP of cultivation soil

Fig. 3-4 shows variations of temperatures, EC, pH and ORP of soil surface (depth: 3 cm) during the cultivation period in blank soil without any additives but supplying chemical fertilizer and soil with supplementation of 1% FBA. As shown in Fig. 3-2, the temperature of soil surface was relatively constant range both blank soil and soil with 1% FBA from 18 °C to 28 °C despite being influenced by outside air (Fig. 3-2a). The EC values in both blank soil and soil with FBA gradually decreased with the cultivation time (Fig. 3-2b). This would be because the electrolyte concentration eluted from the soil was decreased with the exchange of water. The pH in soil was rapidly lowered after spraying FBA (Fig. 3-2c), because the cultivation soil might be acidified by humic acid with decomposition of FBA when filling water on the soil surface [7]. The ORP value performed the extraordinary reduction after supplementation of FBA in the cultivation soil (Fig. 3-2d). This would be because of oxygen consumption by the microorganisms in FBA and/or aerobic microorganisms increasing after FBA spraying, resulting in creating a reduced state [8].

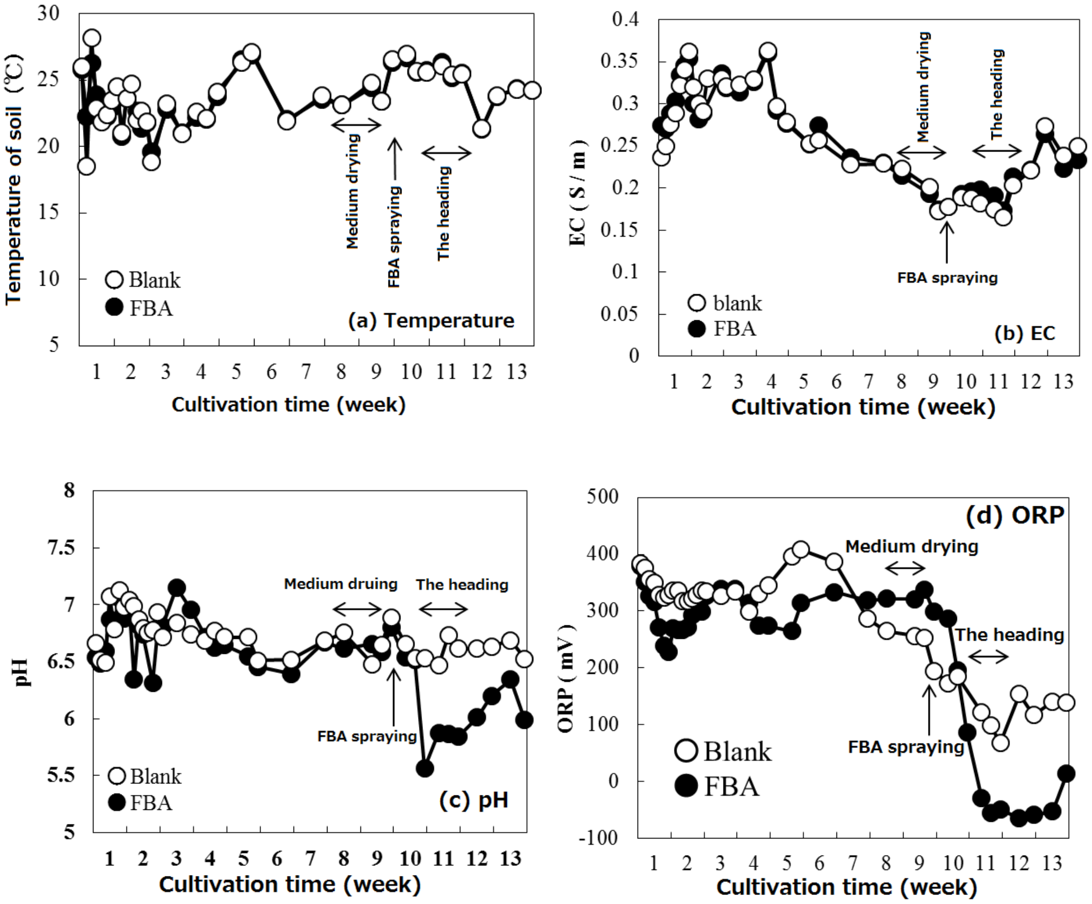

Fig. 3-2 Change in Temperature (a), EC (b), pH (c), and ORP (d) in the cultivation soil of Blank and 1% FBA spreading group.

3.3.4. Uptake of stable Cs by rice in soil with FBA

To evaluate this effect of soil supplementation of FBA on Cs uptake by rice plants, the rice plants were cultivated in the artificially Cs-contaminated soil (Soil #1) with FBA supplemented at 0%, 0.1%, 0.5% and 1%. The Cs (^{133}Cs) contents in brown rice are shown in Fig. 3-3, with those of Cu and Cd tested for comparison. While the uptake of Cu and Cd was suppressed due to FBA application into soil, the trend of Cs uptake was reversed, that is, the Cs content increased in response to the amount of FBA supplementation. The suppression on Cd accumulation was distinct depending on number of FBA addition into soil. The Cd accumulation into rice cultivated in 1% FBA supplemented soil was significantly lower ($P < 0.001$) than rice cultivated without FBA supplementation (i.e., chemical fertilizer only). However, in 1% FBA supplemented soil, the Cs concentration was significantly higher than that of blank ($P < 0.01$), which was opposite response to that of Cd.

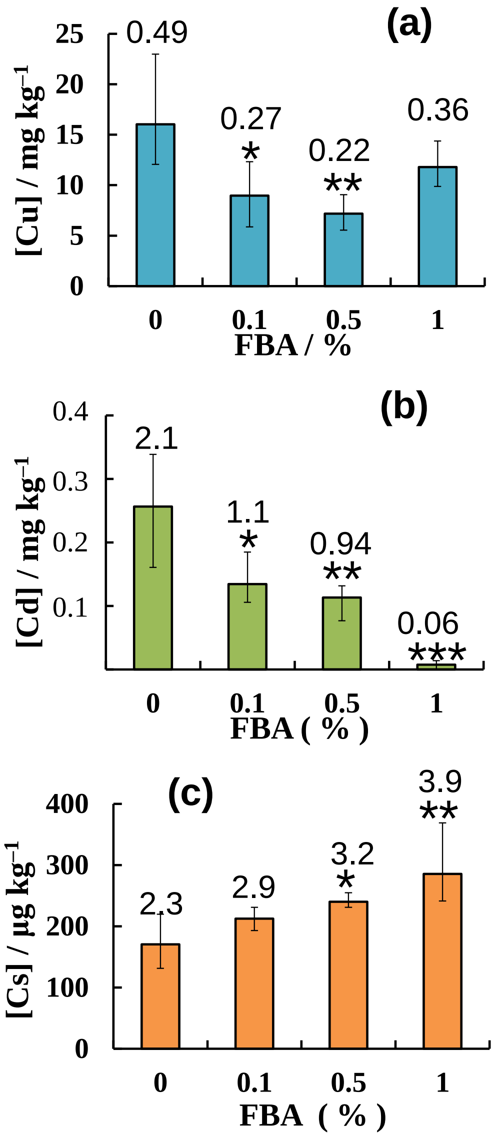

Fig. 3-3 Variations in (a) Cu, (b) Cd and (c) Cs as ^{133}Cs uptakes in response to the amount of FBA applied to cultivation soil. Soil type: #1 (artificially Cs-contaminated soil). Concentrations of analyte metals in Soil #1: [Cu(II)] = 33 ±9 mg kg^{-1}; [Cd(II)] = 0.12 ±0.1 mg kg^{-1}; and [Cs(I)] = 74 ±15 g L^{-1}. Values on the bar graphs are the transfer coefficients (TF = [metal in each part of rice] / [metal in soil after cultivation]). Error bars indicate standard deviation at *n* = 3. Asterisks indicate significant differences between bars at $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***).

3.3.5. Radio Cs uptake by rice in soil with addition of FBA and NH_4^+ salt

Next, I tested the uptake of radio-Cs by rice plants in soil contaminated with relatively high radio Cs content (Soil sample No. 3 with 6800 Bq kg^{-1} ^{137}Cs).

The ^{137}Cs contents in rice plants with 1% FBA application were 1.5-fold higher in roots, 2.5-fold higher in stems, and 2.8-fold higher in brown rice, respectively, if compared with those in soil without FBA (Fig. 3-4). The radio-Cs uptake into rice plants was accelerated by the addition of FBA, although the transfer coefficients (TF) obtained in this experiment were lower than 1.

The uptake of Cs^+ by plants from soil is considered to occur by the same mechanism as NH_4^+ [9-11] and K^+ [12,13]. Here, I compared the radio-Cs contents in rice plants harvested in soil sprayed with 0.1% $(NH_4)_2SO_4$, 1% FBA, and a mixture of 0.1% $(NH_4)_2SO_4$ and 1% FBA. The ^{137}Cs uptake into plants was accelerated by spraying with NH_4^+ salt, indicating a similar response to that of FBA. In the soil sprayed with NH_4^+, the amounts of Cs in the stem and in brown rice were both 1.4-times higher than for those sprayed with FBA alone.

During rice cultivation, the ORP under the above conditions was monitored over time (Fig. 3-5). The ORP decreased substantially in the soil sprayed with FBA, depending on the duration of cultivation. The pH showed variations because of rainfall and sunshine conditions, whereas there was no significant influence of FBA spraying on pH (Fig. 3-6). This mechanism is discussed in sections 3.3.6 and 3.3.7.

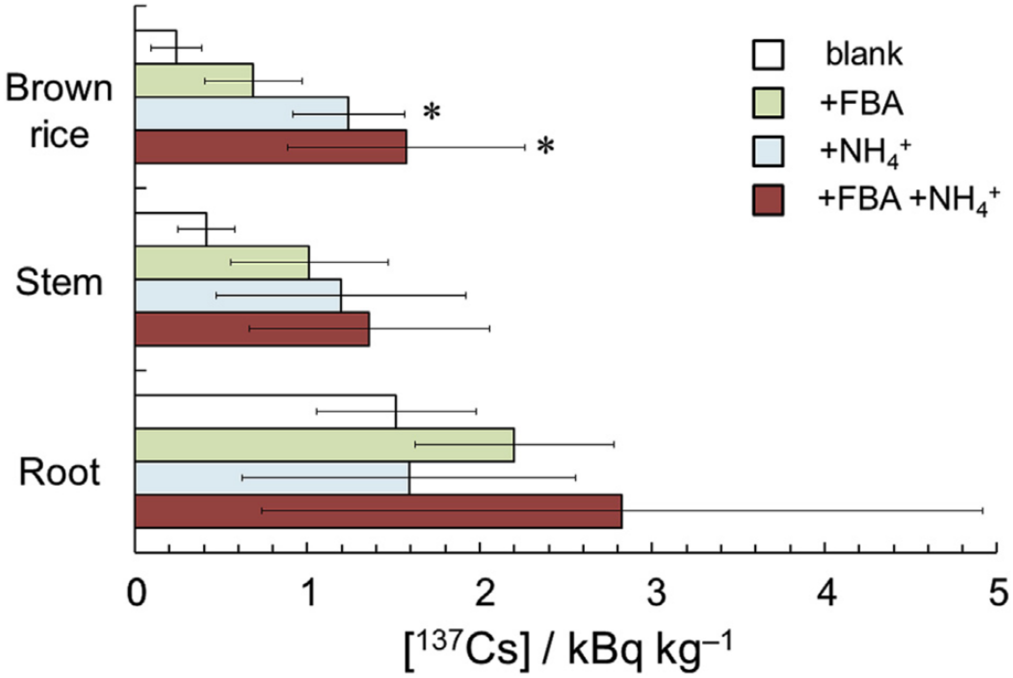

Fig. 3-4 Concentrations and concentration coefficients of ^{137}Cs in the roots, stems, and brown rice of plants cultivated in soil supplemented without any additives (blank), with 1% fermented bark additive (FBA) (+FBA), 0.1% ammonium salt (NH_4^+) (+NH_4^+), and 1% FBA and 0.1% NH_4^+ (+FBA/+NH_4^+). Values on the bar graphs are the transfer coefficient (TF = [Cs in each part of rice] / [Cs in soil after cultivation]). Error bars indicate standard deviation at $n = 3$.

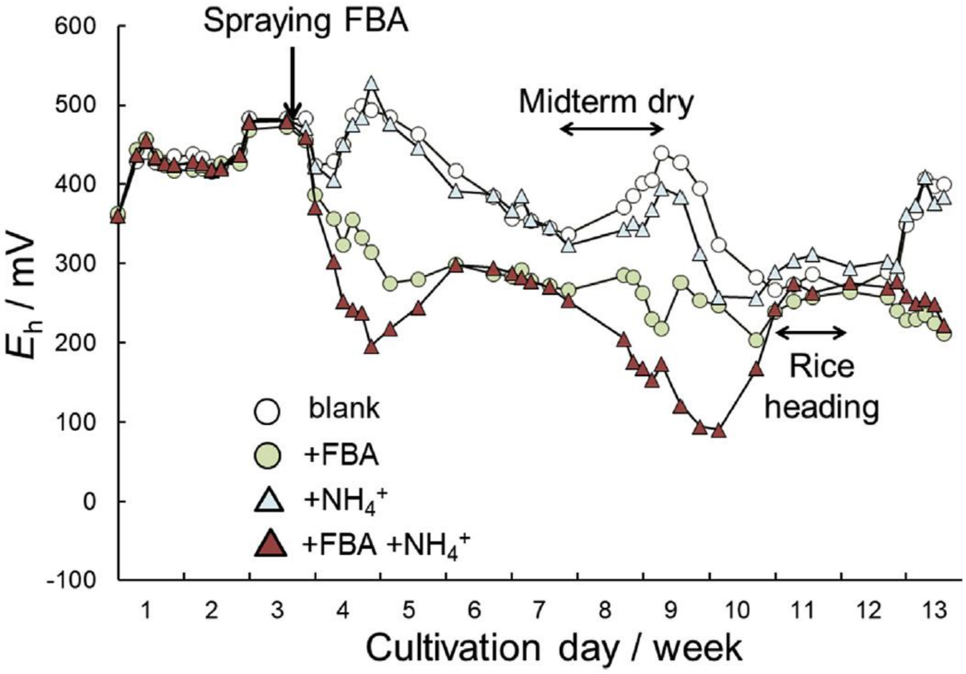

Fig. 3-5 Changes in oxidation reduction potential of soil without any additives (blank), and supplemented with 1% fermented bark additive (FBA) (+FBA), 0.1% ammonium salt (NH_4^+) ($+NH_4^+$), and 1% FBA and 0.1% NH_4^+ ($+FBA +NH_4^+$). Values plotted indicate averages at $n = 3$.

