Article

Analysis of Rare Earth Elements in Pteridophytes from the Historical Mining Area, Southwestern Japan

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Abstract: Recent studies have shown that various fern species can accumulate REEs in the soil and may be utilized as indicator plants. *Athyrium yokoscense*, commonly called hebino negoza in Japan, is a pteridophyte that is used as an indicator of metal-contaminated soils in Japan. Mining and mineral processing increase the potential of mine minerals to oxidize and change their chemical form. A study on the biogeochemical behavior of REEs in the mine area needs to be conducted due to the significant use of REEs as an essential material for various applications in the world. The REE content in sediment and pteridophytes under different mining areas was analyzed by inductively coupled plasma-mass spectrometry. The concentration of REEs contained in *Athyrium yokoscense* was analyzed and then compared to sediment to determine the ability of the plant to absorb and accumulate rare earth elements in the mine area. Sediment and plant samples were collected from three mining areas: Ikuno mine, Akenobe mine, and Tada mine. *Athyrium yokoscense* plants were collected and digested with hydrogen peroxide (H_2O_2) and nitric acid (HNO_3) at a ratio of 2:5. Sediment samples were taken and digested by the alkali fusion method using lithium tetraborate and then processed using the conventional column method. The present study determined that the concentration of REEs varies in every location, and light REEs tend to be more abundant in sediments than heavy REEs. The amount that can be transferred to ferns also varies. *Athyrium yokoscense* in the ex-mining area accumulates proportionately more LREEs than HREEs, specifically in the *Athyrium yokoscense* leaves. The characteristic of the element significantly contributes to the accumulation of REEs in pteridophytes.

Keywords: rare earth element; pteridophyte; sediment; mining

1. Introduction

Recent studies have shown that various fern species can accumulate rare earth elements (REEs) in the soil and may be utilized as indicator plants. *Athyrium yokoscense*, commonly called hebino negoza in Japan, is a pteridophyte that is used as an indicator of metal-contaminated soils in Japan. Mining and mineral processing increase the potential of mine minerals to oxidize and change their chemical form. Due to the increasing usage of REEs for industrial purposes worldwide, research into the environmental biogeochemical soil-plant system behavior of REEs has become the primary issue. This study provides a detailed chemical analysis of REEs to provide information for further studies.

Rare earth elements (REEs) are a group of 17 chemically similar metallic elements—15 lanthanides, with atomic numbers 57 (lanthanum, La) to 71 (lutetium, Lu), together with yttrium (Y, atomic number 39) and scandium (Sc, atomic number 21), as referred to in the International Union of Pure and Applied Chemistry (IUPAC) [1]. The 15 lanthanide elements have been further subdivided into light rare earth elements (LREEs) with lower atomic weights—lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), and europium (Eu); and heavy rare earth elements (HREEs)—gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er),

thulium (Tm), ytterbium (Yb), and lutetium (Lu) [2]. Yttrium is classified with the HREE despite its lower atomic weight due to chemical similarities [3,4].

The unusual chemical and physical properties of REEs have made them useful for a long time. Their natural occurrence is highly reliant on geological conditions and is only found in a few sites in sufficient quantity and concentration, as well as in the right form and setting, to make extraction and exploitation commercially viable. REEs have also been long-established tools for furthering scientific research into the origins of rock and ore geology. In recent years, REEs have had a wide range of high-tech applications, particularly in low-carbon technologies, and demand for them has increased rapidly, a trend that is projected to continue [5]. In the field of medicine, REEs, especially scintillators based on lanthanides, are extremely important for imaging and diagnostics such as CT and PET [6]. At the same time, the future security of their supply, their costs, and any possible risks become a concern on a global scale.

REEs are found in a variety of geological settings, including igneous, sedimentary, and metamorphic rocks. These elements have been found in high concentrations in heavy mineral sand deposits (beach, dune, marine tidal, and channel), carbonatite intrusions, (per)alkaline igneous rocks, iron oxide breccia complexes, calc-silicate rocks (skarns), fluora-patite veins, pegmatites, phosphorites, fluviatile sandstones, unconformity-related uranium deposits, and lignite. Various rock-forming processes, such as enrichment in magmatic or hydrothermal fluids, separation into mineral species and precipitation, and subsequent redistribution and concentration through weathering and other surface processes, all influence the REEs distribution and deliberation in these deposits [7]. In alkaline igneous rocks, layered alkaline complexes, granitic stocks, late-stage dikes, and rarely trachytic volcanic and volcaniclastic deposits also contain REEs mineralization [8]. In addition, REE concentrations in soils are mostly determined by pedogenetic processes and the mineralogy of bedrock and soil. The concentration, speciation, and adsorbed soil water are the main factors influencing the uptake of REEs by plants, rather than only the plants themselves [9].

1.1. REEs in the Mine Area

Mining activities are widely acknowledged to have altered the original environmental conditions and brought about several environmental issues, including ecological destruction, environmental pollution, soil erosion, and geological disasters [10–14]. Due to the disturbed environment, more harmful elements may be released into the environment, causing adverse impacts. Large numbers of waste rocks are generated by mining activities, which are normally placed in large piles around the mine, underground corridors, and vast open pits that can be flooded when mines are abandoned [15,16]. Although significant concentrations of REEs have been reported in near-neutral and alkaline mine waters [17,18], REEs solubility increases under acidic conditions [19]. REEs are naturally omnipresent in soils that mainly originate from the weathering of local parent rocks. Under natural conditions, the concentration of REEs in soils is mainly determined by their parent rocks [20]. Compared to other metal ions, REEs have a limited ability to translocate under natural conditions. The majority of exogenous REEs are fixed on solid surfaces and exist in inert forms, which are typically concentrated in the top layer of soils or deposited in stream sediments [21].

