International Journal of GEOMATE, May 2023, Vol. 24, Issue 105, pp.117-125
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2023.105.sk81
Geotechnique, Construction Materials and Environment

EFFECTIVENESS OF BRYOPHYTES AND CICADA SHELLS AS A BIO-INDEX FOR HEAVY METAL CONTAMINATION OF RIVER WATER AND SOIL

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*Corresponding Author, Received:15 April 2022, Revised: 09 Feb. 2023, Accepted: 12 March 2023

ABSTRACT: Metal concentrations of sampled bryophytes varied widely and increased depending on the metal contamination conditions. The highest values in bryophytes were several 10,000 ppm for Cu and Zn, 100,000 ppm for Pb, several 1,000 for As and a few 100 ppm for W. Bryophytes are therefore considered to be an effective index of Cu, Zn, Pb, As, and W contaminations. However, the heavy metal concentration in bryophytes at a W mine was low and the heavy metal concentration in other plants showed a higher concentration than that of bryophytes. The presence of W is thought to be the cause of the decrease in the heavy metal concentrations in bryophytes. The other possibility is that only bryophytes with a low copper concentration can grow. The highest Zn concentrations in a cruciferous species reached several 10,000 ppm among sampled plants. The Cu and Zn concentrations in both cicada shells and adult cicadas were not always high at metal mines. Therefore, Cu and Zn concentrations of cicada shells were not effective as a Cu and Zn contamination index. Pb and As concentrations in cicada shells at Pb and As mines were higher than those for non-Pb and non-As contaminated areas and were higher than those in cicada adults. Therefore, cicadas were thought to release Pb and As into their shells during molting and maintain high concentrations of Pb and As in their cicada shells. Pb and As concentrations in cicada shells were thought to be an effective contamination index.

Keywords: Cicada shells, Bryophyte, Heavy metals, Bio-index, Metal contamination

1. INTRODUCTION

Metal concentrations in river water and soil were measured to investigate the influence of heavy metals on local plants, insects and animals. Generally, metal concentrations in river water is very low. Moreover, they are changeable depending on the change of flow rate. Heavy metals in soil contain both soluble and insoluble metals, but living organisms use only soluble metals. Not all heavy metals in the soil are used by living organisms. Therefore metal concentrations in river water and soil were not always useful as an index of the influence of heavy metals on life. Then, in this study, bryophytes and cicada shells were proposed as a new index for gauging metal contamination. Bryophytes are easily found around rivers and cicada shells are easy to sample without digging and both contain long term information and bryophytes and cicadas absorbed metal into living body.

Bryophytes were the first green plants to colonize the terrestrial environment [1], and as such had to evolve mechanisms to cope with the much greater amounts of heavy metals present on land than in the water [2]. The absence of a root system indicates the ability of these plants to absorb heavy metals over their entire surface [2,3]. The lack of the cuticle layer, which makes their cell walls easily accessible for metal ions [4,5], pronounced ion-exchange properties [6] and a large surface-to-weight ratio also significantly contribute to this ability [7].

Advantages of bryophyte-performed monitoring, compared to conventional measurements, are cost-effectiveness and easier sampling that results in a much higher sampling density and a larger number of sites that can be included in the survey [3,8]. Due to the great capacity of bryophytes to absorb and retain heavy metals in high concentrations, it is also easier to perform chemical analysis and there are fewer contamination problems [9].

The heavy metal concentration in river insect larvae was found to decrease with weight. In identical river conditions the heavy metal concentrations in different insects, weighing the same as the larvae, were the same. Therefore, bio-concentrations in small herbivorous insect larvae to large carnivorous insect larvae was not found [10]. Heavy metals in river insect larvae were thought to be released with the shells of river insect larvae. Although it is difficult to sample shells of river insect larvae because the molting of aquatic insects is carried out in water, it is easy to sample cicada shells found on trees. Metal concentrations in cicada shells show a positive correlation with soil metal concentrations [11]. Comparing metal concentrations in cicada shells and cicada bodies, Al, Fe, and Pb were enriched in cicada shells and Cu and Zn were enriched in cicada bodies [12]. Thus, cicada shells are thought to be useful as a bio-index for metal contamination. The purpose of this study is therefore to clarify the effectiveness and character of bryophytes and cicada shells as a bio-index for heavy metal contamination of rivers and soils in comparison with plants at the same sampling points and cicada adults.