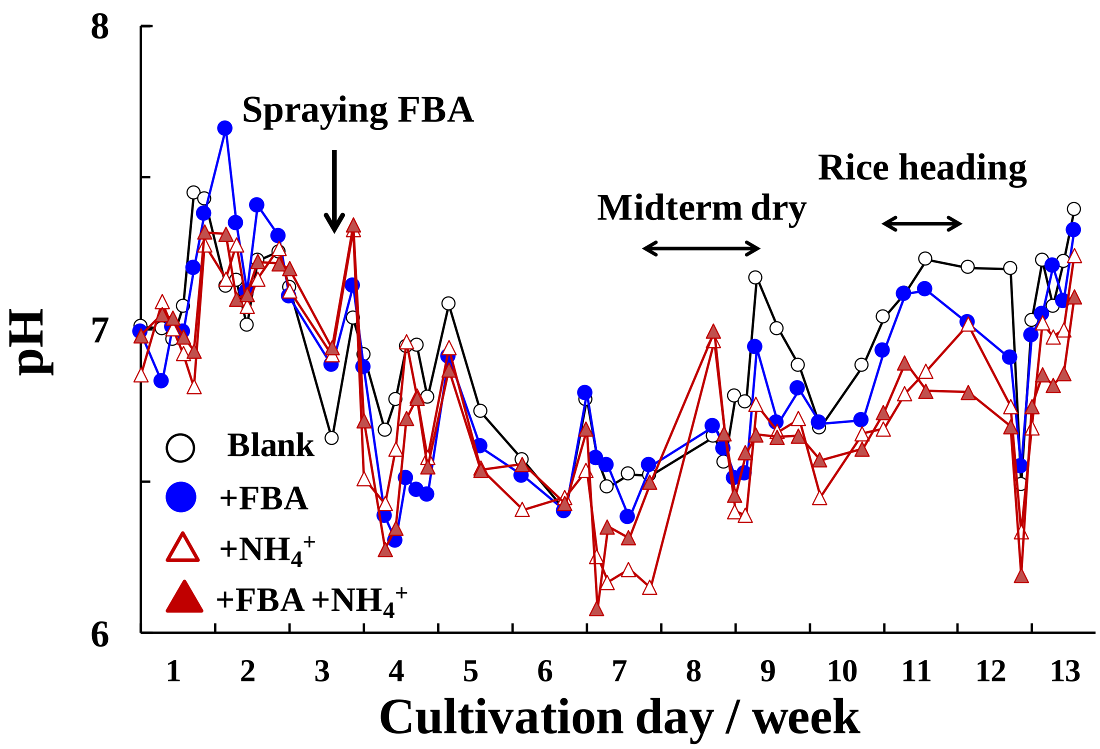

Fig. 3-6 Soil pH during rice growth without any additives (blank), with 1% FBA (+FBA), 0.1% NH_4^+ (+NH_4^+), and 1% FBA and 0.1% NH_4^+ (+FBA +NH_4^+). Values plotted indicate means for $n = 3$.

3.3.6. Relationship between OPR and NH_4^+ concentration

As mentioned above, the reduction in ORP would be because of oxygen consumption by the microorganisms in FBA and/or aerobic microorganisms increasing after FBA spraying, resulting in creating a reduced state [8]. In a previous study [4], the decreases in ORP of soil sprayed with FBA led to the suppression of Cd accumulation into rice, is related to generation of insoluble precipitates with interaction of Cd^{2+} and S^{2-}, and formation of insoluble precipitate results in decreasing ORP [14]. However, Cs^+, which is an alkaline metal, shows limited binding to S^{2-}, which is a soft base.

Therefore, I focused on the chemical form of nitrogen compounds that might be changed with water or soil conditions. To evaluate the chemical form of nitrogen with reduce conditions in soil, the concentration ratio of NH_4^+ and nitrate ions ($[NH_4^+]/[NO_3^-]$) in soil was calculated by the Nernst equation (3) following:

- $\mathrm{NO_3^-} + 10\mathrm{H^+} + 8\mathrm{e^-} \rightarrow \mathrm{NH_4^+} + 3\mathrm{H_2O} \qquad E^\circ = 0.883\ \mathrm{V}$

$$E = E^\circ + 0.0074 \log\left(\frac{[\mathrm{NO_3^-}][\mathrm{H^+}]^{10}}{[\mathrm{NH_4^+}]}\right) \tag{3}$$

where E means reduction potential, E° means standard potential. Calculations were performed under experimental conditions using soil with a pH of 6 at 25 ℃ (T = 298 K).

The $[NH_4^+]/[NO_3^-]$ ratio increased by $10^{13.5}$-fold with a decrease in ORP of 100 mV. Therefore, reducing conditions in soil promoted the production of NH_4^+. The majority of nitrogen compounds was considered to exist as NH_4^+. Accordingly, it was predicted that Cs^+ in the soil exchanged with NH_4^+ and were taken up by rice, as ORP decreased with spraying of FBA.

Additionally, the ratio between Exchangeable Cs eluted by NH_4Cl and Total radio-Cs using exchangeable fractionation basing on the sequential extraction procedures decreased after cultivation in soil with FBA (1% spray) (Table 3-4).

The fraction of exchangeable radio-Cs (64.5%) was expected to be absorbed to the rice plant, while the rate of the exchangeable Cs in soil with 1% FBA was 0.171 before cultivation and 0.063 after cultivation.

Table 3-4 Change in the concentrations of total and exchangeable forms of radio-Cs in soil before and after cultivation [a]

	Blank		Supplementation with 1% FBA[b]	
Rice cultivation	Before	After	Before	After
Exchangeable form/Bq kg^{-1}	853±143	758±58	1120±60	397±25
Total / Bq kg^{-1}	6470±220	6070±350	6540±220	6300±290
Exchangeable-to-total ratio	0.132	0.125	0.171	0.063

[a] The soil sample was Soil C as described in section 2.5.1.

[b] FBA: fermented bark amendment.

To estimate the exchange efficiencies of NH_4^+ to release Cs^+ from soil, the radio Cs-contaminated soil from Mt. Akagi in Gunma Prefecture was reacted in salt solution with metals (NaCl, KCl, NH_4Cl and $MgCl_2$), since the three different types of soils (A–C) used in other parts of this study were consumed through the rice cultivations. In this experiment, the FBA was not mixed with the soil sample.

The Cs leached fractions by NH_4^+ and K^+ salts were found to be two times higher than that of Na^+ salt, and to be 12 times higher than Mg^{2+} salt (Table 3-5). This is because the ion exchange capacity of Cs^+ to negatively charged soil particles are comparable with those of K^+ and NH_4^+, due to the similar ion size with Cs^+ [15].

Table 3-5 Concentrations of radio Cs leached from a contaminated soil by metal salts[a]

	NaCl	KCl	NH_4Cl	$MgCl_2$
Leached fraction / Bq kg^{-1}	112 ± 10	229 ± 57	239 ± 23	19 ± 8
Total / Bq kg^{-1}	2590 ± 90	2430 ± 140	2520 ± 120	2620 ± 90
Ratio of [leached]/[total]	0.043	0.094	0.095	0.007

[a] Soil samples collection were from contaminated site on Mt. Akagi of Gunma Prefecture (Japan).

3.3.7. Mechanism of Cs accumulation in rice plant

Based on above, I estimated a mechanism for Cs accumulation into rice cultivated with supplementations of FBA. Fig. 3-7 illustrates the mechanism predicted here.

Cs is alkali metal and is electrostatically absorbed on negatively charged soil particle (silicate, alumina silicate, etc.). In soil conditions, Cs is replaced by coexistent alkali metal cations such as Na^+, K^+ and NH_4^+.

On the other hand, there are numerous of nitrogen components exist as nitrate (NO_3^-), nitrite (NO_2^-) and ammonia (NH_4^+) in the soil [16]. And under an extremely reduced state, the majority of nitrogen compounds is considered to exist as NH_4^+ [17]. Accordingly, when the ORP in soil decreased with spraying of FBA, Cs^+ on the soil particle is replaced by NH_4^+ and Cs later taken up into rice. Also, if the ammonium salts are directly sprayed into soil, the Cs accumulation is occurred even without the reduction of ORP of soil surface (Figs. 3-4 and 3-5). In this study, I used ammonium ion as partner to exchange with Cs, and the similar accumulation of Cs would occur even if potassium salt was used as the partner as summarized in Table 3-6.

Therefore, the FBA may generate ammonium necessary amount to move Cs into plant, simultaneously result in the reduction of ORP with the supplementation of FBA. Further investigations will be required to demonstrate the mechanism.

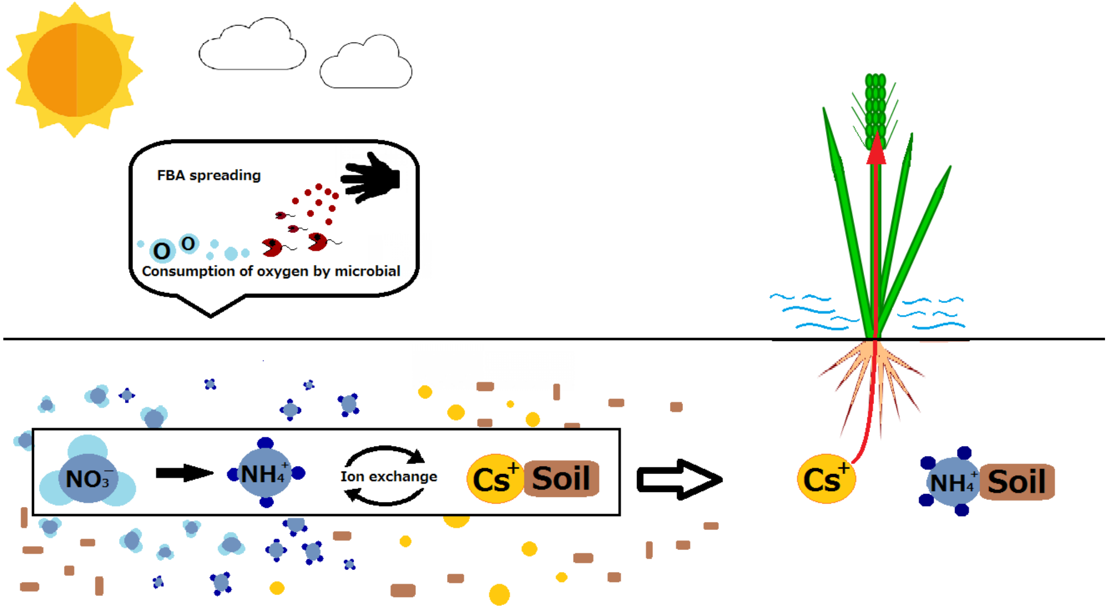

Fig. 3-7 Estimated mechanism of Cs accumulation into rice plant by spreading FBA.

3.3.8. Feasibility of phytoremediation by rice with FBA and NH_4^+ salt applications

Finally, I evaluated the feasibility of phytoremediation by rice under the present soil conditions, calculating the transfer coefficient (TF) through Fig. 3-4.

In this case, the Cs concentrations of soil during the TF calculation were estimated from total ^{137}Cs concentrations. The maximum TF was 0.42 under soil conditions with 1% FBA and 0.1% NH_4^+, which were the most concentrations in soil additives applied here (Table 3-6). There are very few cases of Cs phytoremediation by plants so far. According to Endo et al. (2013), the TFs in chaff, rice bran, brown rice, polishing rice cultivated on a farmland 22.5 km away from FDNPP were 0.049, 0.10–0.16, 0.013–0.017, and 0.005–0.013, respectively. Therefore, it might still be difficult to judge the feasibility of phytoremediation by rice, but the proposed technique can certainly enhance the TF values by supplementation of FBA and NH_4^+ into soil.

In the remediation of the contaminated soil by other method, Mao et al. (2016) [18] reported that soluble and exchangeable Cs in contaminated paddy soils was significantly removed by electro-kinetic remediation coupled with phytoremediation. The technique proposed here might be a more environmentally friendly procedure if compared with that of electro-kinetic remediation, because it only requires the spraying of FBA and NH_4^+ salt after planting rice seedlings.

Table 3-6 Transfer coefficient (TF) of ^{137}Cs taken from radio-Cs contaminated soil into the whole rice plant (*n* = 3).