Mining and mineral processing generate large volumes of waste rock and tailings, which may act as sources of persistent toxic metals long after mining operations cease [22]. The important thing about closed mining, especially small abandoned mines in Japan, is that no one is monitoring its mining activities and how they are managed. Some of the mine minerals could be oxidized and change their chemical form. Mining refers to the process of extracting metals and minerals from the earth, and some metals are leached out and carried downstream as water washes over the rock surface. Metal mobility to the environment can be increased following their dissolution and leaching from tailings [23], often due to acid effluents from mines [24,25]. Davis et al. (1994) stated that contamination of surface and groundwater bodies has particularly been experienced in mining and industrial communi-

ties [26]. In recent decades, a large number of studies have focused on the geochemistry of heavy metals and metalloids whose toxic effects have been well understood in mining areas, such as As and Hg [27]. Heavy metal pollutants, such as arsenic, cobalt, copper, cadmium, lead, silver, and zinc, are contained in excavated rock or exposed in underground mines encountering water. However, with the increasing usage of REEs as momentous materials for industrial and agricultural purposes worldwide and the development of mining activities, more studies on the environmental biogeochemical behavior of REEs in mine areas are needed [28]. A study revealed the REE concentrations in sediments from the Linggi River showed enrichment of LREEs. Natural and terrigenous activities (sand mining activities, residential development, and road construction) are the potential source of REE contaminations in Linggi River sediment [29]. Furthermore, the *Mitromorpha iridescens* species, which live close to the seabed, are exposed and accumulate REEs from sediment and were able to accumulate markedly high concentrations of REEs [30]. To interpret the distribution behaviors of REEs, factor analysis was performed to divide several factors that influence REE concentrations in the abandoned mine and the surrounding areas. This study provides a detailed chemical analysis of REE accumulation and transportation in the Ikuno, Akenobe, and Tada mines to provide information for further studies.

1.2. Athyrium yokoscense

Athyrium yokoscense, commonly called hebino negoza in Japan, is a fern species from the family Asipidaceae [31] and is commonly used as an indicator of metal-contaminated soils in Japan [32]. Recent studies have shown that various fern species can rapidly increase the amount of REEs in the soil and may be utilized as indicator plants [33]. A plant is referred to as a hyperaccumulator if it takes up abnormally high levels of contaminants from the soil and accumulates them in its aboveground biomass [34,35]. Hyperaccumulator plants are commonly used in phytoremediation. A hyperaccumulator is defined as a plant in which the concentrations of heavy metals in the aboveground parts are 50–100 times higher than those in plants from nonpolluted environments [36]. However, only a few plants (21 taxa are now recognized) have the capacity to hyperaccumulate more than 0.1% of REEs in their aboveground parts [37,38]. These plants have potential applications in phytoremediation. The term "phytoremediation" refers to the process of removing pollutants from the environment or reducing their toxicity by the use of plants, soil amendments, and agronomic techniques [39,40]. In addition, it can be utilized for agromining for the extraction of REEs from REE-contaminated mine soils [41,42].

REE transfer throughout the ecosystem is now recognized as a potential problem because many studies have shown toxicological effects on bacteria, fungi, plants, and animals [43,44]. These observations are strong arguments for considering REEs as emerging pollutants. Although REEs are not considered essential for plants, it has been widely shown that they can be taken up and transferred to their aboveground biomass in adequate concentrations. Similar to other metals, a few plant species have been reported to accumulate REEs. A study reported that *Athyrium yokoscense* grows predominantly on metal-contaminated soils in Japan.

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 [45]. A study has shown that *Athyrium yokoscense* has the potential for use in the phytoremediation of soils contaminated by heavy metals. It has been reported that the roots of this fern accumulate a large amount of Pb (~22,400 ppm) as does the leaf blade (~2080 ppm) [46]. In addition, an investigation has been conducted to identify the potential plant species hyperaccumulating LREE in a mining area and revealed that *Christella dentata* has a strong capacity to accumulate Ce and has become a promising hyperaccumulator species [47]. Despite the previously useful information, there has not been enough systematic study of the environmental biogeochemical behavior of REEs in soil-plant systems. The concentration of REEs contained in *Athyrium yokoscense* will be analyzed and then compared to sediment to determine the ability of the plant to

absorb REEs in the mine area as well as to expand our knowledge of REEs uptake and control in this plant.