2. RESEARCH SIGNIFICANCE

Advantages of bryophyte and cicada shell performed monitoring, compared to conventional measurements, are cost-effectiveness and easier sampling that results in a much higher sampling density and a larger number of sites that can be included in the survey. Due to the great capacity of bryophytes to absorb and retain heavy metals in high concentrations, it is also easier to perform chemical analysis. Metal concentrations in cicada shells show a positive correlation with soil metal concentrations. Cicada shells are thought to be useful as a bio-index for metal contamination.

3. METHOD

Bryophytes and some kinds of plants (ferns, *cruciferae,* pines, *Boenninghausenia albiflora*, alders, rushs, laurels, and fruit plants) and cicada shells and adults were sampled at metal contaminated and metal non-contaminated areas. Metal contaminated areas were metal mine dumps and the ruins of old factories. Metal mines were Ikuno (Cu, Pb, As, Zn and W), Waidani (Cu, Pb, Zn and As), Tada (Cu, Pb and Zn), Rendaiji (Cu, Pb and Zn), Kiwada (Cu, As and W), Kaneuchi (Cu, As and W), Nishinomaki (As), Dundas (Cu, Pb, Zn and As), Naganobori (Cu and As), Iwami (Pb, Zn), Ningyotouge (U), Nyu (Hg) [13,14]. Nishikino is the ruin of a wire factory. Bryophytes in non-contaminated areas were sampled at the river sides of the Yoshi and Tenjin rivers (Okayama and Tottori prefecture) and a small river on the Izumi and Kongo mountains.

The cicada shells and cicada adults sampled were black cicadas, large brown cicadas, evening cicadas, *Oncotympana maculaticollis* and *Platypleura kaempferi* and *Terpnosia nigricosta* (sampled at only Kusatsu). They were sampled on trees located at metal contaminated areas (trees were on the metal mine dump) and non-contaminated areas. Non-contaminated areas were city parks in Osaka, Wakayama and Tokyo, Serpentinite Hill (Ryumon, Kamogawa (Mineoka), Nakase and Ogose, basalt lava (Fujinomiya) and a hot spring area (Kusatsu).

In the laboratory, the sampled bryophytes, plants and cicada adults and shells were first rinsed with ultra pure water and then desiccated by a dryer. After drying, they were dissolved with concentrated nitric acid and filtered with a membrane filter with a 0.45 micrometer pore size. After filtering, the W, Cu, Pb, Zn and As concentrations in plants and cicada were measured by ICP-OES (AMETEK, Inc., SPECTRO ARCOS) in the laboratory at Wakayama University. The actual detection limit of ICP-OES is 0.001ppm for W, Cu, Pb, Zn and As. Plants, excluding bryophytes, were divided into leaves, stems and roots and each part was separately analyzed.

4. RESULTS

4.1 Bryophyte

Firstly, Cu, Pb, Zn, As and W concentrations of bryophytes were measured by studying the differences of metal concentrations in bryophytes between metal contaminated and non-contaminated areas. Bryophytes in Ikuno, Waidani, Tada, Rendaiji, Kiwada, Kaneuchi, Nishinomaki and Dundasu mines were sampled as metal contaminated areas. Yoshi River (Okayama prefecture) - Tenjin River (Tottori prefecture) and Izumi Mountain (Wakayama and Osaka prefecture) - Kongo Mountain (Osaka and Nara prefecture) areas were non-contaminated areas.

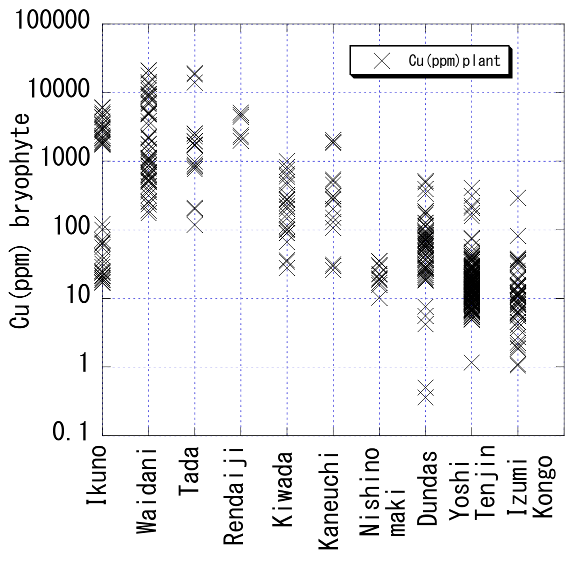

Fig. 1 Cu concentrations of bryophyte for metal contaminated and non-contaminated areas.

