Heavy metals accumulation by *Athyrium yokoscence* in a mine area, Southwestern Japan

Hendra Prasetia\(^1\), Masayuki Sakakibara\(^{1,2}\), Akinari Takehara\(^{1,3}\), Yuri Sueoka\(^1\).
\(^1\) Graduate School of Science and Engineering, Ehime University, Matsuyama 790-8577, Ehime, Japan
\(^2\) Faculty of Collaborative Regional Innovation, Ehime University, Matsuyama, Ehime, Japan
\(^3\) Fukuizumi Junior High School, Sakai City, Osaka, Japan

**Abstract.** Mine dumps pose environmental problems worldwide. Phytoremediation is a remediation technique that uses plants to clean polluted environments. *Athyrium yokoscence*, a fern of the family Aspidiaceae, is a well-known Cd hyperaccumulator used in phytoremediation. The aim of the current study is to determine the extent of heavy metal absorption by *A. yokoscence* and evaluate its potential use in phytoremediation. The shoots, stems, and roots of *A. yokoscence* were analysed by inductively coupled plasma–optical emission spectrometry (ICP–OES) to determine the concentrations of Cd, As, Pb, Cu, and Zn, yielding maximum concentrations in the shoots of 851, 215, 192, 60.9, and 769 mg/kg-DW, in the stems of 481, 140, 881, 28.9, and 495 mg/kg-DW, and in the roots of 1210, 1868, 6473, 2484, and 5446 mg/kg-DW, respectively. The results indicate that *A. yokoscence* is a hyperaccumulator of Cd and can be used for the translocation of As, Pb, Cu, and Zn within the shoot tissue. The roots translocate heavy metals through the stems and they finally reside within the shoot tissue. The results suggest that *A. yokoscence* has considerable potential use as an environmental assessment tool for Cd, As, Pb, Cu, and Zn, and in helping to remediate mine dumps.

**Keywords:** hyperaccumulator; phytoremediation; translocation, maximum concentration

1. Introduction

The toxicity of heavy metals is a serious problem for the integrity of environmental, ecological, and nutritional systems [1]. Heavy metal pollution has been recognized as a global issue for decades. The pollutants are introduced to the environment directly as a result of accidental spills during transportation, leakage from waste disposal sites, and industrial contamination [2]. The metals contaminate air, water, and soil. The most common heavy metal contaminants are Cd, Cr, Cu, Hg, Pb, and Zn [3].

Various techniques and chemical methods are used to remediate heavy metal contamination, such as soil washing, soil vapor extraction, land farming, soil flushing, solidification or stabilization, thermal desorption, biopiles, bioslurry systems, bioventing, encapsulation, and aeration [4].

Biological methods, utilizing living organisms can be applied to assess the degree of heavy metal contamination within an environment [5,6]. Phytoremediation is a well-known, cost effective, green technology that uses plants to remediate heavy metal contamination in water, air, and soil. This technology consists of two components: root-colonizing microbes and the plants themselves [7].
Hyperaccumulator plants are commonly used in phytoremediation. A hyperaccumulator is defined as a plant in which the concentrations of heavy metals in the above-ground parts are 50–100 times higher than in plants from non-polluted environments [8]. A plant’s translocation factor (TF) is the ratio of a metal or metalloid’s concentration in the shoots to that in the roots [9], and this value can be used to determine if a species is a hyperaccumulator, as well as being an important parameter in studies of heavy metal uptake by plants [10,11].

A plant’s species and genotype determines its tolerance to heavy metals [12,13] *Athyrium yokoscence* is a fern of the family of Aspidaceae [14] that is used as an indicator of metal-contaminated soils in Japan [15]. Nishizono *et al.* [16] reported that *A. yokoscence* grows predominantly on metal-contaminated soils in Japan and is a non-habitual condenser. The fern accumulates high levels of Cu and Zn in the roots, and Cd in the shoots, when grown on soils contaminated with heavy metals [16]. Previous studies have shown that *A. yokoscence* has potential for use in the phytoremediation of soils contaminated by heavy metals. In this context, the aim of this study is to determine the potential use of *A. yokoscence* for phytoremediation in mine dumps.