Soil conditions	TF
No additives	0.159 ±0.057
+ 1% FBA	0.287 ±0.078
+ 0.1% NH_4^+	0.300 ±0.065
+ 1% FBA + 0.1% NH_4^+	0.424 ±0.043

3.4. Conclusions

According to the above, the supplementation of FBA to radio Cs-contaminated soil resulted in the promotion of Cs accumulated into brown rice. Furthermore, the addition of FBA and ammonium salt further increased Cs uptake into rice. This is likely because of the additional NH_4^+ was generated with decreasing soil ORP from the FBA application. The rice cultivation in this study demonstrated that FBA and ammonium salt could be useful for enrichment soluble radio Cs from the contaminated soil.

This supplementation of FBA may be applied to phytoremediation of soluble-form Cs. However, further investigations will be required to confirm these results. In addition, this research is important from the viewpoint of effective utilization of waste woody biomass either restoration of radioactive contaminated soil.

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Chapter 4

Effect of magnetic nanoparticle powder and fermented bark amendment for suppression of As and Cd uptake by radish sprouts grown in agar medium

4.1. Introduction

Arsenic and cadmium are known to cause serious damage to the human body following their excess ingestion in foods or polluted drinking water. As and As compounds are used in the production of pesticides, treated wood products, herbicides, and insecticides [1]. As eluted from soil contaminates the groundwater and consequently affects human health across the world [2]. In contrast, ingesting excessive Cd causes genomic instability in living organisms, which raises concerns regarding the inhibition of plant growth [3]. In agro-ecosystems, presence of genotoxic compound significantly decreased yield and quality of crops [4,5]. In Japan, damage of individuals who ate rice cultivated with Cd-contaminated water was reported in 1955 [6].

Therefore, as countermeasures against As and Cd, many researchers have devised methods for inhibiting As and Cd uptake using modified biomass-based materials, synthesized organic reagents, and inorganic adsorbents. For example, Li et al (2018) [7] published a suppression on Cd uptake into paddy plants using the adsorptivity of red mud-based passivation against toxic elements. Xu et al [8] suppressed As uptake into paddies using manganese and iron oxides.

Several researchers have reported that supplementary modifiers such as adsorbents or biochar simultaneously suppressed As and Cd uptake into crops [9]. Nanoparticles comprised of iron oxides or iron hydroxide, which are well known to strongly adsorb inorganic As species and have been used to specifically eliminate environmental As [10-13]. This is because iron compounds such as hematite, goethite, and iron oxide-coated materials, or granular ferric hydroxide are preferred for As adsorption due to leach adsorbed As at a slow rate [14]. Very recently, coating iron oxide particles with adsorbents such as nickel binary oxide or zirconium binary oxide has been aggressively studied and found to enhance the adsorption capability for As in water or soil [14-16].

In contrast, the uptake of soft metals such as Cd by plants is effectively suppressed by converting insoluble forms, such as the sulfide form (e.g., CdS), or organic compound–bound form. To decrease environmental impact of Cd at low cost, supplementing soil with incineration ash, wood, livestock waste materials, and plants has effectively functioned in environment restoration and suppressing Cd uptake by plants [17-21].

However, Honma et al. (2016) [22] showed that it was hard to decrease As and Cd accumulation simultaneously using a single countermeasure, such as water management practice or soil amendment application. For example, with As and Cd uptake by rice plants, although flooding of paddy fields reduced the Cd concentrations in grain, the anaerobic conditions in paddy soil lead to As mobilization, which could consequently increase As the uptake by rice [23]. Thus, it has become widely recognized that there is a trade-off between the uptake of As and Cd by rice [24]. Recently, to overcome the problem, several waste materials have been reported that can suppress the uptake of not only Cd but also As by plants [25-32]. However, most of studies were not considered on the influences of plant growths.

In this study, I investigated whether there was trade-off between As and Cd uptake using a single amendment, and I attempted to mix multiple compounds to decrease uptake about As and Cd simultaneously, considering influence during plant growth. I decided to achieve the suppression of inorganic species uptake by plant performed using magnetic nanoparticle powder (MNP, α-Fe_3O_4) (not reported) and fermented bark amendment (FBA) [33]. MNP can adsorb As species, but it may be insufficient for supplying ample nutrition for plant growth. In contrast, basically, FBA is an organic compost consisting of bark and food waste, it is expected to promote plant growth.

In this study, Japanese radish as the model plant was adopted, as about 90 % of seed can germinate quite fast among 24 h, and resulting sprout can grow up very well in a simple agar medium. During the cultivation process, MNP and FBA were supplied into the agar medium. The paper describes to simultaneously suppress the uptake of As and Cd into radish sprouts by MNP and FBA in agar, after their adsorptivities to targeted analytes were evaluated in an aqueous medium.

In Chapter 4, our findings demonstrate that the cultivation system developed here, using multiple additives, could suppress the uptake of different toxic species, while accelerating plant growth.

4.2. Experimental

4.2.1. Reagents

Most reagents used here were from Wako Pure Chemical Industries Co. (Osaka, Japan). Pure water obtained from a Milli-Q® Integral Water Purification System (Merck Millipore, Billerica, MA, USA) was used to prepare the solutions. Test solutions with

As(V) or As(III) were obtained by diluting a standard 1000 mg/L As solution (for atomic absorption spectrometry; As_2O_3 in nitric acid), or by dissolving potassium As(V) (90%) in pure water, respectively. Agar powder was used as the medium to cultivate radish seeds (Raphanus sativus var. Longipinnatus) provided by Murakami Farm Co., Ltd. (Hiroshima, Japan). Tris(hydroxymethyl) aminomethane solution was used as buffer to adjust pH of agar medium to 7. And pH of tested solutions was adjusted with 5 mol L^{-1} HCl or 0.1 mol L^{-1} NaOH.

4.2.2. Apparatuses

X-ray diffraction (XRD; RINT2200VF, Rigaku, Japan) was employed to analyze crystalline structure of MNP, using CuKα radiation as the X-ray source. XRD patterns were obtained in the 2θ range from 10°~70 °. The Brunauer–Emmett–Teller surface area was estimated by a NOVA 2200e Surface & Pore Size Analyzer (Quantachrome Instruments, Boynton Beach, FL, US), in dry conditions. A light-scattering apparatus (Zetasizer Nano ZS, Malvern, UK) was used to measure the zeta potential and average hydrodynamic diameter of MNP over a pH range of 3~10. The components of major elements in sediment samples were determined using energy-dispersive X-ray spectroscopy (EDX, SEA1200VX, Hitachi−Hightech, Corp., Tokyo, Japan). Solution pH measurements were performed using a D-51 pH meter (Horiba, Kyoto, Japan). Drying of the harvested radish samples was performed in a DX402 drying oven (Yamato Scientific, Japan). Mixing solutions for the adsorption experiments was carried out using an SR-2w shaker (Taitec, Koshigaya, Japan). The separation of sorbent and solution was carried out using an H-200 centrifuge (Kokusan, Tokyo, Japan). The As, Cd, phosphorus (P) and potassium (K) in the sample solutions for adsorption testing of FBA and MNP were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES, FMX26, Hitachi, Japan). As species in the radish sample were determined by ICP-mass spectrometry (ICP-MS, Agilent 7700-ICP-MS, Agilent Technology, Santa Clara, CA, USA). Measurements of target elements by ICP-MS with an auto-sampler were obtained in collision mode with He-gas mode to remove the interference of $^{40}Ar^{35}Cl$ when analyzing ^{75}As. Data acquisition and post-acquisition analysis were executed using G201A Mass-Hunter Workstation Software, A.01.02 (Agilent Technology). All samples were filtered with a 0.45-μm syringe (Advanced Microdevices, India) before injecting into the instrument. The relative standard deviations (RSD) of all triplicate analyses were within 99.5%.

4.2.3. Preparation of MNP

MNP was prepared by Kanto Denka Kogyo Co., Ltd. (Shibukawa, Japan) (not published). Briefly, 0.6 mol $FeCl_2$ and 4.8 mol $FeCl_3$ were added to 1.0 L of pure water. Next, 500 mL 0.1 M NaOH aqueous solution was added, after which a colloid suspension was obtained. During this procedure, nitrogen gas was flowed over the mixture at rate of 10 L min^{-1} at 60 ℃, following air was then bubbled into the sample at 20 L min^{-1} to oxidize the iron species for 6 h. The suspension was separated into particles and aqueous solution by centrifuging at 3000 rpm for 3 h. The nanoparticles were washing with 200 L of deionized water at 60 ℃. The obtained MNP was dried at 120 ℃ under a nitrogen stream.

4.2.4. Preparation of FBA

As raw materials for preparing FBA, recycled bark was purchased from the Forestry Association in Gunma Prefecture. Rice bran and okara containing white rot fungus were obtained from Shirane Co., Ltd. (Niigata, Japan). The raw materials were mixed in the equal amounts with a moisture content of 60% in a fertilizer-production facility (Sasutera Co. Ltd.). The mixing process was performed for one week until the moisture content decreased to 15%. The carbon and nitrogen levels in the FBA were analyzed by Micro Corder JM10 Element Analyzer (J-Science Lab., Ltd., Kyoto, Japan). Cation-exchange capacity (CEC), phosphorus (P as P_2O_5), and potassium (K as K_2O) in the FBA were measured procedures as described in Chapter 2.

4.2.5. Influence of pH on the adsorption of As and Cd

In adsorption tests of the MNP, 1.25×10^{-2} g MNP was decanted into 50 mL of solution containing target ion (1.0×10^{-5} M) dissolved in 0.01 M CH_3COOH/CH_3COONa (pH 4–5), 0.01 M 2-morpholinoethanesulfonic acid (MES; pH 6), 0.01 M 4-(*N*-morpholino)-butanesulfonic acid (MOPS; pH 7–8), or 0.01 M sodium tetraborate decahydrate (pH 9–10). The suspension was shaken at 200 rpm for 1 h. After collection of MNP by ferrite magnet (**Fig. 4-1**), the residual liquid was filtered with a 0.45-µm syringe filter.

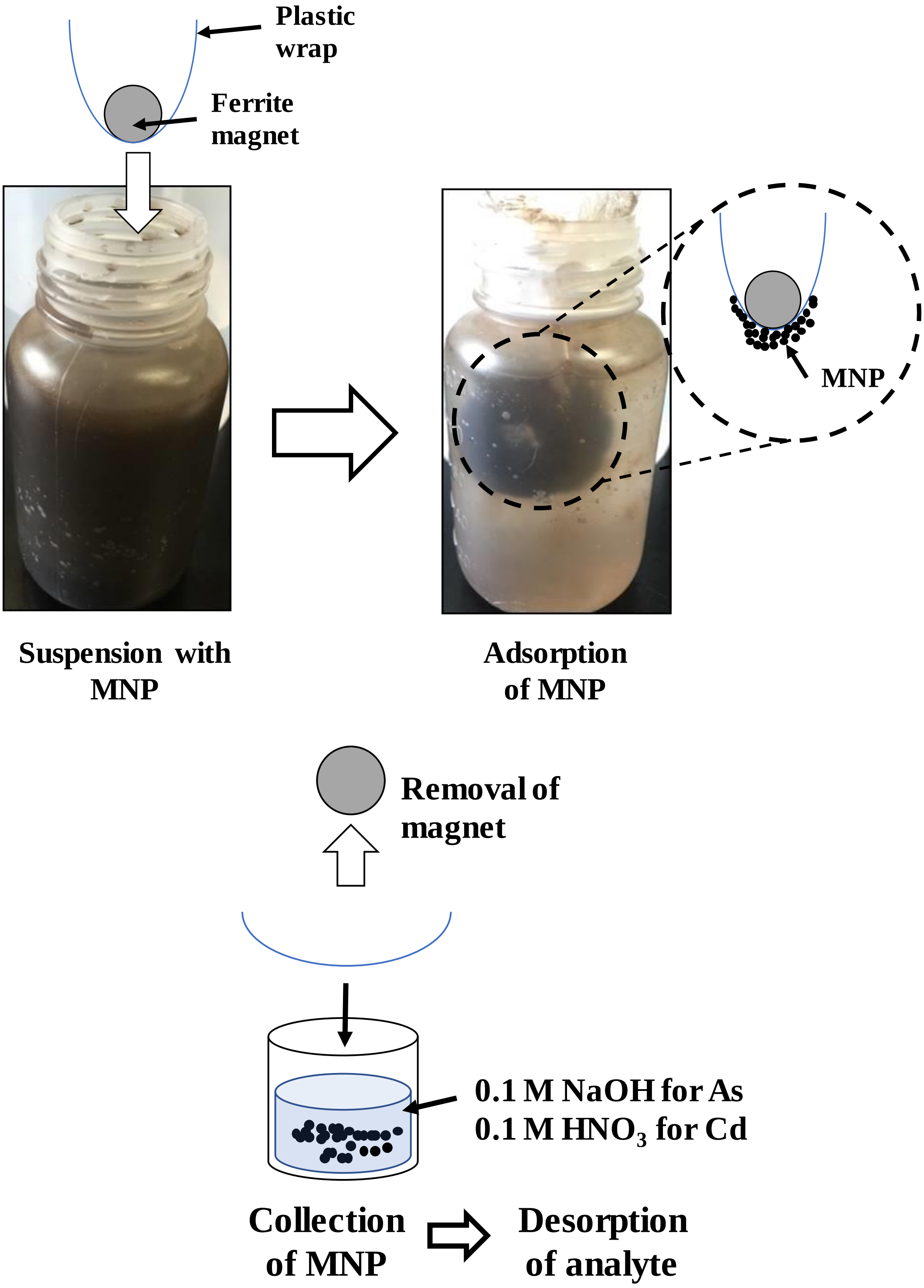


Fig. 4-1 Schematic illustration of MNP from suspension using ferrite magnet.
Upper trace: Removal of MNP using magnet from suspension with MNP.
Bottom trace: Collection of MNP into aqueous solution.