Figure 1 shows Cu concentrations in bryophytes for metal contaminated and non-contaminated areas and their variations were several 0.1 ppm to several 10,000 ppm. Except for Nishinomaki mine, mainly Cu ore was produced at the mines and their Cu concentrations were over 100 times higher than those for non-contaminated areas, mainly several to several tens ppm. Cu concentration at the Nishinomaki mine was the same as those at the non-contaminated area. Even at the same mine, Cu concentrations varied with sampling points. Under the same non-contaminated conditions, those sampled at Yoshii - Tenjin River area were higher than those sampled at Izumi - Kongo area. The Cu concentration in bryophytes varied widely by location and particular sampling points. The highest Cu concentration of bryophytes reached over 10,000 ppm at Waidani and Tada mines and these values were also recorded by past studies [10,11,15,16,17,18,19]. Therefore, the Cu concentrations in bryophytes was very sensitive to metal contaminated conditions. However, Cu concentrations for Ikuno, Kiwada and Kaneuchi mines accompanied with W mineral were not much higher than those for non- contaminated areas.

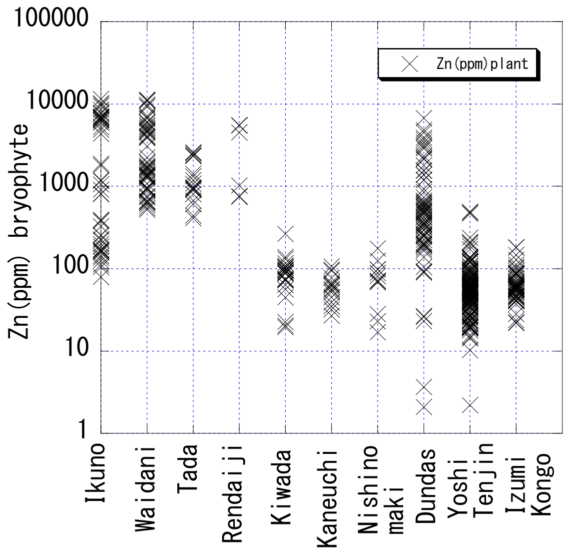

Fig. 2 Zn concentrations of bryophyte for metal contaminated and non-contaminated area.

Figure 2 shows Zn concentrations in bryophytes for metal contaminated and non-contaminated areas and the variation was several ppm to a few 10,000 ppm. Except Nishinomaki, Kiwada and Kaneuchi mines, Zn concentrations in bryophytes at Zn mines were 10 to 100 times higher than those for non-contaminated areas whose concentrations were mainly several 10 to several 100 ppm. Zn. Bryophytes sampled at metal non-contaminated areas were the same as those at the Nishinomaki, Kiwada and Kaneuchi mines. Even at the same mine, Zn concentrations varied with sampling points. However under the same non-contaminated conditions, Zn concentration variations between the Yoshii - Tenjin River area and the Izumi - Kongo area were small. Zn concentrations in bryophytes varied widely by location at particular sampling points. The highest Zn concentrations in bryophytes reached10,000 ppm at the Waidani and Ikuno mines and these values were the largest values compared with past studies [11,15,16,17,18,19]. Therefore, the Zn concentration in bryophytes was very sensitive to the level of metal contamination.

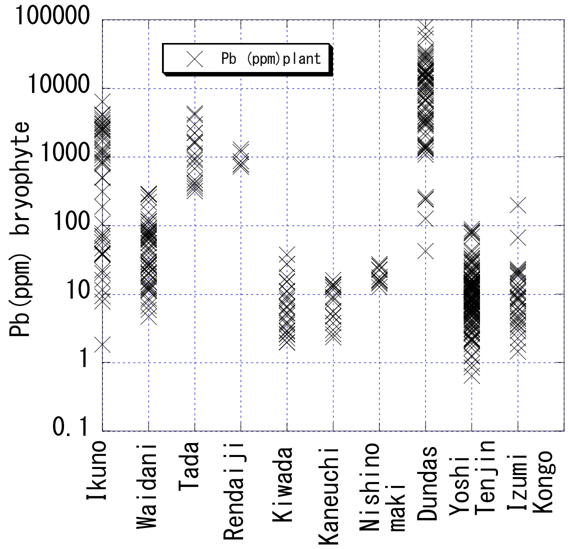

Fig. 3 Pb concentrations of bryophyte for metal contaminated and non-contaminated areas.

