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## Determination of Cu, Pb, Fe, and Zn in Plant Component Polymers of a Hyperaccumulating Plant

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Phytoremediation is an innovative technology that utilizes the natural properties of plants to remediate hazardous waste sites. For more cost-effective phytoremediation, it is important to utilize a hyperaccumulating plant after phytoremediation, *i.e.* the recovery of valuable metals and the production of useful materials. In this work, the determination of metals in plant component polymers in a fern, *Athyrium yokoscense*, as a hyper-accumulating plant was established using steam explosion, Wayman's extraction method, and ICP emission spectrometry. After *A. yokoscense* plants were treated by steam explosion, the steam-exploded *A. yokoscense* were separated into four plant component polymers, *i.e.* water-soluble material fraction, holocellulose fraction, methanol-soluble lignin fraction, and residual lignin fraction. The concentrations of Cu, Pb, Fe, and Zn in these plant component polymers and the dry weights of plant component polymers were measured. These analytical process determining metals in the plants will contribute to not only the evaluation and the efforts of phytoremediation using a hyperaccumulating plant, but also to the development of more effective phytoremediation.

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### Introduction

Soil contaminated with harmful metals has caused the metal contamination of natural water and drinking water, resulting in negative effects on human society.<sup>1-4</sup> The treatment of harmful metal-contaminated soil is a major concern of the environmental community.<sup>5</sup> Phytoremediation, the use of green plants to clean up metal-contaminated environments, has attracted attention as an environmentally friendly useful metal-extraction technique for treating toxic contaminated soil.<sup>6-9</sup> This technology has utilized hyperaccumulating plants that absorb harmful metals (Cd, Pb, As, *etc.*) from contaminated soil, and accumulate in the harvestable above-ground biomass.<sup>10,11</sup> Moreover, the recovery of valuable metals and the production of energy resources, *i.e.* methane or ethanol, and functional resin from a hyperaccumulating plant are necessary for cost-effective phytoremediation.<sup>12</sup>

On the background, techniques for estimation of the metal concentration in hyperaccumulating plants are required for evaluating the degree of metal accumulation of plants. A steam explosion degrading and removing lignin that covered holocellulose (cellulose and hemicellulose) has been reported as an useful pretreatment for the effective utilization of plant biomass, *i.e.* wood, potato, and bamboo.<sup>13-16</sup> Furthermore, the steam-exploded product was easily converted into sugars and ethanol by using enzymes and yeast. The steam explosion technique reasonably allows the plant body to be liquefied and separated into plant component polymers.

This work improved the determination technique of the metals (Cu, Pb, Fe, and Zn) in plant component polymers of a hyperaccumulating plant, *Athyrium yokoscense*, by using various methods, *i.e.* Wayman's extraction method, and the analytical method with ICP emission spectrometry. The Wayman's extraction method liquefied and separated the *A.*

*yokoscense* fern into four plant component polymers, *i.e.* the water-soluble material fraction, the holocellulose fraction, the methanol-soluble lignin fraction (low-molecular-weight lignin), and the residual lignin fraction (high-molecular-weight lignin). The metal concentrations in plant component polymers were measured using ICP emission spectrometry for clarifying the distribution of metals in *A. yokoscense* fern.

### Experimental

#### *Hyperaccumulating plant sample*

A fern plant, *Athyrium yokoscense*, was collected from Ogoya abandoned mine area (Komatsu City, Ishikawa, Japan). The dry plants were separated into the aboveground part (leaves, leaf blade, petiole, and rhizome) and the underground part (roots).

#### *Pretreatment method*

The plant samples were hydrolyzed using a steam explosion method. The apparatus for the steam explosion (Japan Chemical Engineering and Machinery, Osaka, Japan) consisting of a steam generator, a high-pressure reactor, a receiver, and a condenser with a silencing action.<sup>12-16</sup> The solid and liquid products of the exploded fern were recovered in a cyclone at the bottom of the receiver, and the gaseous products were passed from the top of the receiver into the condenser. Steam explosions were conducted under a steam pressure of 2.55 MPa (225°C) and steaming times of 3 min.