2. Materials and Methods

2.1. Study Area

There are three areas for this research: Ikuno, Tada, and Akenobe mines, all of which are located in Hyogo Prefecture, southwestern Japan (Figure 1). The Ikuno mine is a silver mine located in the area of Asago City. The Ikuno and Akenobe areas consist of Late Cretaceous volcanic rocks (rhyolite, biotite rhyolite, dacite, and andesite); plutonic rocks of the Paleocene-Early Eocene, Carboniferous, and Permian (granodiorite, diorite, and gabbroic) accretionary complex; sedimentary rocks of early-middle Jurassic accretionary complex; late Pleistocene-Holocene gravel, sand, mud sandstone, and shale; and many mineral occurrences in this area. Related to lithology, this area is divided into two parts: from south to middle and middle to north. From the south to the middle part, felsic volcanic rocks (rhyolite) and sedimentary rocks (gravel, sandstone, and shale) are dominant. They are distributed from the eastern to the middle part of the research area. A small number of gabbroic rocks are also distributed at the center of felsic volcanic rocks in the eastern part. Some mafic volcanic rocks, felsic plutonic rocks, and sedimentary rocks in the accretionary complex, and small amounts of mafic plutonic rocks are distributed from the western to central parts of this first area. The second area (from the middle to the northern part) is more complicated. All of the bedrock types are distributed in this area, including limestone of the Yakuno complex, granitic rocks, rhyolite, andesite, granodiorite, and sedimentary rocks (shale, gravel, sand, and sandstones).
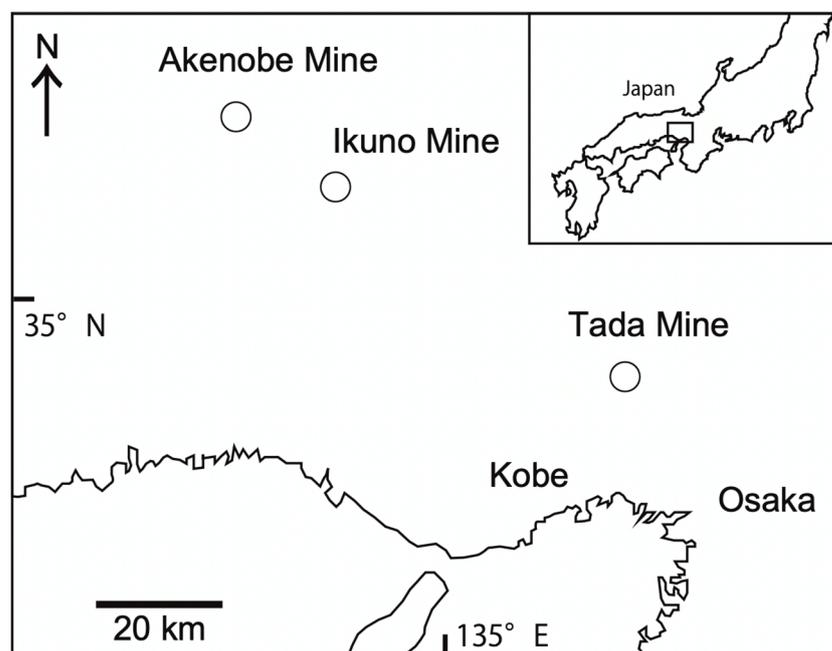

Figure 1. Research Area in Hyogo Prefecture, southwestern Japan.

The Tada mine is a silver and copper mine located in Inagawa town and has become one of the oldest mining areas in Japan. The Tada silver and copper mine is a closed mine in this area. The metal concentrations in the soil samples of the Tada mine site were sufficiently high to be considered heavy metal contaminated (metalliferous) according to research by Bert et al. (2002), in addition to exceeding several legislative thresholds [48]. This site is contaminated with both cadmium and zinc, and accordingly, the Tada mine showed a high level of zinc hyperaccumulation [49]. *Athyrium yokoscense* plants are abundant in this area and are traditionally used as an indicator species to find ore deposits in Japan [50].

Similar to *Athyrium yokoscense*, *Arabidobsis halleri* is also abundant in this area as another hyperaccumulator plant. The Akenobe mine is a copper-zinc-tin-arsenic mine located in Yabu City. The Akenobe mine has become the highest-producing source of tin in Japan. All three mines have been abandoned in recent years.

2.2. Methods

Inductively coupled plasma-mass spectrometry was used to determine the REE concentrations of sediment and pteridophytes under different mining locations. The ability of the plant to absorb and accumulate REEs in the mine area was evaluated by analyzing the concentration of REEs present in *Athyrium yokoscense* and comparing it to sediment. Sediment and plant samples were collected from each mine, except for the sediment sample from the Tada area.

The sampling positions were determined to represent the respective drainage areas (Figures S1–S3). The typical sampling site is confluent near the junction with the main river. The samples were collected at the exits of drainage basins over the area. For the sediment sample, fine-grained stream sediment that passed through an 80-mesh (0.177 mm) stainless steel sieve was collected in wet conditions from active tributary streams from drainage basins throughout the study area. The collected sediments were rinsed with water to separate organic compounds and filtered by filter paper.

In the ex-mining area, *Athyrium yokoscense* is abundant and grows naturally, and the plant grows along the watershed and the surrounding areas. The sample was taken from a plant that was approximately 20–30 cm in height. Approximately five to ten plants are taken specifically from the lamina. The leaves were dried at ~80 °C for twenty-four hours in a ventilated oven. Once dried, the samples were separated between the pinna and pinnule (segment). The pinna part was then pulverized using a powder mill for 30 min. The sample continued to be ground again with an agate mortar and pestle until it became a fine powder. A strainer was used for sieving the fine powder to obtain a homogenous sample. Each 100 mg sample of leaves was weighed and then digested with a solution of nitric acid (HNO_3). By only using nitric acid, the sample is not completely digested and still leaves lumps on the sample. Therefore, another solution was used. In this case, 30% hydrogen peroxide (H_2O_2) and EL grade nitric acid (HNO_3) were placed into a digestion beaker at a ratio of 2:5 and then heated on a hot plate at 140 °C for two hours. The digested sample was transferred to a container and filtered by an injection filter of 0.45 μm. The small pore size of the filter was used to reduce the insoluble particles in the solution. The REEs content was analyzed by ICP-MS.