2. Materials and Methods

This is a preliminary study that investigates the use of *A. yokoscence* to assess the degree of contamination in an abandoned mine site and evaluates its potential use in phytoremediation and as an indicator of environmental toxicology. The organic and mineral contents (%vol) of soil from the site were measured to a depth of 5 cm, using a soil moisture kit, to determine the relationship between the composition of the soil, in which the plant takes root, and heavy metal uptake by the plant tissue.

2.1. Sampling plots

A field survey was completed at an abandoned mine dump in Southwestern Japan on 10–11 May 2014. The dump is located at an altitude of 400–500 m above sea level, in a warm-temperate zone. Copper and Pb were the principal metals to have been smelted at the abandoned mine site, between AD 808 and 1972. The surface of the waste dump consists mainly of slag fragments and tailings composed of andesite, mudstone, and limestone. Solidified and coherent slag has become partially exposed on the slag and tailings dump. *A. yokoscence* grows naturally on this mine dump, and 12 live *A. yokoscence* plants were collected for analysis from randomly selected sites across several areas of the mine dump (Figure 1).

2.2. Analytical methods

The fern samples were first washed with tap water and separated into shoots, stems, and roots. After separation they were rinsed in an ultrasonic frequency cleaner with ultrapure water for 5 min intervals until clean. The samples were then dried at ~80 °C for two days in a ventilated oven. Once dried, the samples were pulverized using a powder mill (Varian PM 2005 m, Osaka Chemical). Each 20 mg sample of shoots, stems, or roots was digested with a solution of H2O2, HF, and HNO3 at a ratio of 2:5:10. Heavy metal concentrations were determined by inductively coupled plasma–optical emission spectrometry (ICP–OES) (Varian Optima™ 8300 series, PerkinElmer, Kanagawa, Japan) at the Integrated Centre for Sciences, Ehime University, Matsuyama, Japan. The different operating wavelengths of metals were used to identify the element in the sample [17,18].

2.3. Translocation Factor

The TF is defined as the ratio of the heavy metal concentration in the shoots to that in the roots [19,20] as follows [21,22]:

\[
TF = \frac{C_{\text{shoot}}}{C_{\text{root}}} \tag{1}
\]

where \(C_{\text{shoot}}\) is the metal concentration in the shoots and \(C_{\text{root}}\) is that in the roots. The TF is calculated using the heavy metal concentrations and is used to evaluate the phytoextraction ability of a plant, in particular the plant’s ability to translocate heavy metals from the roots to the shoots [22].
2.4. Statistical analysis
Statistical analyses were performed using OriginPro 9.0 for Windows. The Shapiro–Wilk test was used to check the normality of the heavy metal concentrations in the shoots, stems, and roots. The data were log normally distributed, so the Kruskal–Wallis ANOVA test was used to test for significant differences, with $p < 0.05$ considered statistically significant.

![Figure 1. Distribution map of A. yokosence and sampling point in an abandoned mine site](image)

3. Results
The organic and mineral contents of the soil samples ranged from 3.80 to 31.6 and 0.20 to 25.3 (%vol) (Table 2), respectively. These results indicate that there is no significant relationship between the organic and mineral contents of the soil and heavy metal uptake into the plant tissue. The total concentrations of Cd in the plant shoots ranged from 60.9 to 851 mg/kg dry weight (DW) (Table 1). The total concentrations of Cd in the stems and roots ranged from 111 to 481 and from 184 to 1210 mg/kg DW (Table 1), respectively. These results suggest that A. yokosence can accumulate high concentrations of Cd, at levels that exceed the standard concentration for hyperaccumulators of Cd, at this abandoned mine site.