#### *Determination of plant component polymers*

The materials of steam-exploded *A. yokoscense* plants were separated into four categories, such as a water-soluble material fraction, a holocellulose fraction, a methanol-soluble lignin fraction (low-molecular-weight lignin), and a residual lignin

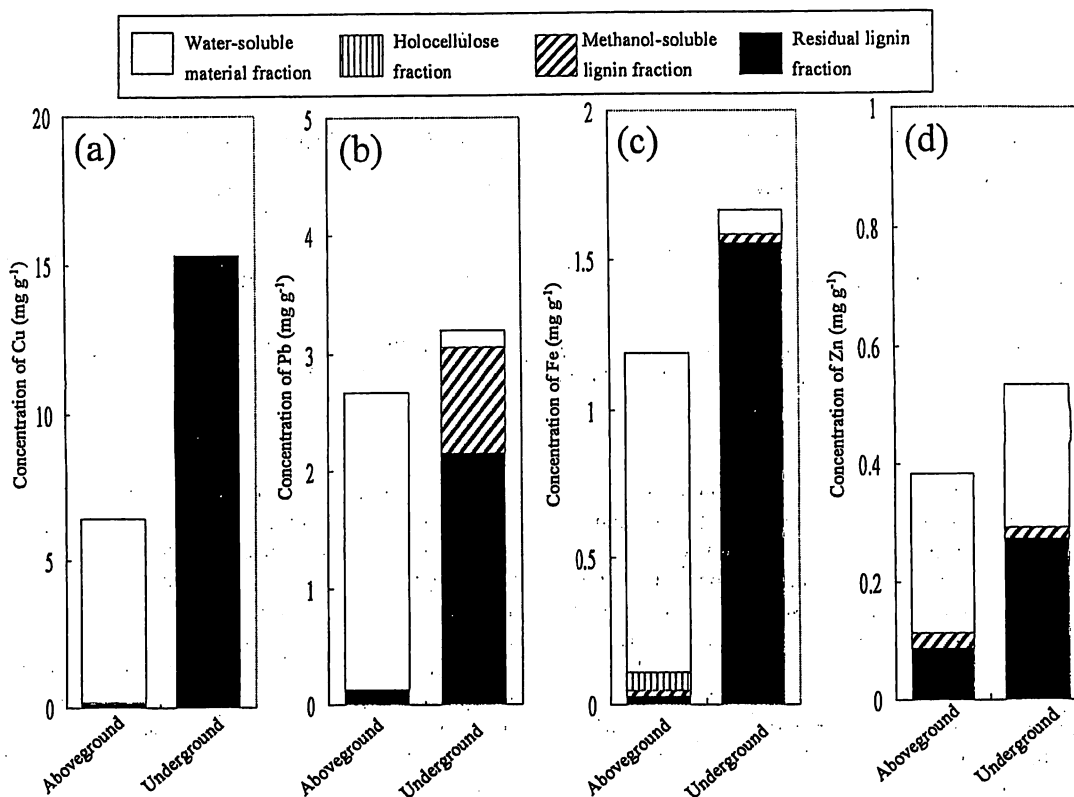


Fig. 1 Concentrations of Cu (a), Pb (b), Fe (c), and Zn (d) in unit per gram of steam-exploded *A. yokoscense* plants.

fraction (high-molecular-weight lignin), using Wayman's extraction method.<sup>17</sup> After water in the steam-exploded *A. yokoscense* plants was sublimated by a freeze-dry method, 5 g of steam-exploded dry *A. yokoscense* plants and 300 ml of distilled water were mixed for 12 h at room temperature and filtered by a Buchner-type funnel with a glass filter (40–100  $\mu\text{m}$  pore size, Shibata Scientific Technology Co. Ltd., Tokyo, Japan). The filtrate was dried, and this solid was a water-soluble material fraction. The residue was dried rapidly, and 1 g of dry residue was extracted by a Soxhlet extractor (WB-6S, Shibata Scientific Technology Co. Ltd., Tokyo, Japan) with 100 ml of 100% (v/v) methanol for 12 h. The dry materials extracted by methanol were the methanol-soluble lignin fraction (low-molecular-weight lignin). The saccharide contained in the residue of methanol extraction was removed using a 72% (v/v) sulfuric acid treatment. A residual lignin fraction (high-molecular-weight lignin) was obtained as a residue after a sulfuric acid treatment. The filtrate was the holocellulose fraction.