For the sediment, collected samples were dried at 110 °C in the oven for approximately 24 h. To reduce the variation in the estimated element concentrations, all samples in this study were homogenized and filtered through an 80-mesh (0.177 mm) sieve again. The next preparation was digested using alkali fusion methods, which took approximately 2.5 g of sediment. The sample was ground with an agate mortar and pestle for approximately 20 min. For making a glass bead, the sediment sample was mixed with the reagent $Li_2B_4O_7$ with a sediment concentration of 2 g and a reagent concentration of 3 g. The mixture was placed in a platinum dish and heated at 1000 °C for 12 min in the TR AutoBead-1000-S machine.

Glass beads were ground with an agate mortar and pestle to make a powder. Approximately 12.5 mg of powder was processed using the column method for analyzing the REEs. At this point, approximately 20 mg of the outcome was ready to analyze. Concentrations of REEs in the diluted digestion plant solutions and sediment samples were measured by inductively coupled plasma-mass spectrometry (Agilent ICP-MS 7500). The precisions of the ICP-MS measurement were typically less than 5% for the sediment sample and less than 10% for the plant sample.

3. Results and Discussion

3.1. REEs in Sediment

The analysis revealed that all sediment samples contain REEs (Table 1). The highest concentration of REEs in the Ikuno area is cerium (Ce) (50.85 mg kg^{-1}). Within sampling sites, the concentration of Ce was higher than that of any other REEs, which could be affected by the characteristics of Ce. Cerium in the oxidized form Ce^{4+} has a higher hydrolysis ability than other trivalent REEs. Ce^{4+} is likely to precipitate and remain in the original surface soil, resulting in its higher abundance there [51].

Table 1. The average REEs concentration in sediment samples collected from the Ikuno mine and Akenobe mine.

Element	Ikuno		Akenobe	
	mg kg^{-1}	Std	mg kg^{-1}	Std
Y	24.09	7.15	24.99	6.12
La	24.18	7.77	18.9	5.2
Ce	50.85	15.58	43.35	13.32
Pr	5.4	1.61	4.53	1.24
Nd	20.35	5.92	18.08	5.08
Sm	3.97	1.09	3.88	1.1
Eu	0.93	0.29	1.08	0.43
Gd	3.81	1.02	3.97	1.15
Tb	0.65	0.17	0.69	0.17
Dy	4.15	1.12	4.48	1.19
Ho	0.88	0.29	0.96	0.23
Er	2.86	0.78	2.98	0.73
Tm	0.42	0.11	0.43	0.09
Yb	2.91	0.78	2.98	0.65
Lu	0.45	0.11	0.46	0.1
LREE	109.49		93.79	
HREE	36.41		37.97	
REE	145.9		131.76	

Std = Standard deviation.

Sediment from the Ikuno mine also has higher concentrations of lanthanum, yttrium, and neodymium, while holmium, terbium, lutetium, and thulium were found in low concentrations. This pattern was observed not only in the Ikuno area but also in the Akenobe area. In the Akenobe area, yttrium, lanthanum, and neodymium were found in quite high amounts; on the side, holmium, terbium, lutetium, and thulium showed small concentrations. This study showed that both locations tend to have a higher concentration of LREEs than HREEs, except for yttrium. It is known that LREE concentrations in sediment are generally greater than HREE concentrations. As stated by Kabata and Mukherjee (2007), the concentrations of REEs in the soil are associated with the geological origin of parent rocks and mineral composition. There are several factors that influence the rare earth element contents in soils: (i) parent materials and organic matter contents, (ii) soil texture, (iii) pedogenic processes, and (iv) anthropogenic activities [52].

The REE concentrations in sediment from different mining sites are shown (Figure 2). The REE concentration in soil differed widely even in the same sampling location. The result tends to follow the Oddo-Harkins rule. According to the rule, the concentrations of REEs with even atomic numbers were higher than those with odd atomic numbers [53]. The graph also showed a general peculiarity; with an increase in atomic weight, the contents tended to decrease.
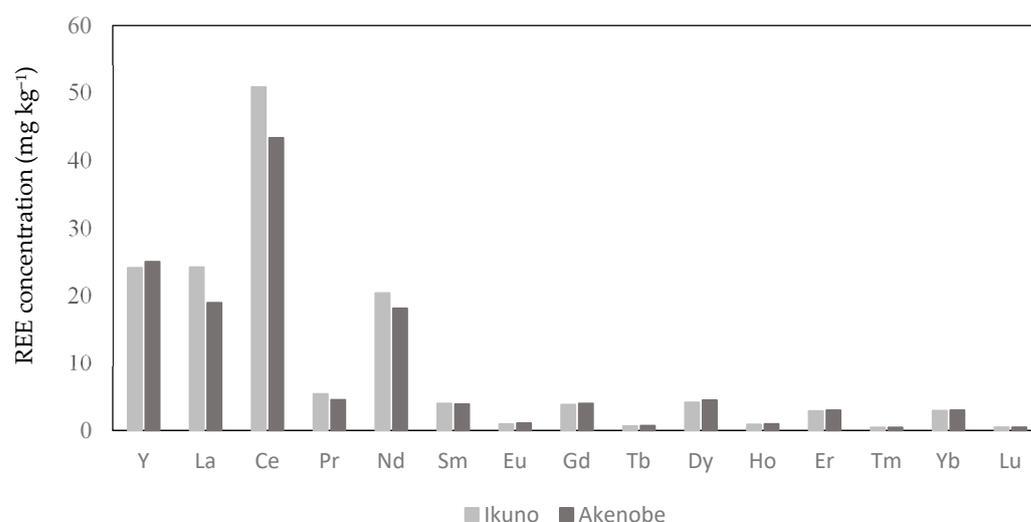
Figure 2. REE concentrations in sediment samples collected from the Ikuno mine and the Akenobe mine.