In experiments involving the reuse of MNP, desorption and adsorption were repeated five times. The As and Cd ions adsorbed on MNP were desorbed washing by 100 mL 0.1 M NaOH, and 0.1 M HNO_3, respectively, with shaking at 200 rpm for 3 h. The MNP collection was using ferrite magnet after washing several times by pure water, which was reused for adsorption testing.

In adsorption tests with FBA, 2.50 $\times 10^{-2}$ g FBA was added into 100 mL solution with 1.0 $\times 10^{-5}$ M target ion dissolving in 0.01 M CH_3COOH/CH_3COONa (pH 4), 0.01 M MES (pH 6), 0.01 M MOPS (pH 7–8), or 0.01 M sodium tetraborate decahydrate (pH 9). The other experimental conditions were same as in those mentioned above for MNP.

The As species in the filtered solution were quantified by ICP-AES. The targeted element absorption rate was calculated using Eq. (4-1):

$$Adsorption\ rate\ (\%) = \frac{[C]_{\mathrm{int.}} - [C]_{\mathrm{pH}}}{[C]_{\mathrm{int.}}} \times 100 \qquad (4\text{-}1)$$

where $[C]_{\mathrm{int.}}$ was the initial concentration of analyte before adding additive, and $[C]_{\mathrm{pH}}$ was the concentration of analyte remained in the solution after mixing with additive at each of pH value. The error bars for each plot were calculated by standard deviations of triplicate.

To determine the adsorption-saturation capacities of MNP and FBA for the targeted elements, Eq. (4-2) describing a Langmuir adsorption isotherm was modified to Eq. (4-3), in which $1/q_e$ was plotted against $1/C_e$, yielding a linear plot that was extrapolated to intersect the y-axis provide the intercept:

$$q_e = \frac{Q^0 b C_e}{1 + b C_e} \qquad (4\text{-}2)$$

$$\frac{1}{q_e} = \frac{1}{Q^0 b}\frac{1}{C_e} + \frac{1}{Q^0} \qquad (4\text{-}3)$$

where q_e is the amount of the heavy metals adsorbed per unit weight of the adsorbent (mol/g), C_e is the equilibrium concentration (M) of the heavy metal bulk solution, Q^0 is the monolayer adsorption capacity (mol/g), and b is the constant related to the free energy or net enthalpy of adsorption ($b \propto e^{-\Delta H/RT}$). Accordingly, Q^0 calculated from Eq. (4-3) indicates the adsorption-saturation capacity.

4.2.6. Determination of the As and Cd concentrations in radish sprouts

Radish sprouts were cultivated in agar medium, according to cultivation method of Hatano et al (2016) [34], as **Fig. 4-2** and **Fig. 4-3**.

Three components of radish cultivations were performed for each condition. First, the FBA and/or MNP, 0.2 g agar powder, and 25 mL Tris(hydroxymethyl) aminomethane buffer containing both arsenic(III) and Cd(II) (100 ppb) was heated to 90 ℃ and then solidified cooling to 25 ℃. MNP and FBA were added in range of 0.025–2.5 g per 25 mL agar, that is, the solid-to-liquid ratio (g/mL) was 0.001–0.1.

After implanting the radish into agar medium, radish was cultivated in an artificial meteorological equipment (LH-60(S)FL3/12-DT manufactured by Nippon Medical Chemical & Instruments CO.LTD., Japan) at 20~25 ℃. During the cultivation period, to simulate nature environment UV-Vis.lamp in the greenhouse is turned on from 6:00 to 18:00 and kept dank from 18:00 to second 6:00 (as in **Fig. 4-3**). The radish seeds were cultivated in agar medium for 18 days and then harvested.

The radishes produced were dried using oven at 80 ℃ for 3 hours, following length measurements for the roots and edible portions. The dried radish samples were mashed using an agate mortar and completely dissolved in nitric acid. As or Cd levels in dissolved solution were quantified by ICP-MS.

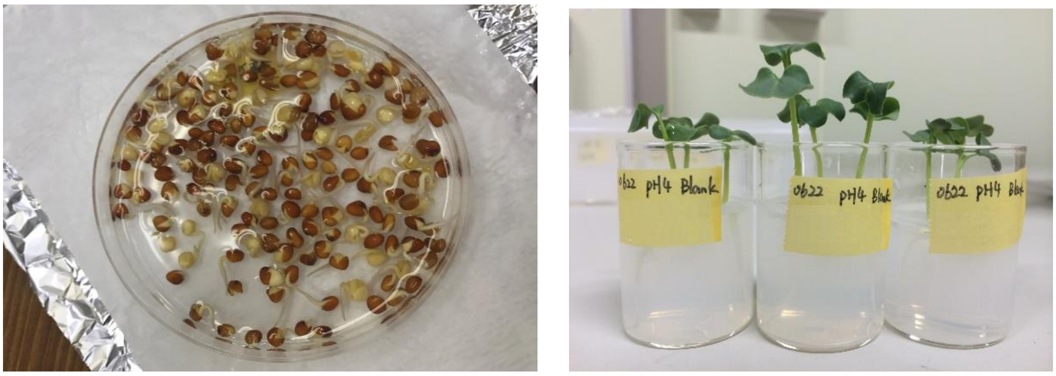


Fig. 4-2 Sprouting of radish bean seed (left) and implant of the sprouted radish into agar medium (right).

Fig. 4-3 Cultivation of radish in an artificial meteorological equipment.

4.2.7. Statistical analysis

Differences in As and Cd concentrations among radish samples harvested from the agar with and without MNP and FBA were evaluated using a *Student's* *t*-test at a significance level of 0.05 [35]. Descriptive statistics were calculated in Microsoft Excel 2010.

4.3. Results and discussion

4.3.1. Characterizations of MNP

The MNP was characterized by common items used on that of inorganic particles. The coercive force and degree of saturation magnetization of the MNP were estimated by Kanto Denka Kogyo Co., Ltd.

XRD spectral data revealed that the crystal phase of MNP was a typical α–Fe_3O_4 structure basing on the findings of Kazeminezhad and Mosivand (2014) [36], as shown in **Fig. 4-4**.

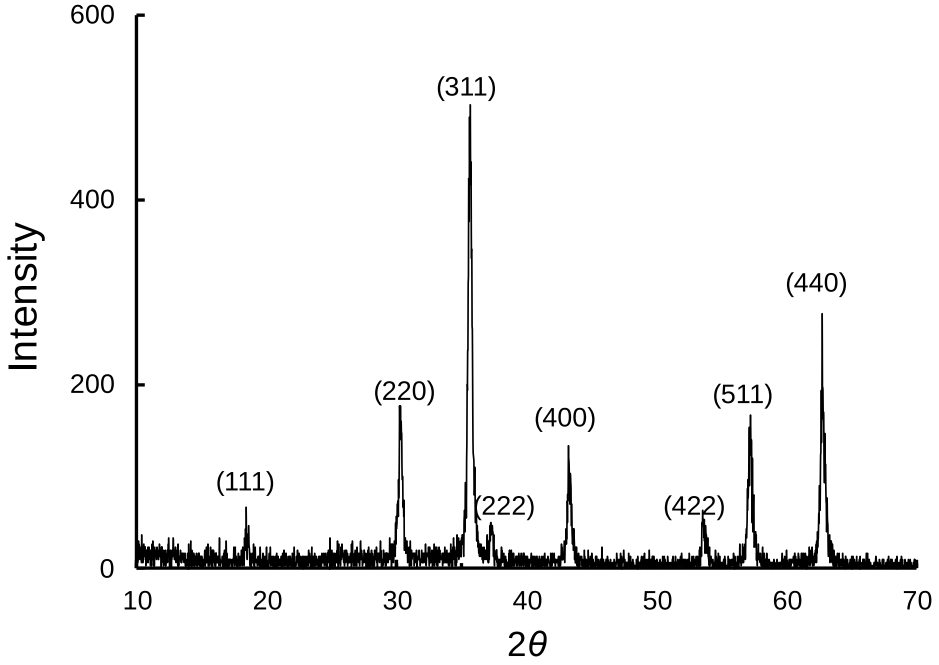


Fig. 4-4 XRD pattern of the MNP used in this study.

The other characteristics of the MNP were as follows: the pH that indicates point of zero charge (pH_{pzc}): 6.8 ±0.1 ($n = 3$), the average hydrodynamic diameter of MNP particle at pH 6: 45.1 ± 2.5 nm ($n = 3$), specific surface area: 25.0 ± 3.2 m^2/g ($n = 3$), coercive force: 16.7 kA/m ($n = 1$), and saturation magnetization amount: 78.1 Am^2/kg ($n = 1$) (Table 4-1). The chemical composition of MNP was 97.9 wt % Fe (as F_2O_3) and 0.22 wt % Al (as Al_2O_3), as determined by EDX.

Table 4-1 Physical property of MNP used in this study

Average particle diameter (nm)	Specific surface area (m^2/g)	Coercive force (kA/m)	Saturation magnetization amount (Am^2/kg)
45	25.0	16.7	78.1

Measurement on physical properties was conducted by Kanto Denka Co., Ltd.

4.3.2. Characterizations of FBA

The FBA obtained after 7 days of fermentation was characterized in terms of its C/N ratio and the contents of N, P, K. Total N and C levels in bark (a reference material for FBA) were 0.10 ± 0.02% and 51.8 ± 0.2%, respectively; thus, the C/N ratio was 518. In contrast, total N and C values of FBA were 2.92 ± 0.10% and 35.9 ± 0.4%, respectively, resulting in a C/N ratio of 12.3. The C/N ratio of FBA was distinct lower than that of bark, application of FBA in large quantities causes a reduction in number of N available to the crop, since the average C/N ratio of commercial chemical fertilizers is approximately 0.1. N, P, and K contents in FBA were 2.9%, 1.5%, and 2.8%, respectively, giving a total content of 7.2%. Considering that the recommended total contents of N, P, and K in commercial chemical fertilizers is 30%, the FBA had significantly lower N, P, and K levels and was, thus, a special organic fertilizer [37]. The CEC of FBA was analyzed in accordance with the criteria stipulated by the Japan Bark Compost Association (JBCA). The standard levels of bark compost cited were those officially released by the JBCA (http://www.bark-assoc.jp/products2.html). The CEC was 71.5 cmol/kg, which was within the standard ranges (>70 cmol/kg) established by JBCA.

4.3.2. As(V), arsenic, and Cd adsorption capabilities

Fig. 4-5a shows the adsorption capability of MNP for As(V) and As(III) as a function of solution pH, relative to adsorption capability of Cd(II). The adsorption behaviors were different between As(V) and As(III), and variations of As species also differed from that of Cd(II). Because the pH_{pzc} of MNP obtained in this study was 6.8, the MNP was electrostatically attracted to negatively charged species such as As(V) and repulsed by positively charged species such as Cd(II), under an acidic pH. In contrast, under an alkali pH, the adsorptivity of As(V) was reduced owing to repulsion with the negatively charged MNP. The adsorptivity of Cd(II) rose with increased solution pH, but the precipitation of the insoluble hydroxyl form might be included when considering the solubility product constants (K_{sp} at 25 ℃: $[Cd^{2+}][OH^{-}]^2 = 2.8 \times 10^{-14}$). As(III), which is a very weak acid ($pK_{a1} = 9.1$ as $HAsO_2$), indicated the quantitative adsorption at all pH values tested (adsorption rate: 99.7% to 100% in pH 4 to 10). MNP, which consisted of iron oxide, could interact with As(III), based on the specific surface area and sorption sites [38].

Additionally, the reusability of MNP were tested by repeating the adsorption and desorption of analyte ions for five times. All analyte ions could be quantitatively desorbed from MNP by procedures described before. The relative standard deviation (RSD, $n = 5$) was within 10% for all analytes as summarized in **Table 4-2** in supplementary material.

The adsorptivities of FBA for As(III), As(V), and Cd(II) were also investigated, following conditions mentioned above. In **Fig. 4-5b**, adsorption rates of As(III) or As(V) to FBA as function of solution pH were lower than those for MNP. Bark or its decomposed products, which are components of FBA, include lignin with binding sites for metal cations, such as oxygen or nitrogen atoms. FBA would predominantly adsorb heavy metal cations.

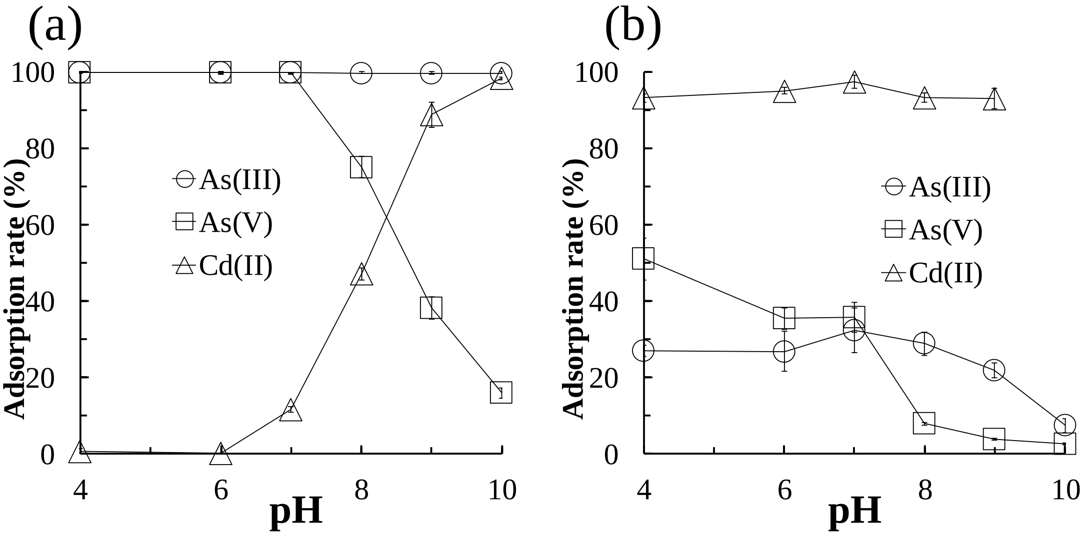


Fig. 4-5 Changes in adsorption rates of As(III), As(V), and Cd(II) to (a) 0.25 g/L MNP and (b) 0.25 g/L FBA as a function of the solution pH. Initial concentration: 1.0 × 10^{-5} M.