#### Determination of metals

The plant component polymers were dried at 90°C for 12 h, and heated at 500°C for 6 h using an oven (SH-OMT, Nitto Kagaku Co. Ltd., Tokyo, Japan). The mineralized samples without organic matters were acidified with a 10% (v/v)  $\text{HNO}_3$  solution. The metal concentrations in the treated samples were determined by an ICP (inductively coupled plasma) emission spectrometry OPTIMA 3300XL (Perkin Elmer, Massachusetts, USA).

## Results and Discussion

#### Determination of metals

Figure 1 shows the concentrations of Cu, Pb, Fe, and Zn unit

per gram of *A. yokoscense* plants. After the hyperaccumulating plant was treated by the steam explosion method, the metal concentrations in the plants were determined using an ICP-AES. The metal-determination technique showed that both the aboveground and underground parts of *A. yokoscense* plants especially contained Cu at higher concentrations of 6.4  $\text{mg g}^{-1}$  and 15  $\text{mg g}^{-1}$ , respectively, compared with other metals, such as Pb, Fe, and Zn. Morishita and Boratynski reported that the accumulation of heavy metals in plant increased with an increase of the concentrations of metals in soil.<sup>18</sup> Presumably, the soil components of the Ogoya copper mine, where *A. yokoscense* plants were collected, includes dominantly Cu, and the plants would uptake the copper from the soil. The metal-determination technique for hyperaccumulating plants could not only monitor the amounts of accumulated metals, but could also be used to indirectly investigate the degree of metal contamination in the environmental soil. A variety in the distribution tendency of heavy metals was observed. The metal-determination technique could reveal the characteristic distribution of heavy metals in plant-component polymers. In our previous works, each plant component polymer could be converted into useful materials and products, *i.e.* fuel materials (ethanol or methane) from the water-soluble material fraction, pulp from the holocellulose fraction, and high-functional resin from the methanol-soluble lignin fraction (low-molecular-weight lignin).<sup>12-16</sup> Therefore, the metal-concentration data obtained from the metal determination-technique will be applied to effective phytoremediation.

In the aboveground part, the water-soluble material fraction and the residual lignin fraction included Cu, while little Cu was detected in the methanol-soluble lignin fraction and the holocellulose fraction. The Cu in the water-soluble material fraction indicated a concentration two orders higher than that in

Table 1 Dry weights of plant component polymers in biomass

Biomass	Part	Plant component polymers/g g <sup>-1</sup>				Ref.
		Water-soluble material fraction	Holocellulose fraction	Methanol-soluble lignin fraction (low-molecular-weight lignin)	Residual lignin fraction (high-molecular-weight lignin)	
Wood ( <i>Eucalyptus</i> )	Trunk	0.18	0.44	0.23	0.15	15
Bamboo ( <i>Phyllostachys heterocycla</i> )	Trunk	0.16	0.53	0.049	0.26	16
Hyperaccumulating plant ( <i>Athyrium yokoscense</i> )	Aboveground	0.42	0.24	0.13	0.21	This work
	Underground	0.19	0.31	0.13	0.37	This work

the residual lignin fraction. The leaf cell walls prevent metals from maintaining to attach to the cell surfaces.<sup>19</sup> The Cu attached to the cell walls seems to be easily dissolved into the water-soluble material fraction during this pretreatment. In the underground part, Cu was detected only in the residual lignin fraction. On the contrary to the case of leaves, the root cell walls entrapped metals on the cell surfaces.<sup>19</sup> It seems that the Cu entrapped in the root cell walls bound to the residual lignin fraction (high-molecular-weight lignin) because the concentration of Cu in the residual lignin fraction was very high. Morrison *et al.* and Nishizono *et al.* reported that the heavy metals in the root cell walls were bound to the structural materials of the root cell walls, such as cellulose and lignin.<sup>20,21</sup> From our results, it was clarified that the heavy metals were bound to lignin rather than cellulose in the root cell walls.