3.2. REEs in Athyrium yokoscense

The increasing use of REEs worldwide along with environmental contamination, especially around mining areas, raises the question of remediation of these sites. Plant-based remediation techniques can help reduce high REE concentrations in soils through the use of REE-accumulating plants. Relatively few plant species accumulating REEs are currently known; however, most of them are ferns, which could represent a yet un-explored reservoir of REE accumulators. Members of the Athyrium family are widely known as hyperaccumulator plants. The average REE concentrations in the *Athyrium yokoscense* leaves at the different mining sites are shown in Table 2. The study showed that *Athyrium yokoscense* was highly enriched in LREEs. The average total concentration of REEs contained in *Athyrium yokoscense* is much higher than that in other plants, especially for LREEs. The total concentration of lanthanum in the Ikuno area and the Akenobe area showed the highest concentration (11.11 mg kg^{-1}, 7.37 mg kg^{-1}) compared to other REEs. In contrast to the Tada area, the cerium concentration ranged up to 20.28 mg kg^{-1} and became the highest concentration compared to other locations. Terbium, holmium, erbium, and thulium showed very low concentrations in the three different mining areas. Referring to Tyler (2004), non-hyperaccumulator aboveground plant parts have REE concentrations < 5 mg kg^{-1} [54].

Table 2. The average REEs concentration in *Athyrium yokoscense* leaf samples collected from the Ikuno mine, Akenobe mine, and Tada mine.

Element	Ikuno		Akenobe		Tada	
	(mg kg^{-1})	Std	(mg kg^{-1})	Std	(mg kg^{-1})	Std
Y	1.72	3.16	1.59	6.12	1.84	1.61
La	11.11	13.49	7.37	5.20	19.84	16.52
Ce	3.87	4.55	4.19	13.32	20.28	29.17
Pr	1.32	1.82	1.18	1.24	2.40	1.96
Nd	4.51	6.48	4.44	5.08	7.47	5.90
Sm	1.18	2.15	0.73	1.10	5.84	5.16
Eu	0.18	0.21	0.25	0.43	0.13	0.11
Gd	0.55	0.93	0.56	1.15	0.74	0.69
Tb	0.06	0.11	0.06	0.17	0.08	0.07
Dy	0.25	0.44	0.27	1.19	0.30	0.29
Ho	0.04	0.07	0.04	0.23	0.05	0.05
Er	0.10	0.17	0.10	0.73	0.14	0.14
Tm	0.01	0.02	0.01	0.09	0.01	0.02
Yb	0.05	0.08	0.05	0.65	0.07	0.07
Lu	0.01	0.01	0.01	0.10	0.01	0.01

Std = Standard deviation.

There are several reasons that correlate with these phenomena. The first one could be affected by the LREE availability in sediment, which reflects the REE contents in the plant. The second is a general peculiarity that a smaller molecule size is easier for plants to absorb. Šmuc et al. (2012) mentioned that LREEs are found in higher amounts in the environment, with smaller atomic masses, higher solubility, and alkalinity [55]. In contrast, HREEs have higher atomic masses, smaller solubility, and alkalinity. The third reason is the preferential accumulation of LREEs. The preferential accumulation of LREEs has been previously reported in REE-accumulating ferns that grow naturally in mining areas [56,57]. The concentration of LREEs is usually 3 to 60 times more abundant than that of HREEs. LREE enrichment in plants occurs because of the preferential uptake of LREE ions. According to Gao et al. (2003), Eu and La can enter the plant cell and attach to the internal membranes of several organelles. Ion channels may be implicated in the passive diffusion of REEs across the plasmalemma [58]. The study by Tao (2005) showed that LREEs are preferentially absorbed by plants. A previous study showed that at the maturing stage of spring wheat, the contents of REEs in different parts of the wheat followed the order root > leaf > crust and stem. In addition, the ratios of light REEs to heavy REEs in the roots, stems, and crust are close to those in the soil. However, it was relatively higher in the leaves, where the composition was light REEs [28]. This suggests that plants may be able to distinguish between complex and free cations that differ in diameter and charge. In order to comprehend the underlying mechanisms, more study in this area is still needed. The preferential accumulation of LREEs by *Athyrium yokoscense* leaves can also be affected by REE-binding chlorophyll. There are several studies that show magnesium replacement by LREE. Magnesium is the main component of chlorophyll substituted by LREEs. The study showed the effect of cerium (III) on chlorophyll formation in spinach and showed that the Ce^{3+} contents of chloroplasts and chlorophyll were higher than those of any other REEs; it was also suggested that Ce^{3+} could enter the chloroplasts and bind easily to chlorophyll and might replace magnesium to form Ce-chlorophyll [59]. The results also showed that Ce^{3+} could obviously stimulate the growth of spinach. Other research reported by Wei et al. (2005) showed an accumulation of REEs found in the intact chloroplast of *Dicranopteris linearis,* and the study showed an indication of magnesium atom replacement in chlorophylls by REEs in this fern. Chlorophyll binds to LREEs, possibly due to insufficient magnesium concentrations in soil [59]. A study has been conducted by Hong et al. (2002) on spinach where there is no Mg^{2+} in the solution. La^{3+} may substitute Mg^{2+} for chlorophyll formation. The study shows that La^{3+} can obviously promote growth and increase the chlorophyll content and photosynthetic rate of spinach [60]. In addition, depending on the dose, lanthanum chloride resulted in an increase in the mass and the height of citrus plants with an increase of dry matter and changed the physiology of the plant [61].