Table 4-2 Adsorption amount (q_e) of MNP to Cd(II), As(III), and As(V) in five repeated examinations*

	q_e (μmol/g)		
Run number	Cd(II)	As(III)	As(V)
1	1.34	13.4	12.6
2	1.26	13.0	11.8
3	1.32	12.0	10.8
4	1.22	12.4	10.4
5	1.30	11.8	10.0
Average	1.29	12.5	11.1
Standard deviation	0.048	0.672	1.064
Relative standard deviation	3.7%	5.4%	9.6%

* Initial concentration of analyte: 2.0×10^{-5} M. MNP: 100 mg. Solution pH: 6.0±0.1. Reaction time: 1 h.

4.3.3. Adsorption-saturation capacity of MNP and FBA for As(III), As(V) and Cd

Next, the adsorption-saturation capacities of As(III), As(V), and Cd(II) on MNP and FBA (pH 6) were estimated from the adsorption amounts (q_e) reached during the saturated state after mixing for 30 min into the residual concentration (C_e). In **Fig. 4-6a**, calibrations between As(III) and As(V) for MNP were almost the same, that is, the adsorptivity of MNP for As species at pH 6 was independent of chemical form of As. In contrast, adsorptivities for As(III) and As(V) were different for FBA (**Fig. 4-6b**). This difference likely reflects the acid dissociation constants (pK_{a1}) of As(III) (pK_{a1} = 2.2 as arsenite acid) and As(V) (pK_{a1} = 9.2 as arsenate acid) for FBA [39].

The adsorption-saturation capacities (Q) of MNP and FBA were estimated from the intercept of the calibration curve, the Q values of arsenic species of MNP were two-fold higher than Cd(II), and the opposite tendency was observed for FBA, as summarized in Table 1. In addition, when comparing to the previous reports in **Table 4-3**, the Q value of As(III) to MNP was higher than those to the adsorbents cited in this paper[40-42], though that of As(V) was lower than several materials complexing multiple adsorbents[43,44]. The Q value of Cd(II) to FBA indicated much higher than biomass materials presented in reports by several researchers [45-48].

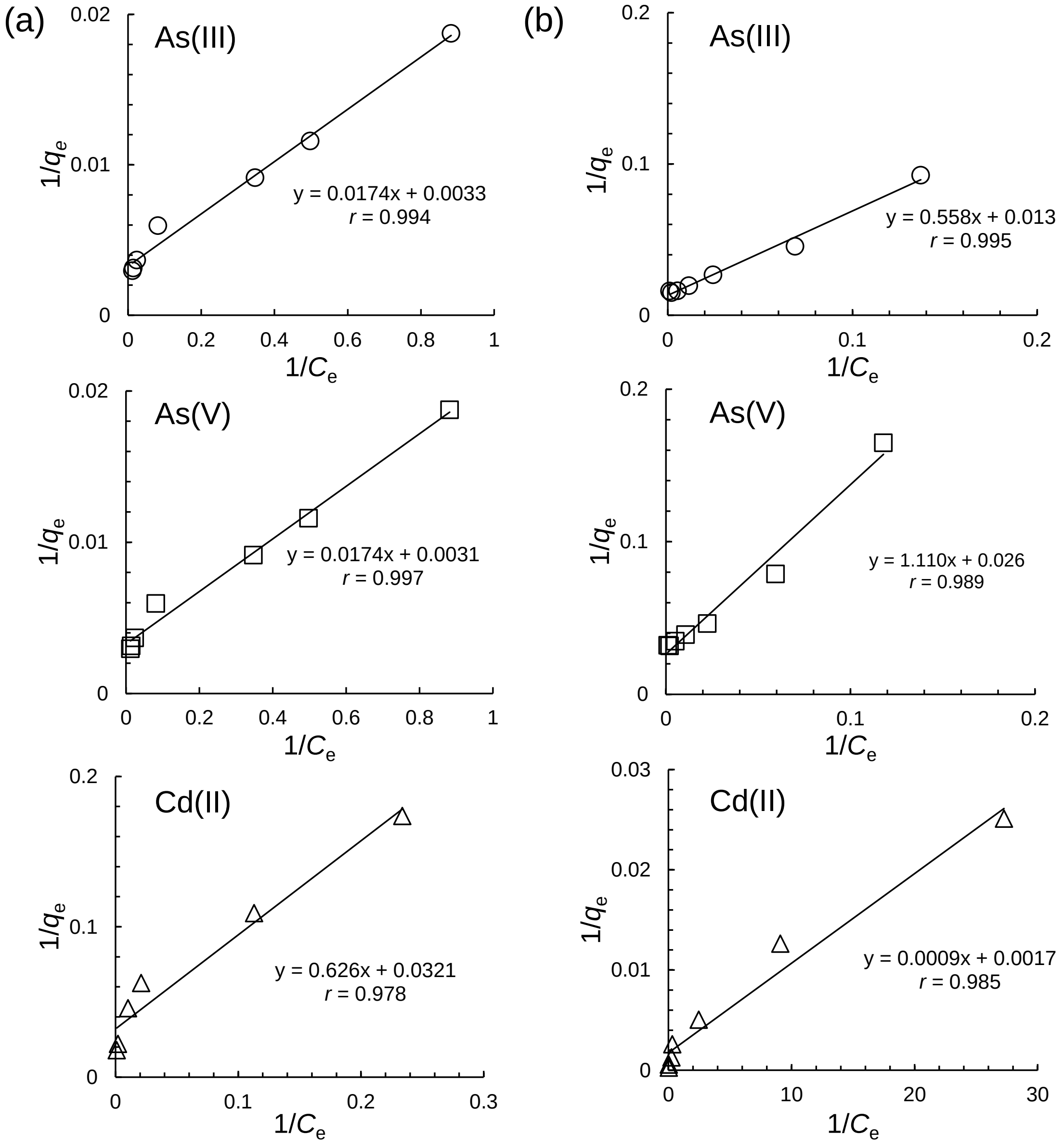


Fig. 4-6 Relationship between the reciprocals of q_e and C_e. Additive: (a) MNP and (b) FBA. Tested initial concentration: 1.0 $\times 10^{-5}$–1.0 $\times 10^{-3}$ M. Solution pH: 6. Mixing time: 30 min at 25 ℃

Table 4-3 Adsorption-saturation capacity (Q) of As and Cd to MNP and FBA and the comparative data by adsorbents obtained from industrial wastes and iron oxide in previous researches

Adsorbents	Analyte	*Q* (μmol/g)	Initial concentration Initial pH	References
MNP	As(III)	303 ±12	0.01–1.0 mM pH 6	This work
FBA		76.9 ±5.3	0.01–1.0 mM pH 6	This work
Pine cone-magnetite		227	5 – 100 mg/L pH 8	Ouma et al. 2018
Hematite-coated Fe_3O_4		13.3	0.05 – 2 mg/L pH 7	Simeonidis et al. 2011
Iron oxide-coated sand		0.38	0.1 – 0.8 mg/L pH 7.5	Gupta et al. 2005
MNP	As(V)	303 ±10	0.01–1.0 mM pH 6	This study
FBA		38.5 ±7.2	0.01–1.0 mM pH 6	This study
Iron oxide-loaded biochar		25.5	0.1 – 5 mg/L pH 7	Duan et al. 2017
Hematite-coated Fe_3O_4		28.0	0.05 – 2 mg/L pH 7	Simeonidis et al. 2011
Granular ferric hydroxide		30.9	0.50 – 0.95 mg/L pH 7.4	Saldaña-Robles et al. 2017
Synthesized magnetite		887	0.1 – 150 mg/L pH 5	Iconaru et al. 2016
Chitosan-coated $Cu(OH)_2$		520	10 – 100 mg/L pH 4	Elwakeel and Guibal 2015

Table 4-3 Continued.

Adsorbents	Analyte	*Q* (μmol/g)	Initial concentration Initial pH	References
MNP	Cd(II)	27.4±1.1	0.01–1.0 mM pH 6	This study
FBA		558±36	0.01–1.0 mM pH 6	This study
Pinus halepensis sawdust		65.6	1 – 50 mg/L pH 9	Semerjian 2010
Magnetic biochar derived from kelp		207	500 – 2000 mg/L	Son et al. 2018
Pyrolyzed coffee residues and clay		527	25 – 250 mg/L pH 7	Boonamnuayvitaya et al. 2004
Papaya wood		15.4	5 – 500 mg/L pH 5	Saeed et al. 2005

Average and standard deviations in MNP and FBA were estimated from three different samples.

4.3.4. Effect of FBA for growth of radish plant

First, to optimize pH of agar medium for radish cultivation, the agar media were prepared of pH 4, 6 and 10, and 10 days for cultivation period. As shown in **Fig. 4-7**, the best growth of radish plants was found in the agar at pH 6.

In pH 4 and pH 10, radish is not survive. In general, the suitable pH for plant growth is from 6 to 7 [49]. Agar medium pH is considered as important variable in cultivation experiment due to it affects many chemical processes. The pH in agar medium affects the absorption of mineral ions by plants, and further affects the osmotic pressure of plant roots or causing water loss. Although the organic matter in the agar can provide nutrients for radish growth, in the medium of pH 4, excessive hydrogen ion inhibits the absorption of potassium, magnesium and calcium ions into plants [50]. For soil, the higher pH adversely affects physical and chemical properties. In agar medium at pH 10, osmotic pressure of the solution increases, which can cause physiological drought of the plant, and makes it difficult for the plant to absorb water, causing the plants to dehydrate and die [51].

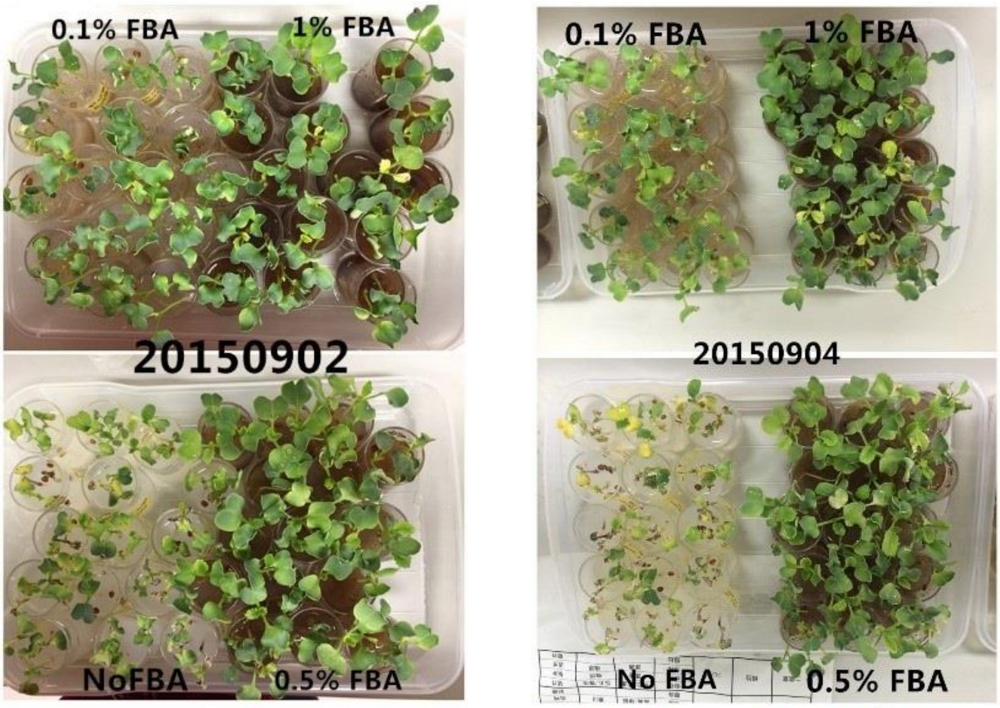
Fig. 4-7 Photos of radish plants cultivated in agar with pH 4, 6 and 10. The FBA was not sprayed into those agars.

Next, I investigated the influence of plant growth with the addition of FBA into the agar media (pH = 6). **Fig. 4-8** shows that each group of radish plants in agar media with 0.1%, 0.5% and 1% FBA in the cultivation period for 10 days. The plant growths were not inhibited by addition of FBA. Also, the pHs in the media were not largely changed by adding FBA.