From the results shown in Fig. 1, the amounts of Pb and Zn per 1 kg of *A. yokoscense* plant were estimated to be 3.1 g and 0.49 g, respectively, because the dry weights of the aboveground part and underground part were 290 and 710 g, respectively (data not shown). The data of Pb and Zn in *A. yokoscense* reported by Ishizawa *et al.* and Honjyo *et al.* were 1.1 and 0.37 g kg<sup>-1</sup>, respectively.<sup>22,23</sup> Our results were almost in agreement with those of previous studies concerning the order. The underground part contained higher concentrations of Pb and Zn at 3.2 mg g<sup>-1</sup> and 0.54 mg g<sup>-1</sup>, respectively, compared with the aboveground part at 2.7 mg g<sup>-1</sup> and 0.38 mg g<sup>-1</sup>, respectively. Regardless of the metal element, the concentration of the metal in the underground part was higher than that in the aboveground part. These results agreed with the data that the concentration of lead of *A. yokoscense* plants increased in the following order: petiol, leaf blade, rhizome, and root.<sup>18,19</sup> Nishizono *et al.* reported that a large proportion of metal was also contained in the root cell wall of *A. yokoscense*.<sup>21</sup> Presumably, the residual metal increased in the root tissues which filtrate water, when the water containing metals were sucked up from the roots to the leaves.

#### Determination of plant component polymers

The dry weights of the plant component polymers to the dry weight of *A. yokoscense* plants were compared with those of bamboo and wood, which were obtained in our previous studies (Table 1). The lignin content, *i.e.* the sum of the methanol-soluble lignin fraction (low-molecular-weight lignin) and the residual lignin fraction (high-molecular-weight lignin), of *A. yokoscense* plants in the aboveground part was 0.34 g g<sup>-1</sup>. This value was larger than that of bamboo (0.31 g g<sup>-1</sup>), but smaller than that of wood (0.38 g g<sup>-1</sup>). The dry weights of the water-soluble material fraction and the holocellulose fraction of *A. yokoscense* plants in the aboveground part were different from

those of bamboo, while these results were in good accord with the data of a rice straw, *i.e.* hemicellulose and cellulose were 0.35 and 0.35 g g<sup>-1</sup>, as reported by Ado.<sup>24</sup> These results indicated that the character of *A. yokoscense* was similar not to woody biomass, but to monocotyledonous angiosperm biomass. The dry weight of residual lignin fraction (high-molecular-weight lignin) of *A. yokoscense* plants in the underground part was higher than that in the aboveground part. If *A. yokoscense* plants were converted into methane, the amount of the methane produced from the aboveground part was estimated to be higher than that from the underground part because the amount of methane produced is inversely proportional to the dry weight of the residual lignin fraction (high-molecular-weight lignin).<sup>16</sup>

#### Conclusion

For the determination of metals in plant component polymers of a hyperaccumulating plant, *A. yokoscense*, steam explosion followed by Wayman's extraction method were examined. Estimations of the concentrations of Cu, Pb, Fe, and Zn indicated that almost metal elements exist in the water-soluble material fraction of the aboveground part (leaves, leaf blade, petiol, and rhizome) and in the residual lignin fraction of the underground part (roots). From the results of measuring the dry weights of the plant component polymers, the dry weights of the water-soluble material fraction and the holocellulose fraction were high in the aboveground part and the dry weights of residual lignin fraction (high-molecular-lignin) and holocellulose fraction were high in underground part. This determination of metals in plant component polymers will contribute to evaluating the efforts of phytoremediation using a hyperaccumulating plant.

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