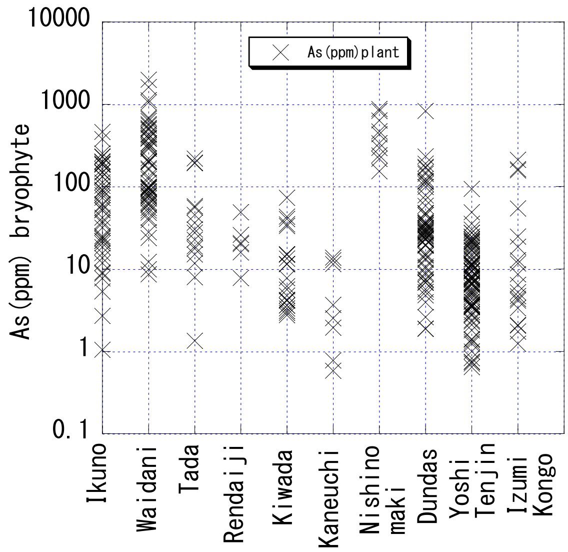

Fig. 4 As concentrations of bryophyte for metal contaminated and non-contaminated areas.

Figure 3 shows Pb concentrations in bryophytes for metal contaminated and non-contaminated areas and their variation was from several 0.1 ppm to about 100,000 ppm. The exceptions being Nishinomaki, Kiwada and Kaneuchi mines where Pb concentrations in bryophytes at Pb mines were from 10 to 1000 times higher than those for non-contaminated areas whose concentrations were mainly several to several 10 ppm. Pb in bryophytes sampled at metal non-contaminated areas were the same as those at the Nishinomaki, Kiwada and Kaneuchi mines. Even at the same mine, Pb concentrations varied by sampling points. Pb concentrations in bryophytes varied widely by location and particular sampling points. The highest Pb concentrations in bryophytes reached several 10,000 ppm at the Dundas mine and these values were the largest values compared with past studies [10,15,16,17,18,19]. Therefore, the Pb concentrations in bryophytes were very sensitive to the metal contaminated conditions.

Figure 4 shows As concentrations in bryophytes for metal contaminated and non-contaminated areas and their variation was from several 0.1 ppm to several 1,000 ppm. Bryophyte As concentrations at Nishinomaki, Ikuno, Dundas and Waidani mines where As ore mineral was extracted were over 100 ppm, 10 to 100 times higher than those for non-contaminated areas. As minerals, Realgar AsS and orpiment As2S3 were found at the Nishinomaki mine. The maximum As concentration in bryophytes reached several 1,000 ppm at the Waidani mine and these values were the largest values compared with past studies [10,11,15,16,17,18,19]. Therefore, As concentrations in the bryophytes were sensitive to the metal contamination condition.

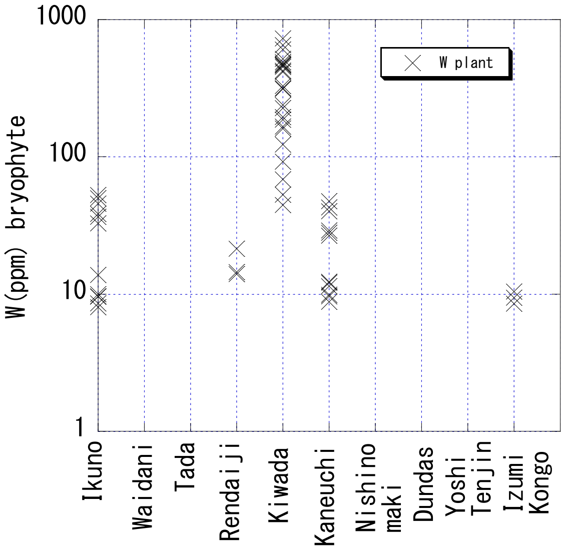

Fig. 5 W concentrations of bryophyte for metal contaminated and non-contaminated areas