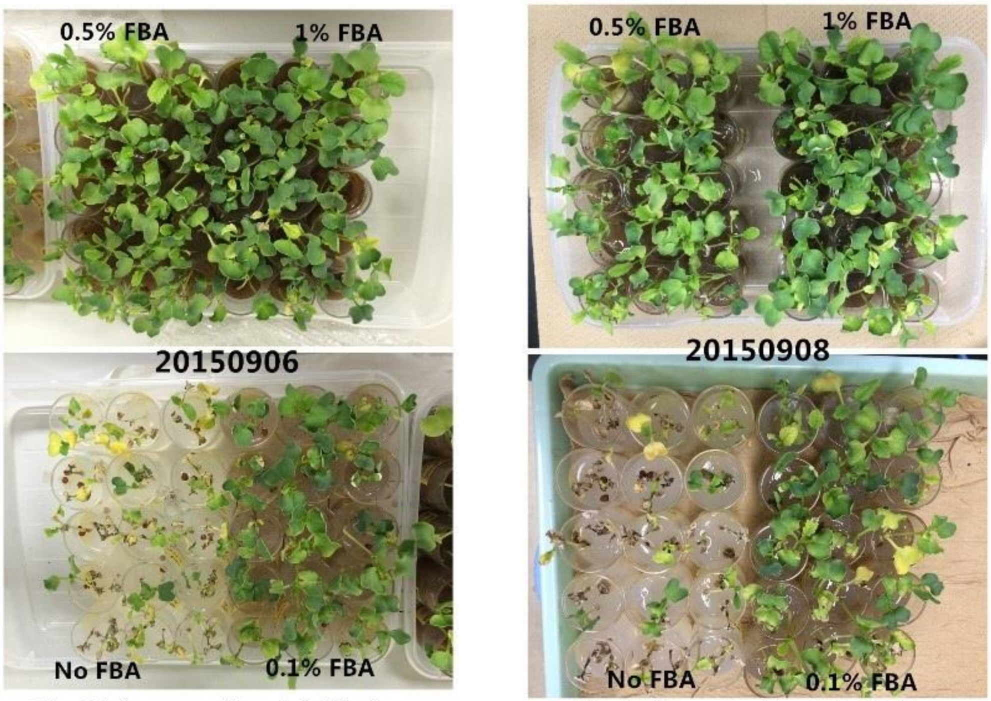
Fig. 4-8 Photos of radish plants cultivated in agar media without FBA and with 0.1%, 0.5% and 1% FBA in cultivation for 10th days.

Furthermore, the radish growths in the agars without and with FBA (0.1%, 0.5% and 1%) powder were investigated for 18 days. At the head 10th cultivation day (**Fig 4-8**), each plant growth was retained. From the 12th day, radish in agars with 0.5% FBA and 1% FBA were grown faster than those in agar without FBA and with 0.1% FBA. At 18th day, radish plants in agar with FBA were much larger growth than that without FBA (**Fig. 4-9**). Consequently, FBA had a good influence on the growth of radish.

The lengths of radish plants harvested after 18 day-cultivation revealed the differences between agar without FBA and with FBA. The longer plants were harvested in the agar medium with higher concentration of FBA in **Fig. 4-10**. Cultivation of the radish in agar with FBA was obviously better than those of the blank conditions and the agar with MNP only (see **Fig. 4-12** in section 4.3.6).



Radish growth at 12th day Radish growth at 14th day



Radish growth at 16th day Radish growth at 18th day

Fig. 4-9 Photos of radish plants cultivated in agar media without FBA and with 0.1%, 0.5% and 1% FBA in cultivation for 12th -18th days.

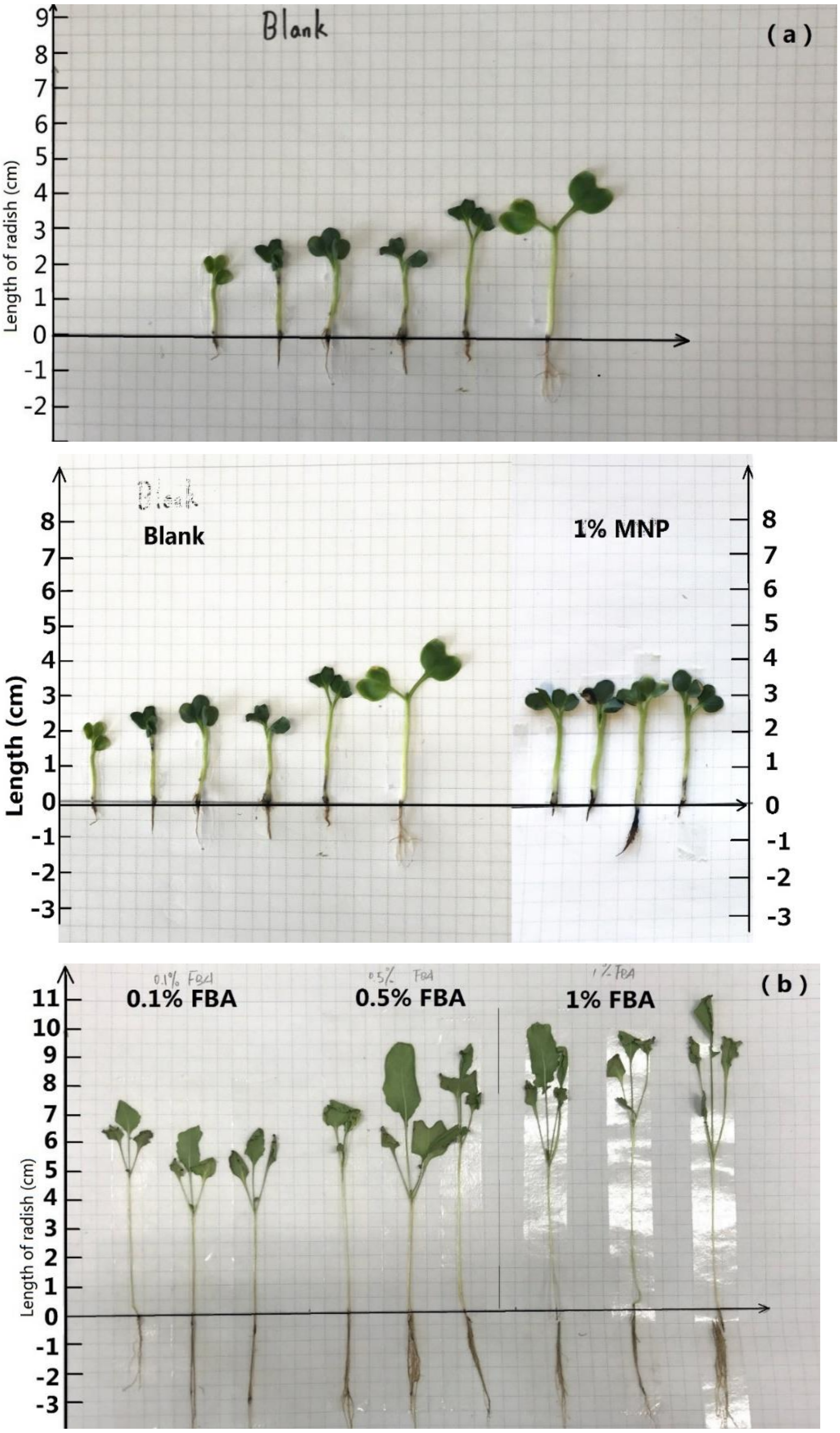


Fig. 4-10 Effect of FBA on radish growth in (a) agar without FBA and (b) agars with 0.1%, 0.5% and 1% of FBA.

4.3.5. Changes in As(III) and As(V) accumulation into radish cultivated in agar with FBA

In the radish planting experiment, radish seeds were planted into the agar medium with As(III) and As(V) prepared in our laboratory. Considering the concentrations of arsenic species commonly found in natural environmental soil, both As(III) and As(V) were added at 10 μg/L, 100μg/L, and 1000 μg/L in the agar. Also, in Cd experiment the same concentrations of Cd as those of As were individually added in the agar. Concentrations of As(III), As(V) and Cd accumulated in radish plants harvested after 18-day cultivations were shown in **Figs. 4-11**.

As shown in **Figs. 4-11a and 4-11b**, uptakes of As(III) and As(V) by radish plants were not successfully suppressed by the cultivation in agar with FBA, compared to that in the blank experiment, although uptakes of As(III) were very slightly suppressed in agars with 10 – 100 μg/L As(III) (initial concentration) and 0.5 – 1.0% FBA.

While FBA can sufficiently provide the nutrients for growth of radish plants, the FBA does not work for the suppressor of As(III) and As(V) uptake by the roots. One of the reasons may be related to chemical species of arsenic species in agar medium. As(III) and As(V) seem to be existed as anionic species in agar like those in water solution. Especially, mobilization of As is similar to phosphorous, which is an element of the same family as As. Therefore, As(V) and As(III) anions would be absorbed into radish like phosphate and phosphite anions, respectively.

In addition, since FBA has many oxygen atom such as phenyl group or carbonyl group. Zeta potential of FBA in the neutral condition were neutral, but the electron pair in oxygen atom in FBA cannot easily give the electron to anionic species. Thus, the arsenic species in the agar medium might not successfully be bound to FBA.

On the other hand, FBA added in agar could suppress the uptake of Cd by radish plant depending on the amount of FBA as shown in **Fig. 4-8c**. When FBA (1%, w/w) was applied, the Cd concentrations in radish in agars with 100 μg/L and 1000 μg/L Cd (initial concentration) were about one-fourth or one-twice lower than those obtained in blank agar with 100 μg/L and 1000 μg/L Cd. This seems because Cd ion, which is dissolve as cationic species in agar, is bound to FBA in the agar by providing a lone pair of electrons on the phenyl group of FBA.

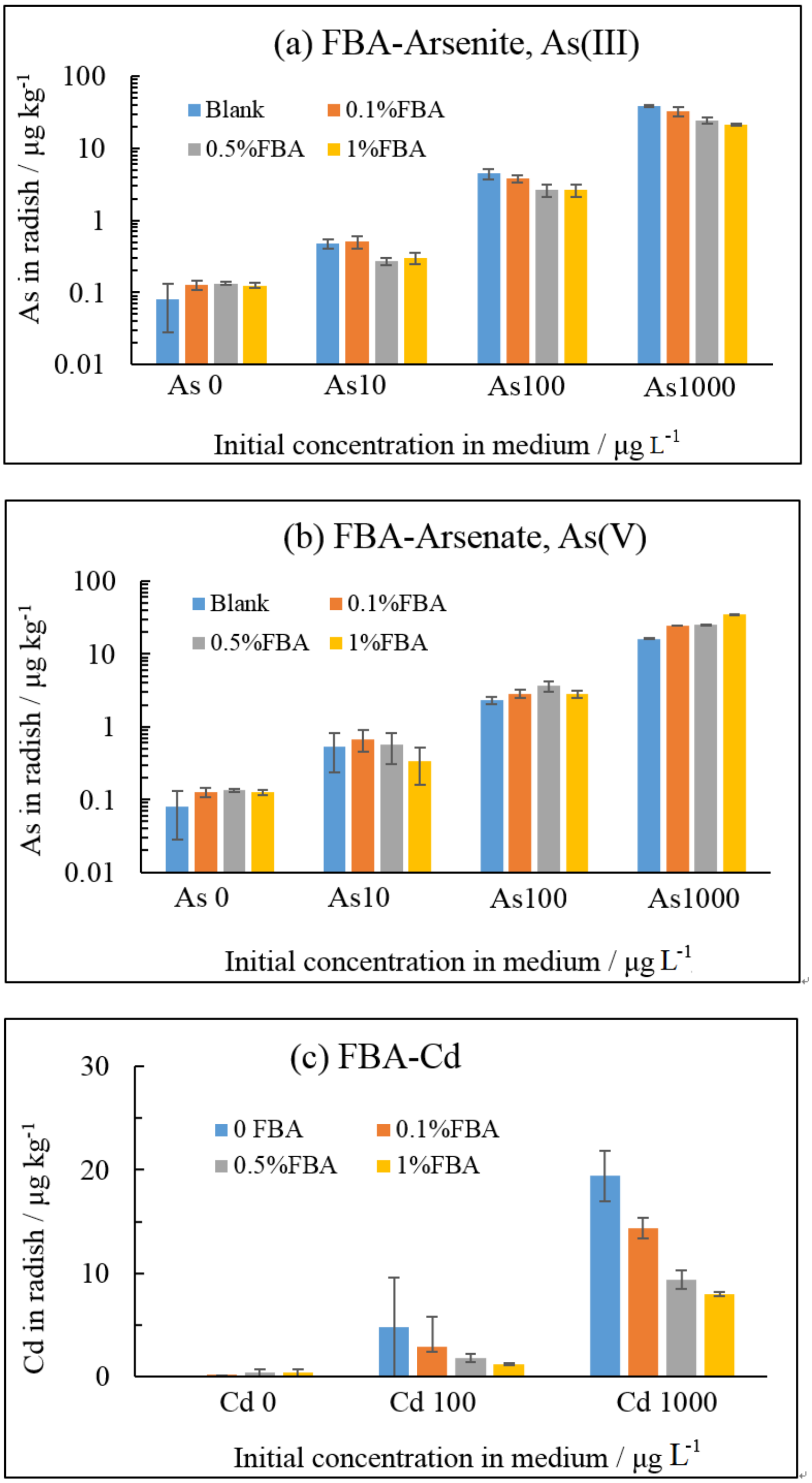


Fig. 4-11 Contents of (a) As(III), (b) As(V) and (c) Cd in radish planted in agar medium with FBA.

4.3.6. Effect of MNP for growth of radish plant

To discuss the effects of MNP added in the agar on radish growth, radish plants were cultivated in agar with 1% MNP. **Fig. 4-12** shows the photos of radish plants harvested after 18 day-cultivation. From this result, the addition of MNP into agar would inhibit the growth of plant, though they did not die. This implies that MNP could not provide sufficient nutrients to the plants.

Fig. 4-12 Effect of MNP on radish growth in (a) agar without MNP and (b) agars with 1% of MNP.

4.3.7. Changes in As(III) and As(V) accumulation into radish cultivated by agar with MNP

During some previous researchers, iron hydroxides and oxides could immobilize As in aqueous solution [8-10, 13, 15, 16, 38, 42, 55]. In this study, MNP containing iron-oxide was expected to bind As in mediums and suppress As uptake in radish.