However, as a comparison, the average concentrations of REEs in the *Athyrium yokoscense* leaves in the Ikuno, Akenobe, and Tada mines are compared with those in the Ashio mine by Miyoshi et al. [62]. As shown in Figure 3, *Athyrium yokoscense* leaves in the Ashio mine most likely have a high concentration of LREE. This shows the same pattern as the Ikuno, Akenobe, and Tada mines. This result supports the REE transport mechanisms in pteridophytes, specifically in *Athyrium yokoscense* leaves that preferentially accumulate LREEs. The characteristic of the element plays a significant role in the accumulation of REEs. In addition, the concentration of HREEs in the Ashio mine is higher than that in the Ikuno, Akenobe, and Tada mines. The reason could be the REE availability in soil that affects REE uptake by plants [62].

Furthermore, Miyoshi et al. (2015) also compared the concentration of REEs in leaves and roots [62]. Clearly, Figure 4 shows that the concentration of REEs in general accumulated more in roots than in leaves. The study suggests that there may be a limited amount of REEs that can be transported aboveground (leaves), which caused the decreasing amount of REEs from roots to leaves. Even so, another study is still required to elucidate more detail about the process of REE transport from the underground to aboveground parts. Fur-

thermore, from the figure, the LREE and HREE concentrations show opposite tendencies. In the underground part (roots), HREEs accumulate in higher concentrations than LREEs. While in the aboveground, LREE concentrations are higher than HREE concentrations. This contrasting absorption by roots and leaves, which amplifies a preferential accumulation of LREEs in leaves, still needs further study.
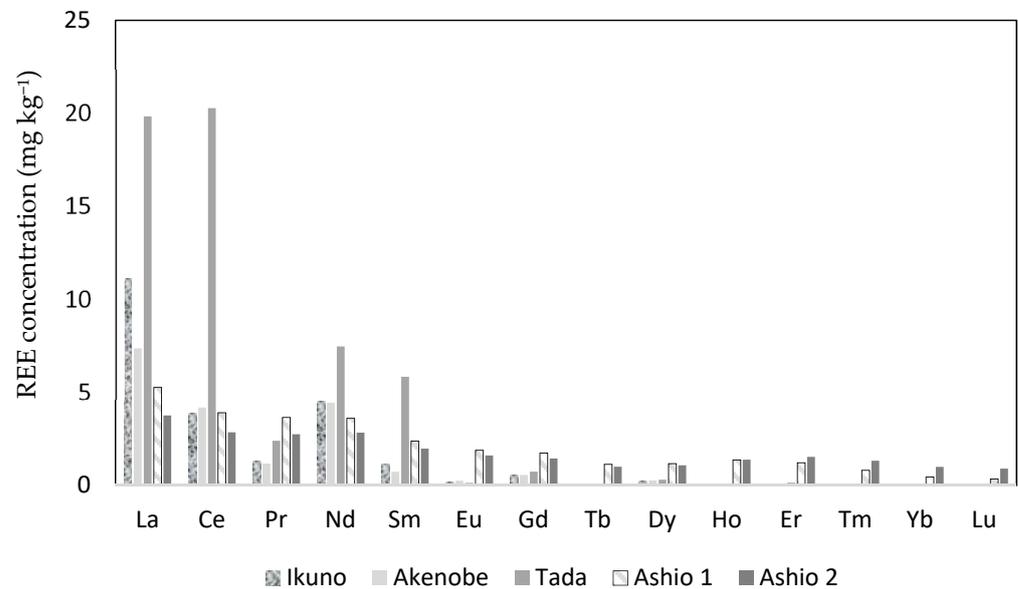

Figure 3. REE concentrations in *Athyrium yokoscense* leaf from the Ikuno mine, Akenobe mine, Tada mine, and Ashio mine were compared (Miyoshi, 2015) [62].

Figure 4. REE concentrations in *Athyrium yokoscense* leaf and root samples from the Ashio mine (Miyoshi, 2015) [62].