| Sample | Cd Shoot (mg/kg-DW)±SD | Stem (mg/kg-DW)±SD | Root (mg/kg-DW)±SD | TF | As Shoot (mg/kg-DW)±SD | Stem (mg/kg-DW)±SD | Root (mg/kg-DW)±SD | TF | Pb Shoot (mg/kg-DW)±SD | Stem (mg/kg-DW)±SD | Root (mg/kg-DW)±SD | TF |
|--------|------------------------|--------------------|--------------------|----|------------------------|--------------------|--------------------|----|------------------------|--------------------|--------------------|----|
| 1      | 487±87.1               | 434±71.5           | 1210±280           | 0.40 | 51.8±9.2               | 61.2±10.1          | 84.2±19.5          | 0.61 | 92.1±16.5              | 257±42.3            | 1147±265           | 0.08 |
| 2      | 230±40.6               | 181±32.4           | 184±31.3           | 1.25 | ND                     | 55.3±9.1           | 833±142            | ND  | 32±23.4                | 441±70.9            | 3876±658           | 0.03 |
| 3      | 301±54.3               | 240±41.2           | 652±120            | 0.46 | 215±38.8               | ND                 | ND                 | ND  | 64.7±11.7              | 55.0±9.44            | 445±82.0           | 0.15 |
| 4      | 425±75.2               | 390±89.2           | 982±167            | 0.43 | 161±28.4               | ND                 | ND                 | ND  | 40.4±7.1               | 454±80.6            | 158±26.8           | 0.26 |
| 5      | 280±48.2               | 234±39.1           | 714±124            | 0.39 | 47.6±8.18              | 26.0±4.32          | 2.31±0.40          | 20.58 | 32.1±5.53              | 246±41.1            | 740±129            | 0.04 |
| 6      | 381±65.9               | 210±39.1           | 917±159            | 0.42 | 104±17.9               | 57.7±10.7          | ND                 | ND  | 24.3±4.2               | 99±18.4             | 422±72.8           | 0.06 |
| 7      | 448±78.4               | 375±64.9           | 448±79.4           | 1.00 | ND                     | ND                 | 1868±331           | ND  | 59.6±10.4              | 150±26.0            | 6473±1148          | 0.01 |
| 8      | 492±87.1               | 333±62.3           | 545±96.4           | 0.90 | 92.8±16.4              | ND                 | 112±19.7           | 0.83 | 114±20.3               | 49.6±9.30           | 3182±563           | 0.04 |
| 9      | 350±51.1               | 305±51.7           | 792±169            | 0.44 | 102±18.7               | 47.1±7.98          | 127±27.0           | 0.81 | 46.2±4.85              | 204±34.7            | 1091±232           | 0.04 |
| 10     | 60±10.6                | 111±19.2           | 232±40.8           | 0.26 | 161±28.1               | ND                 | 545±95.7           | 0.30 | 191±33.5               | 880±152             | 3361±590           | 0.06 |
| 11     | 851±154                | 481±81.7           | 953±171            | 0.89 | 146±26.1               | 119±20.2           | 363±65.2           | 0.40 | 160±29.0               | 373±63.2            | 2236±401           | 0.07 |
| 12     | 458±81.8               | 354±59.2           | 760±135            | 0.60 | ND                     | 140±23.5           | 388±68.9           | ND  | 62.8±11.2              | 175±29.3            | 1032±184           | 0.06 |

DW: Dry Weight; SD: Standard Deviation; ND: Not Detected.
The shoots of *A. yokoscence* accumulated As and Pb at total concentrations ranging from not detected (ND) to 215 and from 24.3 to 192 mg/kg DW, respectively (Table 1). Ranges of Cu and Zn total concentrations in the shoots were from ND to 60.9 and from ND to 770 mg/kg DW, respectively (Table 2). These results suggest that *A. yokoscence* cannot accumulate high concentrations of As, Pb, Cu, and Zn from the roots into the shoots. Ranges of As, Pb, Cu, and Zn in the stems were from ND to 140, 49.6 to 881, ND to 21.5, and 63.9 to 494.7 mg/kg DW, respectively, and in the roots were from ND to 1868, 158 to 6473, ND to 2484, and 508 to 5446 mg/kg DW, respectively (Table 1 and 2). The amount of heavy metals transported from roots to stem show a positive correlation at a logarithmic scale, with the root concentrations (Figure 2). The results indicate that *A. yokoscence* has the ability to uptake heavy metals into its root tissue, but is generally unable to transport them to its stem tissue.