Figure 5 shows the W concentrations in bryophytes for metal contaminated and non-contaminated areas and their variations which were from several ppm to a few 100 ppm. W concentrations in most of the samples were very low and undetectable. However, W concentrations at the Kiwada mine was 100 to 1000 ppm, 10 to 100 times higher than the non-contaminated areas. The sampling points at the Kiwada mine were the stored ore composed of mixed sands and stones with a W content of of about 10%. The plants in this area had grown naturally for over 10 years. At the Ikuno and Kaneuchi mines, W minerals were found but the W concentrations were not extremely high, 10 to 100 ppm. The maximum W concentration in the bryophytes reached several 100 ppm at Kiwada mine. Therefore, the W concentrations in the bryophytes was sensitive to the metal contaminated condition.

4.2 Plants

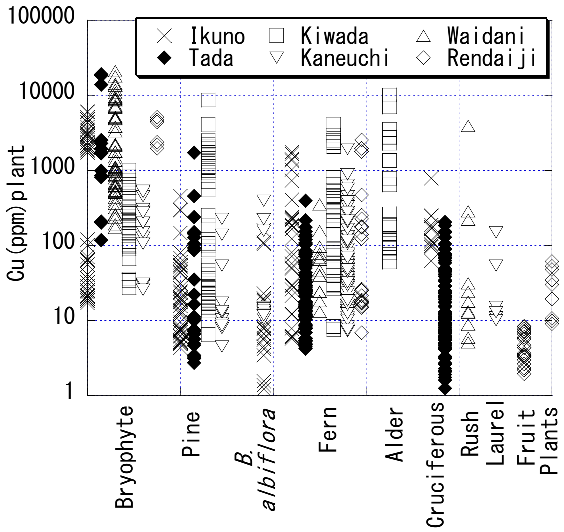

Fig. 6 Cu concentrations of plants for metal mines.

Next, Cu, Pb, Zn, As and W concentrations of the bryophytes and plants were measured to study the difference in metal concentrations between bryophytes and plants for Ikuno, Waidani, Tada, Rendaiji, Kiwada, and Kaneuchi mines. Figure 6 shows Cu concentrations in bryophytes and plants for metal contaminated areas. Cu concentrations in bryophytes were not always the highest value of all plants for each metal contaminated area. For the Kiwada mine, Cu concentrations in alders had the highest values even though their Cu concentrations varied. It’s root concentrations were high. Cu concentrations in bryophytes at the Tada, Waidani and Rendaiji mines were the highest values compared to other plants. Cu concentrations in bryophytes at the Kaneuchi and Ikuno mines were almost the same as those of ferns. Kiwada mine was a W mine and plants and bryophytes were sampled on the W ore sediments. Ikuno and Kaneuchi mines produced W ore accompanied with Cu ore. Tada, Waidani and Rendaiji mines did not produce W minerals. When accompanied with W minerals, Cu concentrations in bryophytes at mines were lowered. Therefore, the W mineral was thought to be important for Cu concentrations in bryophytes.

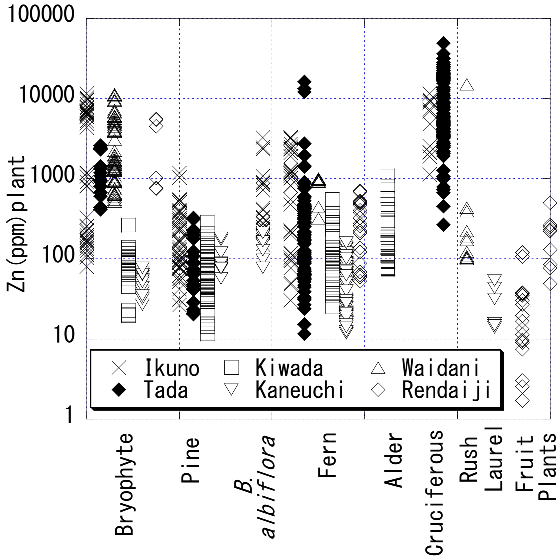

Fig. 7 Zn concentrations of plants for metal mines.