3.3. Bioconcentration Factor

In recent decades, the translocation, bioaccumulation, and bioconcentration of REEs in various environmental samples have been widely discussed [63,64]. REE concentrations in soils are known to be influenced by the parent material of the soil. Many countries, including China, have evaluated their mining area for soil-plant systems and found significantly high levels of REEs in plants through various transport processes compared to natural areas. In order to explore the correlations between elements and to understand the geochemical transfer of REEs, bioconcentration factors (BCFs) in the Ikuno and Akenobe mines are

measured by dividing the REEs concentration of *Athyrium yokoscense* into sediment as shown below:

$$BCF = \frac{C_{A.yokoscence}}{C_{sediment}}$$

As shown in Table 3, almost all *Athyrium yokoscense* samples in the Ikuno and Akenobe areas are enriched with LREEs with BCF values of 0.46 La, 0.3 Sm, 0.24 Pr, and 0.38 of La, 0.26 Pr, and 0.24 Nd.

Table 3. The bioconcentration factor of stream sediment and *Athyrium yokoscense* leaves from the Ikuno mine and Akenobe mine.

Element	Ikuno	Akenobe
Y	0.07	0.06
La	0.46	0.39
Ce	0.08	0.10
Pr	0.24	0.26
Nd	0.22	0.25
Sm	0.30	0.19
Eu	0.19	0.23
Gd	0.14	0.14
Tb	0.09	0.09
Dy	0.06	0.06
Ho	0.04	0.05
Er	0.03	0.03
Tm	0.02	0.02
Yb	0.02	0.02
Lu	0.01	0.01

In comparison with LREEs, the bioconcentration factor of HREEs reveals a different trend (Figure 5). *Athyrium yokoscense* accumulates LREEs and HREEs differently. A preferential accumulation of LREEs by leaves and the transport process from the underground part to the aboveground part has a role in this result.
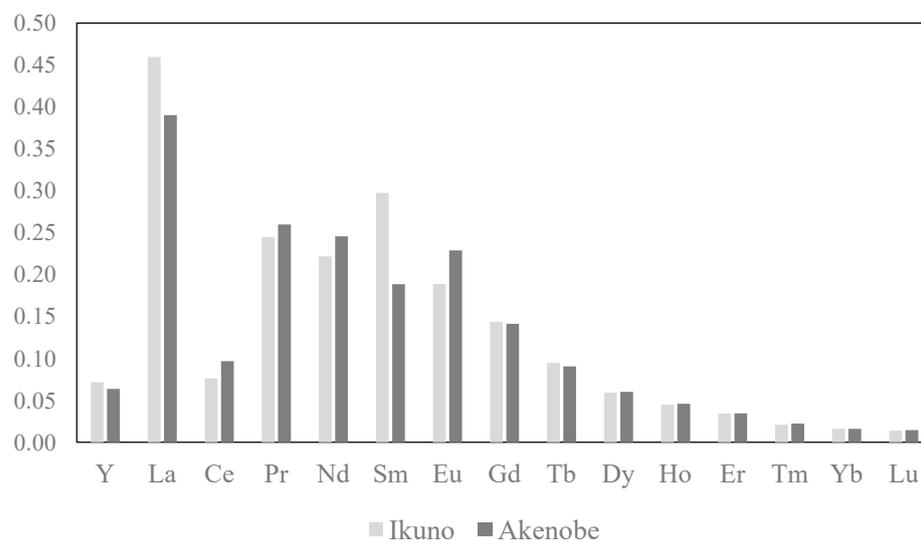

Figure 5. Bioconcentration factors of stream sediment and *Athyrium yokoscense* leaves from the Ikuno mine and Akenobe mine.

In addition, a study by Khan et al. (2017) showed that LREEs were mostly found in the roots and leaves (petiole, lamina). Leaves (petiole and lamina) hyperaccumulate Ce and La (LREE) with small amounts of Sc and Y [65]. However, another fern was reported to accumulate LREEs. *Hypogymnia physodes* in mosses and lichens were found to be good lanthanide accumulators. Bioaccumulation of La, Ce, and Y in root surface and cortical cells was found in the genera *Dryopteris*, *Adiantum*, *Dicranopteris*, and *Asplenium* [56]. This study

has potential limitations. Not all plant samples were collected in the same locations where the plant lived as it should be. They are subjected to not fully represent the absorption ability of REEs to the plant. Further analysis is needed to compare the REEs concentration in other parts of the plant to elucidate more detail about the process of REEs transport from the underground to aboveground parts and a preferential accumulation of each organ. In addition, analyzing the chemical form of REEs as potential sources is needed.

3.4. Chondrite-Normalized REEs Pattern

In this study, REE concentrations in sediment and plants are normalized with CI chondrites. Chondrite normalization is one of the common approaches to normalizing REE concentration with standard REE abundance on Earth according to the Masuda-Coryell plot [66]. The formula to calculate the normalization value using chondrite data is shown below [67]:

$$Normalization\ value = \frac{[REE]_{Sample}}{[REE]_{chondrite}}$$

In this normalization, promethium was not analyzed in this analysis because it is an element that does not naturally occur in nature or on Earth. By applying the equation above, the normalization value of REEs is given below (Figures 6 and 7).
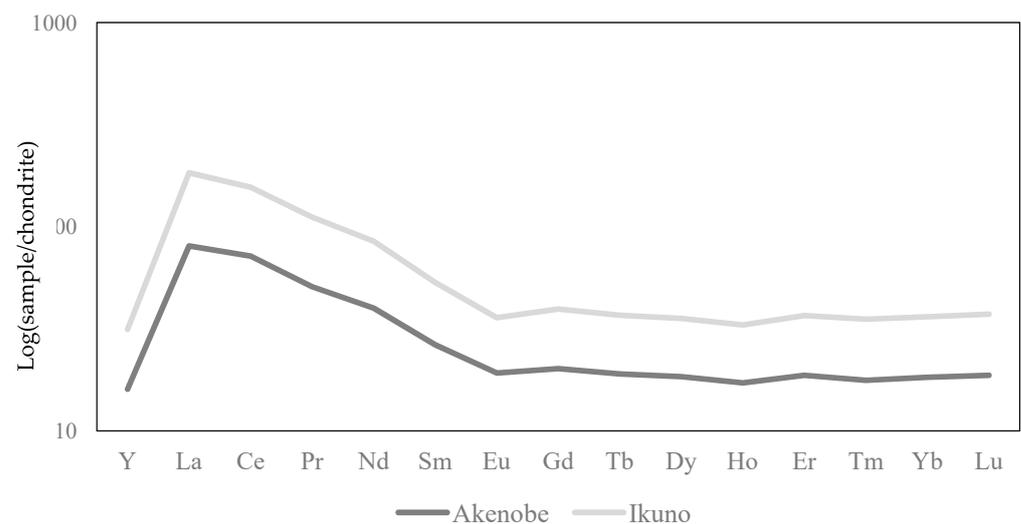

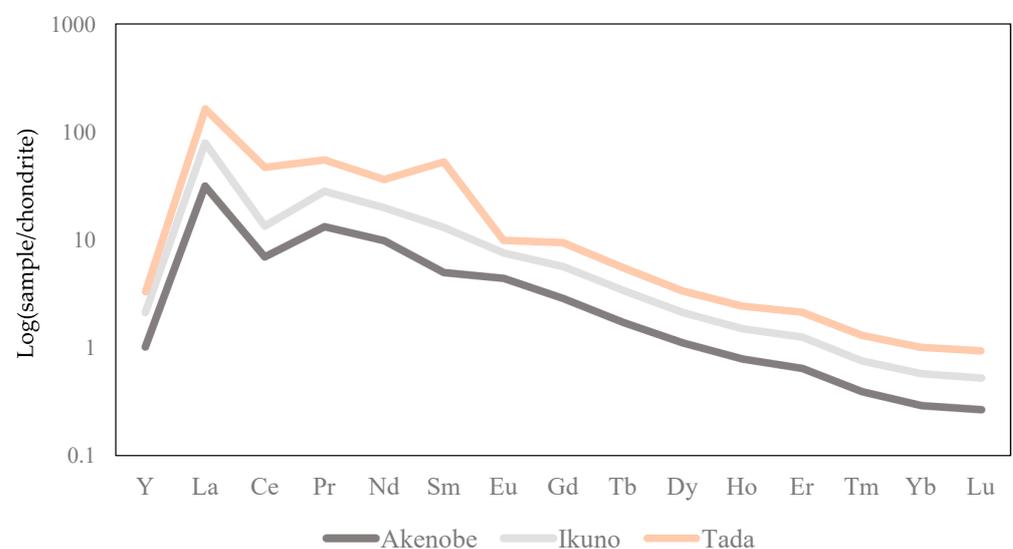**Figure 6.** Sediment-chondrite normalization of the Ikuno and Akenobe mines.

Figure 7. *Athyrium yokoscense* -chondrite normalization of the Ikuno, Tada, and Akenobe mines.

Referring to Nakada (2017), the anomaly may be either positive or negative, depending on whether plagioclase was removed during the rock's formation. The cerium anomaly, Ce^{3+} can be oxidized by O_2 to Ce^{4+}, which is attributed to the weathering and oxidation process in the mining area [68]. In sediments, there are no cerium anomalies, while plant samples show cerium anomalies. The change in oxidation state from dissolved water and absorption by plants could have caused the occurrence of the anomaly. It is also possible that the oxidation state changed inside the plant organ due to the transport process. It is also explained by Braun et al. (1990), as the majority of other lanthanides cerium occurs as Ce^{3+} or in oxidizing conditions as Ce^{4+}. If soluble Ce^{3+} is oxidized to Ce^{4+}, cerium precipitates from the solution as very insoluble CeO_2 and results in the solution showing a negative anomaly [69].

On the other hand, the europium anomaly states all lanthanides form relatively large trivalent (3+) ions. Additional valences (2+) in europium behave differently, referred to as the 'Eu anomaly', in the presence of plagioclase due to Eu^{2+} substitution for Ca^{2+}. The Eu anomaly in the plant sample occurs due to the important role of Ca in the plant. The Eu substitution Eu^{3+} can enter or be absorbed into plant cells through Ca^{2+} channels. Identical or similar mechanisms are involved in Ca and uptake by plants [54].

4. Conclusions

The concentration of REEs appeared to diverge in every location, and LREEs in sediments tended to be more abundant than HREEs. The REE concentration in *Athyrium yokoscense* shows the same pattern as sediment samples, and the study concludes that *Athyrium yokoscense* is a REE accumulator, with concentrations above 5 mg kg^{-1}. The amount of REEs that can be transferred to ferns also varies. Analyzing the bioconcentration factor clearly showed that the LREE absorption ability of *Athyrium yokoscense* is different from that of HREEs. *Athyrium yokoscense* leaves contained proportionately more LREEs than HREEs. The element's property significantly contributes to the accumulation of REEs. Altogether, these results are of great interest for several purposes since they provide new insights into the behavior of the underlying REE transport mechanisms in pteridophytes.

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