8. **Table 2.** The Cu and Zn concentrations and organics and minerals content of soil of *A. yokoscence.*

| Sample | Shoot (mg/kg-DW)±SD | Stem (mg/kg-DW)±SD | Root (mg/kg-DW)±SD | TF | Shoot (mg/kg-DW)±SD | Stem (mg/kg-DW)±SD | Root (mg/kg-DW)±SD | TF | Organic (%vol) | Mineral (%vol) |
|--------|---------------------|-------------------|-------------------|----|---------------------|-------------------|-------------------|----|---------------|----------------|
| 1      | ND                  | 33.2±7.67         | ND                | ND | 497±89.0            | 254±42.0          | 1456±336          | 0.34| 6.40          | 2.43           |
| 2      | ND                  | 741±126           | ND                | ND | 367±66.5            | 103±17.7          | 833±153           | 0.44| 4.80          | 1.00           |
| 3      | ND                  | ND                | ND                | ND | 583±103            | 415±73.6          | 1650±280          | 0.35| 15.3          | 10.40          |
| 4      | 60.9±10.8           | 28.9±5.13         | ND                | ND | 225±58.7           | 151±25.3          | 1109±193          | 0.20| 18.9          | 13.7           |
| 5      | ND                  | ND                | ND                | ND | 213±37.0           | 157±29.2          | 946±164           | 0.23| 5.60          | 1.6            |
| 6      | ND                  | ND                | ND                | ND | 18.7±3.24          | 495±85.6          | 5447±966          | 0.13| 23.7          | 18.2           |
| 7      | ND                  | 21.5±4.03         | ND                | ND | 694±122            | 332±62.3          | 5026±888          | 0.13| 15.5          | 10.7           |
| 8      | 20.1±3.56           | 2484±439          | ND                | ND | 659±117            | 509±108           | 103±17.7          | 0.39| 18.1          | 12.7           |
| 9      | ND                  | ND                | ND                | ND | 7.21±1.53          | 145±24.6          | 790±140           | 0.39| 15.6          | 9.40           |
| 10     | ND                  | ND                | ND                | NP | 200±36.6           | 145±24.6          | 509±108           | 0.39| 15.6          | 9.40           |
| 11     | 37.2±6.74           | 904±162           | ND                | 0.04| 770±139           | 368±62.5          | 3275±588          | 0.23| 12.2          | 7.47           |
| 12     | ND                  | 486±86.5          | ND                | 0.24| 456±81.6          | 242±40.5          | 1904±340          | 0.24| 17.3          | 12.4           |

**DW:** Dry Weight; **SD:** Standard Deviation; **ND:** Not Detected.

### 4. Discussion

4.1. Heavy metal absorption and transportation from the roots to stem of *A. yokoscence*

Heavy metals are absorbed into plant tissue through the phloem, in conjunction with nutrient absorption. The heavy metals are absorbed in the form of essential macro- and micronutrients, induced by the selective uptake of ions by roots, or by the diffusion of elements in the soil [23,24]. The ability of a plant to accumulate heavy metals is considered a detrimental trait in the long term [25], because even micronutrients can become toxic for plants when they exceed threshold values [24]. The composition of the soil substrate and its water content may influence a plant’s ability to uptake heavy metals [26]. The separation of metals from their source, by uptake to a root, is complicated by the mediating effect of soil properties and plant processes [23]. Heavy metal uptake by plants depends on several plant factors, such as root intrusion, water, and ion flux, the kinetics of metal solubilization in soils, biological parameters, and a plant’s ability to adapt metabolically to metal stresses in the environment [23]. This study includes some observations of soil composition, but more detailed analysis would be required to fully understand the role of the soil substrate in the absorption and transport of heavy metals by *A. yokoscence*.

Cadmium represents a serious environmental hazard because it can be absorbed through the alimentary tract, it penetrates through the placenta, and damages membranes and DNA [27]. The preferential uptake of Cd, as observed in this study, is of particular concern because it demonstrates that Cd can be present at elevated levels in plant tissues not considered to be phytotoxic. This could pose a threat to humans and animals if the plants form part of their food chain [24]. Iron deficiency promotes the uptake of Cd in *Thlaspi caerulescens* Ganges ecotype [28]. In *Arabidopsis halleri*, Cd transfer from the growing medium to the root xylem is partially shared with Zn and/or Fe transport [29]. On the other hand, a manganese-enriched medium promotes the uptake of Cd in *Lactuca sativa* [30].
Figure 2. TF and transporting of heavy metals of *A. yokoscence*; ND: Not Detected; DW: Dry Weight.