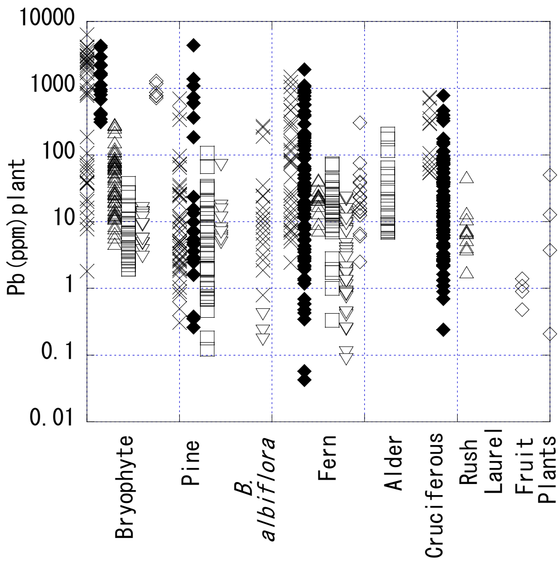

Fig. 8 Pb concentrations of plants for metal mines

Figure 7 shows Zn concentrations in bryophytes and plants for metal contaminated areas. The highest Zn concentrations were in cruciferous plants at the Tada and Ikuno mines. They were several 10,000 ppm, and the highest of the sampled plants as well as the ferns at the Tada mine. The highest bryophyte Zn concentrations at the Ikuno and Waidani mines reached 10,000 ppm. Therefore, the Zn concentrations in bryophytes were not always the highest values among the plants for each metal contaminated area. However, the Zn concentrations in pines and other plants were lower than those of the bryophytes. For the Kiwada mine, the highest Zn values in the bryophytes, pines, ferns and alders increased from several 100 ppm to 1,000 ppm in that order. Zn concentrations in the bryophytes for the Kiwada mine was almost the same as non-Zn contaminated areas. When accompanied with W minerals, Zn concentrations in bryophytes at mines were lowered. Therefore, the W mineral was thought to be important for Zn concentrations in bryophytes.

Figure 8 shows Pb concentrations in bryophytes and plants at metal contaminated areas. Pb concentrations in bryophytes were always the highest value among plants in each of the metal contaminated areas except for the Kiwada and Kaneuchi mines. The highest Pb concentrations in bryophytes reached several 1,000 ppm. Pb concentrations in pine roots, ferns and cruciferous plants were over 1,000 ppm, although Pb concentrations in pines, ferns and cruciferous plants were variable. The highest Pb concentrations were, several 100 ppm, in the alders, ferns and pines at the Kiawada mine which were higher than the several 10 ppm, in the bryophytes at the Kiwada mine. The highest Pb concentrations in pines and ferns at Kaneuchi were several 10 ppm, almost the same as those in the bryophytes at Kaneuchi. Without the W mineral, the highest Pb concentrations in bryophytes reached several 1,000 ppm. When accompanied with W minerals, Pb concentrations in bryophytes at mines were low. Therefore, the W minerals are thought to be important for Pb concentrations in bryophytes. Without W, the bryophytes' Pb concentrations reached the higher values.

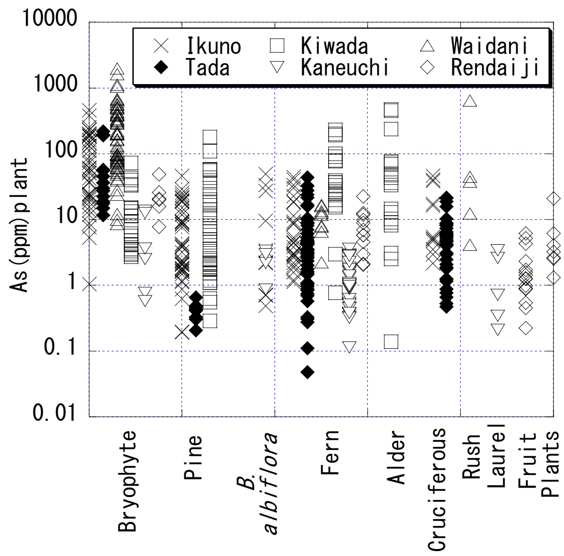

Fig. 9 As concentrations of f plants for metal mines.

Figure 9 shows As concentrations in bryophytes and plants for metal contaminated areas. As concentrations in bryophytes were always the highest values among plants for each of the metal contaminated areas except for the Kiwada W mine. The highest As concentrations in bryophytes reached a few 1,000 ppm. The highest As concentrations in pine roots, ferns and alders were over 100 ppm, although the As concentrations were variable. The highest As concentrations, were several 100 ppm, in alders, ferns and pines at the Kiawada mine which were higher than the several 10 ppm, in the bryophytes at the Kiwada mine. Without a lot of W minerals, the highest As concentrations in the bryophytes reached a few 1,000 ppm. When accompanied with a lot of W minerals, As concentrations in bryophytes at the mines was low. Therefore, W minerals were thought to be important for As concentrations in bryophytes.