Considering the concentrations of arsenic species commonly found in natural environmental soil, both As(III) and As(V) were added at 10 μg/L, 100μg/L, and 1000 μg/L in the agar. Also, the same concentrations of Cd as those of As were individually added in the agar. Furthermore, 0.01 – 1% MNPs were added into agar with As(III), As(V) or Cd, respectively. Concentrations of As(III), As(V) and Cd accumulated in radish plants harvested after 18-day cultivations were shown in **Figs. 4-13**.

As shown in **Fig. 4-13a**, the addition of 0.01% MNP in agar was considered insufficient to immobilize As(III) in the medium, comparing to that in blank agar. The absorption of As(III) into the radish was efficiently suppressed in the agar media when 0.1–1% MNPs were added. The agar medium with 10 μg/L As(III) (initial concentration) did not provide the obvious differences of suppression effect of As(III) by addition of MNP with that in blank, but the obtained As concentrations were lower than that in Environmental Quality Standards for Water in Japan (< 0.01 mg/L) [52]. When the As(III) concentration in agar were increased to 100 μg/L and 1000 μg/L, the suppression effect was quitly obtained. The As(III) accumulation in radish was reduced to about one-tenth of that obtained in blank. The addition of 0.1% MNP could significantly suppress uptake As(III) as much as 1000 μg/L As(III) as the initial concentration in the agar.

As shown in **Fig. 4-13b**, the effect of MNP for suppression of As(V) uptake by radish was somewhat different with that for As(III) uptake. When the initial concentration of

As(V) in agar was 1000 μg/L, the suppression effect of As(V) by radish were reduced depending on MNP amounts in agar. This might be because the adsorption amount of As(V) per unit mass of MNP under agar condition used in this study were smaller than that of As(III) per unit mass of MNP. The adsorption of MNP to arsenic species is described also in Chapter 3.

On the other hand, the suppression of Cd uptake by radish plant was obtained in the 1000 μg/L Cd-contaminated agar with 0.5% and 1% MNP. The Cd concentrations in the 1000 μg/L Cd-contaminated agar with MNP were half of that in the contaminated agar without MNP. The suppression effect of MNP for Cd might consequently be lower than that for As. However, the Cd concentration in radish (20 μg/L) was largely reduced even in cultivation by agar without MNP, considering that the initial concentration of Cd in the agar were 1000 μg/L. Namely, a combination of MNP and agar are concluded to be possible to synergistically suppress the Cd uptake by radish plant, though it does not accelerate the plant growth as described in section 4.3.6.

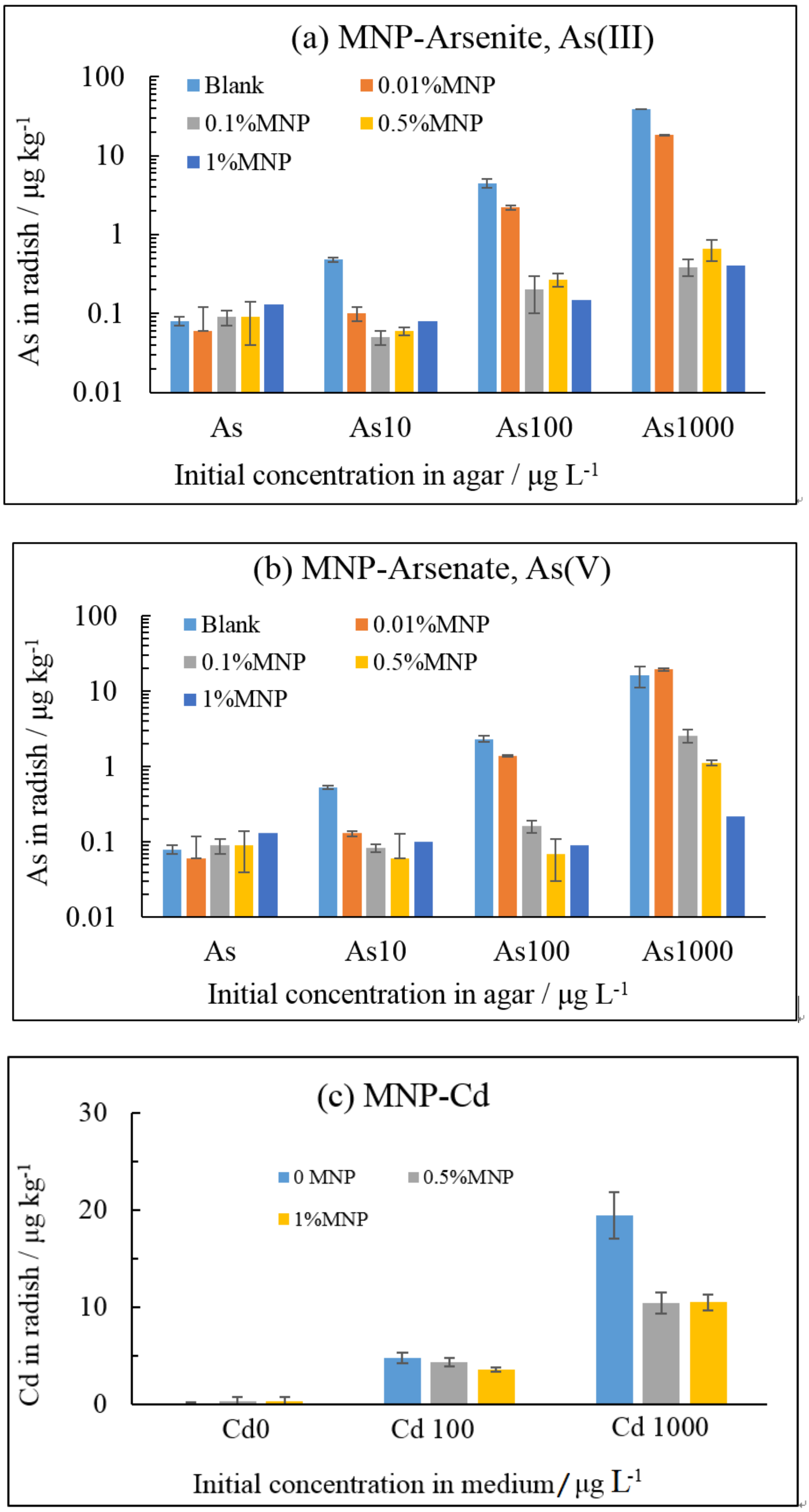


Fig. 4-13 Contents of (a) As(III), (b) As(V) and (c) Cd in radish planted in agar medium with MNP.

4.3.8. Effect of a mixture of FBA and MNP for growth of radish plant

The combination of MNP and FBA was found to be effective for the plant growth. In fact, the elongations of root and stem in radish cultivated in agar with FBA were larger than those in agars without FBA or with MNP only (**Fig. 4-14a**). As shown in **Fig. 4-14b**, in addition of the 0.5% MNP only, the growth of radish was obviously defected compared to other conditions, that is, the MNP might suppress the absorption of As but cannot supply the nutrients such as potassium and phosphorus concerning the growth of plant. Also, the average length of stem and root cultivated in the agar with 0.5% FBA and 1% FBA was respective 2.7-fold and 3.4-fold longer than these without additive. The potassium concentration in the radish was enhanced in the agar conditions with FBA, as summarized in **Table 4-4**, because the FBA supplies the nutrients that accelerate the radish growth. In contrast, the addition of MNP in the agar medium leaded to a low concentration of phosphorus, which is homologous element with As, because MNP would perform high affinity for phosphorus as well as As. Even though, potassium concentration would clearly contribute to the growth of the radish sprouts.

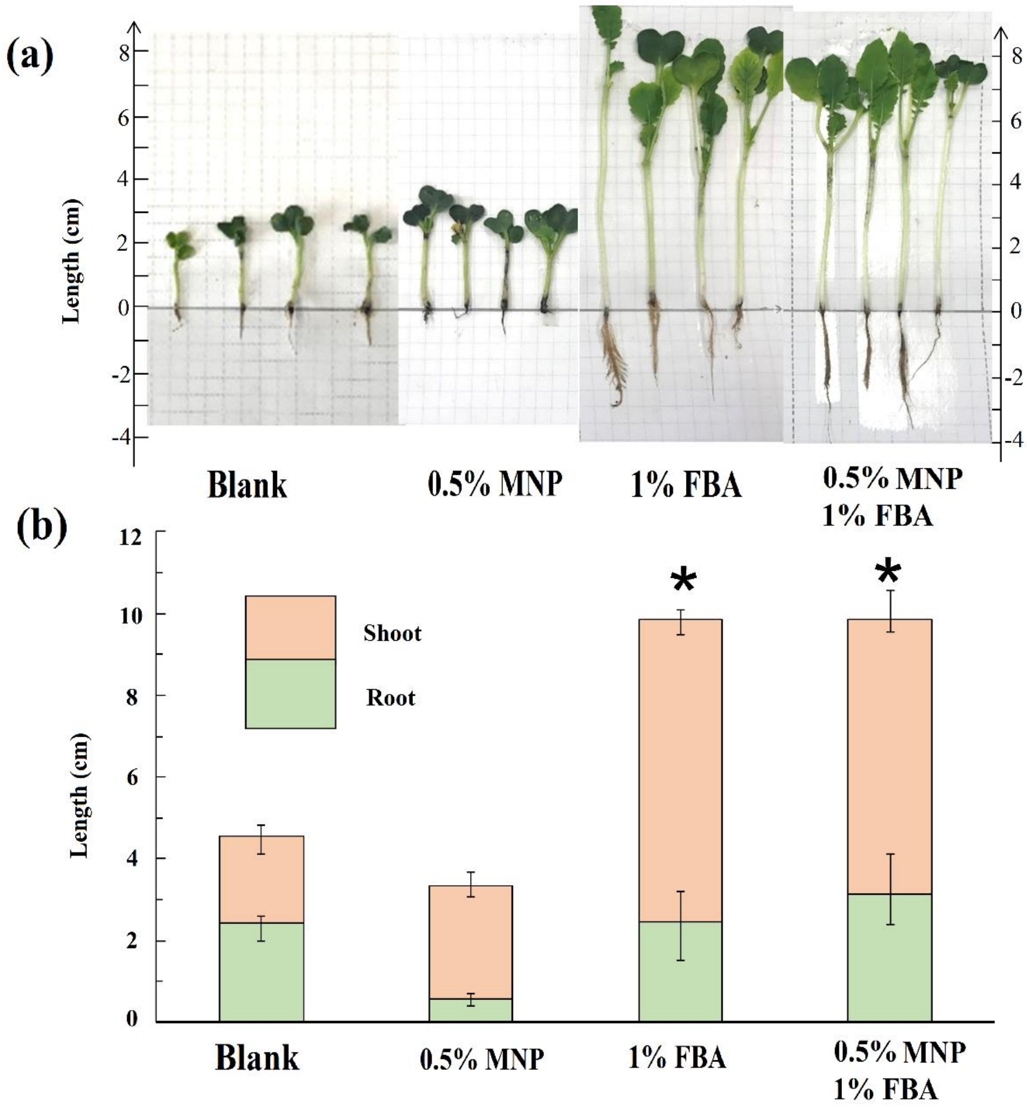


Fig. 4-14 Growths of radishes harvested after growth in blank medium, or medium containing 0.5% MNP, 1% FBA and 0.5% MNP, and 1% FBA. Trace: (a) photographs and (b) bar graphs. Significant differences ($p< 0.05$, $n = 4$) between the results of the blank and those of the treatments with MNP and/or FBA are indicated by asterisk above bar. Blank

Table 4-4 Concentrations of potassium and phosphorus in the whole radish sprouts harvested after growth in agar in the absence or presence of the indicated additives

Additives	K concentration (mM)	P concentration (mM)
Blank	15.0±6.1	0.43±0.03
1% MNP	10.5±8.7	0.09±0.01
1% FBA	56.8±11.1	1.12±0.18
0.5% MNP + 0.5% FBA	44.5±12.1	0.18±0.05

Average and standard deviations were estimated from three different samples.

4.3.9. Changes in As(III) and As(V) accumulation into radish cultivated by agar with a mixture of FBA and MNP

When the radish sprouts were grown in the agar media with As and Cd (each initial concentration: 100 μg/L for As(III) and Cd), the effects of MNP and FBA added to the medium were compared by determining the amounts of As and Cd absorbed in the sprouts. In this experiment, As(III) and Cd were co-mixed in the agar media. The amount of FBA and MNP added to the agar media ranged at 0.1~1.0%.

As shown in **Fig. 4-15**, the absorption of arsenic into the sprouts was almost quantitatively suppressed by adding MNP in the medium at concentrations higher than 0.1% MNP. In addition, the arsenic content decreased by approximately 50% in the blank condition by the addition of 1% FBA. And the amount of Cd absorbed by the sprouts in the agar medium with FBA was reduced by approximately 5-fold than blank. Interestingly, Cd uptake by the radish was also reduced by 60–85% by adding MNP, although Cd adsorption on MNP in aqueous solution was much lower than As adsorption on MNP.

The cation-exchanger or chelating complex site in FBA (which contains oxygen or nitrogen atoms) promoted by decomposing bark by activation of white rot fungus would have relation in biding to metals. According to a report by Baker et al [53], the carboxyl and phenolic hydroxyl groups in humus binds to metal cations via forming complexes with the adsorption site. As uptake by the radish sprouts in the agar media with FBA was higher than that of Cd. This is because of the low binding capability for anionic species like As to materials with carboxyl groups and phenolic hydroxyl groups [54].

Many researchers provided that iron hydroxides and oxides could efficiently bind and remove As from aqueous solution [8,12,13,15,16,51]. In this study, MNP composed of iron oxide could bind negatively charged As ions; thus, the binding mechanism of MNP to As species is comparable to that for iron oxide.