Cd concentrations in the shoots of *A. yokoscence* may exceed 100 mg/kg DW, indicating this is a hyperaccumulator plant [31]. Total Cd concentrations observed in shoots, in previous studies by Van et al. [15] and Morishita and Boratynski [32], were <1200 and 996 mg/kg DW, respectively. In the present study, Cd uptake to the stem tended to increase and stabilize when Cd was present at high concentrations in the roots (Figure 2). A positive correlation between Cd transport to the stem and root concentrations indicates that *A. yokoscence* has the potential to accumulate high concentrations of Cd in its shoot tissue (Figure 2). This suggests in turn that *A. yokoscence* can be used for phytoremediation and as a preliminary step in environmental assessments of Cd contamination.

In the present study, *A. yokoscence* also accumulated As, Pb, Cu, and Zn (Figure 2). Other studies have reported that As accumulation is influenced by soil properties [33]. Plants with concentrations of As in their shoots exceeding 1000 mg/kg DW have been classified as As hyperaccumulators [34].

Lead is highly mobile in the soil and accumulates at depths of up to 20.32 cm [3]. The amount of Pb in a plant is controlled by the pH, organic matter content, and phosphorus concentration of the soil [3]. In this study, no relationship was observed between Pb concentrations and the organic content of the soil, which suggests that Pb uptake and accumulation are not controlled by organic matter content in this case.

In root tissue, Cu is almost always present in complexed forms; however, it is most likely that the metal enters the root cells in dissociated forms [35]. The rate of Cu uptake by plants differs widely...
according to speciation [35]. The concentration of Cu in root tissue affects a plant’s capacity to retain Cu against transport to the shoots [35].

Zinc uptake is controlled by plant metabolism [34]. When Zn concentrations in contaminated soil exceed the level at which Zn is required as a nutrient, it may cause phytoextraction [1]. The accumulation and absorption of Zn into the shoots is necessary, given its role as a micronutrient [26]. The positive correlation between the concentration of metals in the stem and roots, as observed in this study (Figure 2), indicates that A. yokoscence has potential both as a metal-tolerant plant and in preliminary environmental assessments of As, Pb, Cu, and Zn at the level of an individual park. The uptake of these metals and their transport from roots to stem tend to increase and become stabilized at high concentrations in the root tissue. In the present study it is possible that leaching occurred during washing and sample preparation, which may have caused a bias in the data and the very wide range in concentrations observed in this study. A logarithmic scale was used to determine the correlations between the concentrations of Cd, As, Pb, Cu, and Zn in the roots and stems, and in roots and shoots, given the wide range in values.

4.2. Translocation Factor
The TF is a measure of a plant’s ability to translocate heavy metals from the roots to the shoots. While some species are able to accumulate heavy metals in their shoots, most plants have a low TF value, which indicates they have limited ability to translocate heavy metals. In this study, the TF values of Cd, As, Pb, Cu and Zn range from 0.26 to 1.25, ND to 20.6, 0.01 to 0.25, ND to 0.04, and ND to 0.44, respectively. When a TF value exceeds 1, it indicates the plant is a hyperaccumulator. Mycorrhizal fungi may influence TF values [9]. The TF values in this study indicate the hyperaccumulation of Cd and As by A. yokoscence (Figure 2). However, the TF results differ from the concentration results, especially for As (Figures 2). This finding indicates that a plant should not be classified as a hyperaccumulator based solely on TF values, but that concentration data for the metal of interest in the shoots should also be considered in the classification, and vice versa.

5. Conclusions
The present results show that A. yokoscence steadily accumulates Cd, As, Pb, Cu, and Zn, and translocates high concentrations of Cd to its shoots. The positive correlation between heavy metals in the shoots, stem, and roots indicates that A. yokoscence can be used to determine contamination by these metals in a preliminary environmental assessment. The results indicate that the hyperaccumulator classification should reflect a combination of shoot accumulation and TF. These factors indicate that A. yokoscence has potential for the phytoextraction of Cd at abandoned mine sites.

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