Furthermore, in the cultivation in the blank condition without any additives, the Cd content absorbed by radishes was 10-fold lower than the initial concentration added in the agar media. Because agar includes sugars, saturated fatty acids, and polyunsaturated fatty acids possessing carboxyl or hydroxyl groups [56], the agar itself in the media might suppress Cd uptake but the suppression capability obviously inferior compared to those in the media with MNP and FBA.

Japanese radish was cultivated in the agar medium containing a mixture of 0.5% MNP/0.5% FBA or 0.5% MNP/1% FBA, the concentrations were obtained after harvest. As shown in **Fig. 4-12**, the uptakes of As and Cd were suppressed by the presence of the mixed additive. For example, the As and Cd contents in the sprouts cultivated in the agar medium containing both 0.5% MNP and 0.5% FBA were reduced to 90% and 82%, respectively, relative to the corresponding levels found in the blank condition. However, the capability of the mixture for suppressing analyte uptake was slightly lower than found for As with 1% MNP alone and Cd with 1% FBA alone. This would be because the adsorption capabilities of MNP and FBA for analytes was offset. Indeed, the suppression of analyte uptake did not change even when the amount of FBA was increased to 1% and mixed with 0.5% MNP to further reduce the Cd contents in radishes. Inversely, when the MNP amount was increased to 1% and mixed with 0.5% FBA, As content was not different to that with 0.5% MNP and 0.5% FBA but the radish growth was largely suppressed.

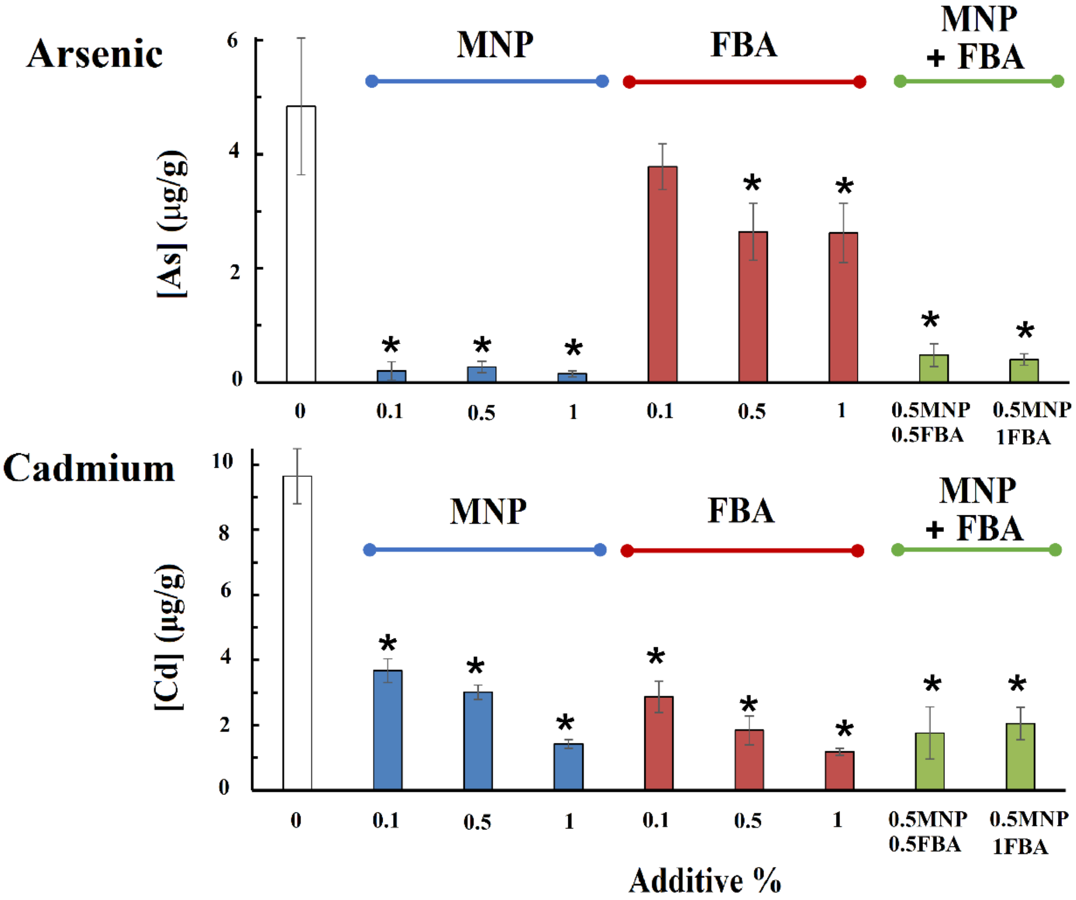


Fig. 4-15 Contents of As (upper trace) and Cd (lower trace) absorbed into radishes planted in agar medium supplemented with MNP, FBA, and a mixture thereof (0.5% MNP/0.5% FBA or 0.5% MNP/1% FBA). The initial concentrations of As(III) and Cd(II) in the agar were 100 μg/L. The error bars in bar graphs were estimated from the standard deviations of analytes in radishes, obtained in triplicate experiments. Significant differences ($p < 0.05$, $n = 3$) between the results of the blank and those of the treatments with MNP and/or FBA are indicated by asterisk above bar.

4.3.10. Mechanism of As and Cd accumulation in radish

According to a report by Du et al [57], when both iron oxide and dissolved organic matter (DOM) co-exist in medium, the Cd preferentially binds to functional carboxyl groups on aromatic rings in humic acid than to iron oxide. DOM generated by decomposing FBA may adsorb to both heavy metals and As(III) on mineral surfaces [27]. Sharma et al and Bauer et al [10,58] demonstrated to reduce the As content when iron and DOM coexisted in aqueous medium. As adsorption might be due to the formation of Fe-bridged As−Fe−DOM complexes and surface complexation of As on DOM-stabilized Fe colloids [59-61], as an estimated mechanism in **Fig. 4-16**.

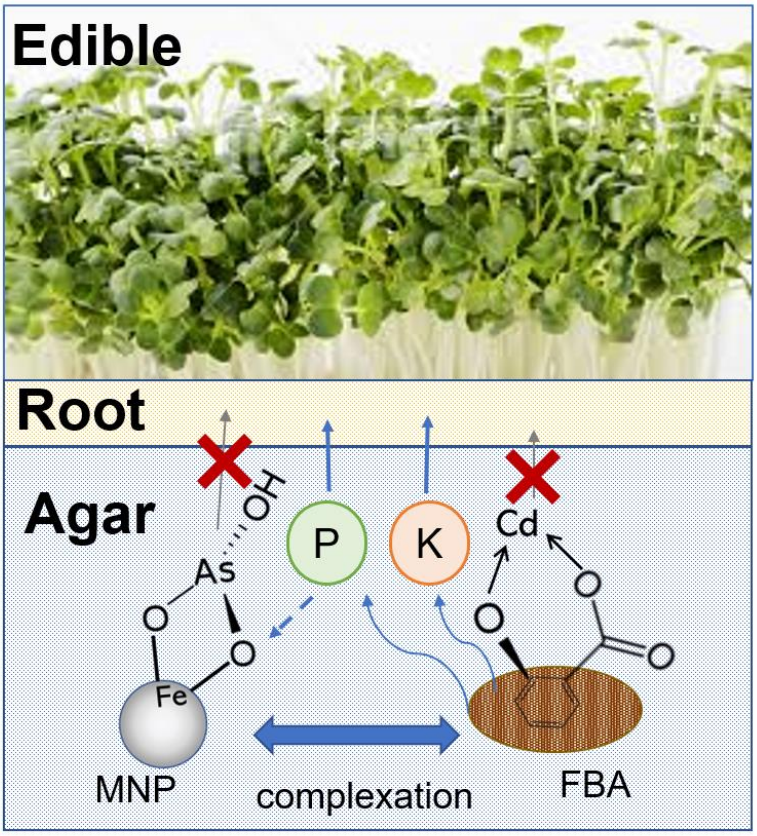


Fig. 4-16 Estimated mechanism of As/Cd accumulation into radish by spreading MNP and FBA.

4.4. Conclusions

Addition of MNP and FBA into the agar medium could simultaneously suppress As and Cd uptake by the radish sprouts and also assist in their growth. Combining inorganic and organic materials with different properties did not significantly reduce the performance of MNP and FBA, that is, it becomes cultivation technique to solve the problems of trade-off between the uptake of As and Cd by plant. Further investigations will primarily be interested to obtain effect of MNP and FBA in real toxic element-contaminated farmland soil.

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Chapter 5

Conclusion and future prospect

5.1. Conclusions

In this study, I effectively suppressed Cs, As and Cd uptake into a plant using fermented bark amendment (FBA) or magnetic nanoparticle powder (MNP) in agar or soil medium.

FBA, which ferments bark by aerobic microorganisms (white rot fungus) and food wastes, enhanced the adsorption capacity for heavy metals. The addition of FBA into the cultivation medium such as agar would suppress the absorption of heavy metals by radish. Inversely, the supplementation of FBA into radioactive substance-contaminated soil accelerated uptake of radio-Cs by rice plant. Furthermore, when MNP was co-existed with FBA in agar medium, the accumulation of As and Cd by plant (radish) were simultaneously suppressed while the plant growth was accelerated. Accordingly, our research results will be important references to elucidate absorption characteristics of harmful elements to plants.

This chapter summarizes the conclusions in chapters 2, 3 and 4 as described below.

Chapter 2 described the characteristics and properties of FBA developed in our laboratory. The low C/N of FBA means to easier decompose in soil than bark and FBA has a higher metal adsorption capacity. The total number of N, P, K contents of FBA was 7.2%, which is identified as a low-chemical fertilized capacity. CEC values of FBA was higher than bark. The decomposition of original bark by microorganisms would improve the adsorption abilities for heavy metals. These results were expected that the properties of FBA might play the roles to effectively suppress the mobility of heavy metal in soil.

Chapter 3 described that application of FBA in radio-Cs (radio-Cs) contaminated soil in pot experiment promoted radio-Cs in brown rice, and the expected mechanism was discussed. The factor of the Cs uptake accelerating was concluded to be reduction of oxygen-reduction potential (ORP) with supplementation of FBA. Furthermore, the addition of ammonium salt led to the same effect, i.e., acceleration of radio-Cs uptake by rice, as the FBA supplementation. This research could obtain the important opinions on effective recycle of waste woody biomass and its utilization to restoration reagent for radioactive contaminated soil.

Chapter 4 described application of FBA and MNP in cultivation of radish in agar medium experiment. Addition of MNP and FBA into the agar medium could simultaneously suppress As and Cd uptake by the radish sprouts but also assist in their growth. Combining inorganic and organic materials with different properties did not significantly reduce the performance of MNP and FBA, that is, it becomes cultivation technique to solve the problems that there is a trade-off between the suppression of As and Cd by plant. Further investigations will primarily be interested to obtain effect of MNP and FBA in real toxic element-contaminated farmland soil.

5.2. Future prospect

Recycle of biomass like FBA used in this study has been a subject of special interests in environmental treatment research and renewable energy. This attention has been expected to be even greater in future. Through this study, I am considering that FBA can be applied to 1) suppression of various heavy metals uptake by plant beside Cd and As and 2) utilization of new woody waste amendments.

1) Base on adsorption rates on heavy metal ions to FBA (chapter 3), FBA is expected to express good adsorption capacity on Cu and Pb. At this stage, the cultivation experiment is considered that the target element would be Cu or Pb, because they are focused as pollutants of croplands [1].
2) Raw material of FBA, cedar bark, is treated as waste in Japan, while in other countries, the type of wood waste varies depending on the plant species. Our next study will develop new FBWA (fermented bark waste amendment) provided as raw material different types of woody wastes in other countries, especially for developing countries with large populations [2].

To achieve the future prospects as mentioned above, my group will go on application of FBA to suppress the absorption of Cu and Pb by plants, and development of new bark waste amendments based on the manufactural procedures of FBA, such as cornstalks in China [3].

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Acknowledgment

I would like to express my heartfelt thanks, appreciation and gratitude to the promoter of the dissertation, Prof. Dr. ***Hideyuki Itabashi*** and Prof. Dr. ***Masanobu Mori.*** Prof. Dr. ***Hideyuki Itabashi***, Graduate School of Science and Technology Gunma University, encouraged me a lot to continue my dream when I first time came to Japan. Prof. Dr. ***Masanobu Mori***, Faculty of Science and Technology Kochi University, gave me many preciousness suggestions and guidance for this work, and also paid attention to my personal life and growth just like family. It is indispensable motivation for me to persevere in this research.

I wish to express my gratitude to Assoc. Prof. Dr. ***Ken-ichi Hatano*** and Assoc. Prof. Dr. ***Hiyama***. Assoc. Prof. Dr. ***Ken-ichi Hatano*** gave me valuable suggestions and constructive opinions during the planting process. With his help, I succeeded to plant the healthy plants although I cannot even plant potted flowers before. Assoc. Prof. Dr. ***Hiyama*** patiently guided Research Proposal for me.

I would also like to thank Mr. ***Seiko*** and Ms. ***Amata***, Takasaki Advanced Radiation Research Institute Pamphlet, with their help I was able to obtain good data. At the same time, please allow me to represent my group to express thanks for workers in The Water Department General Administration Division of Kiryu City, fortunately with their support and help, I can complete the measurement of radioactive elements.

In addition, I would also like to thank Ph. D ***Kobayashi Shizusa*** of Hokkaido University for completing the planting experiment of radioactive elements together. I will value our friendship across borders. During the long process of the experiment, my husband and friends gave me great support in my daily life and shared the gladness or

discourager with me. They will all be the wealth of my life.

Last but not least, my deepest gratitude and appreciation are for my parents, who at great sacrifice made it possible for me to have the luxury of length education. Everything I am owning now thanks the love from them.

July, 2019, Gumma, Japan

Xiaotong Sun