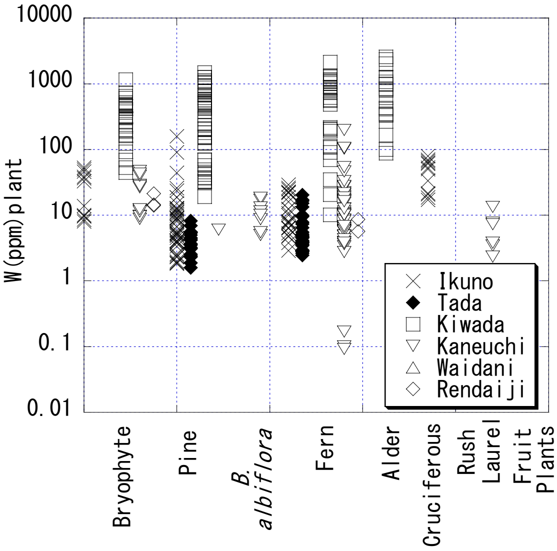

Fig. 10 W concentrations of f plants for metal mines.

Figure 10 shows W concentrations in bryophytes and plants for metal contaminated areas. W concentrations in bryophytes were not always the highest values among plants for each of the metal contaminated areas. For the Kiwada mine, the highest values in bryophytes, pines, ferns and alders increased from 1,000 ppm to several 1,000 ppm in that order. The maximum W concentrations in bryophytes, pines, ferns and alders in the Kiwada mine were more than 10 to 100 times higher than those in the Ikuno and Kaneuchi mines and the W concentrations in plants depended on the W concentrations in the soil.

4.3 Cicada Shells and Adults

Cu, Pb, Zn, As and W concentrations in cicada shells and adults were measured by studying the difference in metal concentrations in cicada shells and cicada adults comparing metal contaminated and non-contaminated areas.

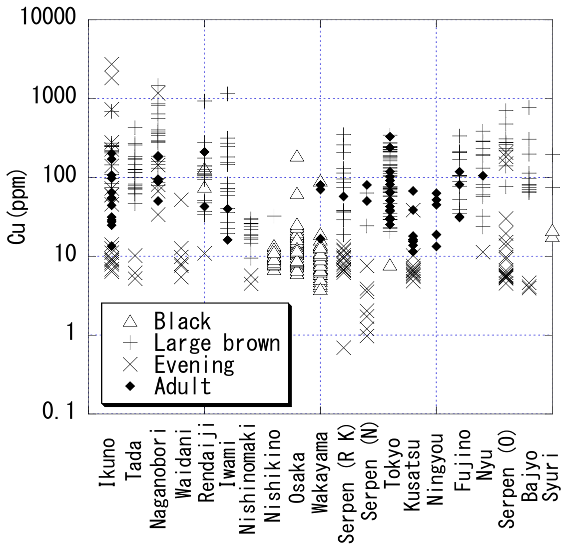

Fig. 11 Cu concentrations of cicada shell and cicada adult.

Figure 11 shows Cu concentrations of cicada shells and cicada adults which were very variable. From several 0.1 ppm to several 1,000 ppm for cicada shells and from several 10 ppm to over 300 ppm for cicada adults. The mark of "Large brown" showed large brown cicadas and *Oncotympana maculaticollis* as well as the following figures. They were the same sizes. The mark of "Evening" showed evening cicadas, *Platypleura kaempferi* and *Terpnosia nigricosta* (sampled at only Kusatsu) as well as the following figures. They were the same sizes. Black cicadas (the mark of Black) were sampled in city parks and ruins in various cities in West Japan. Their shell Cu concentrations were mainly several to several 10 ppm and lower than those of the other cicada shells. Cu shell concentrations of large brown cicadas and *Oncotympana maculaticollis* were from 10 to several 1,000 ppm and higher than those of other cicada shells. Cu shell concentrations of large brown cicadas and *Oncotympana maculaticollis* for Cu non-contaminated areas, serpentinite, Tokyo parks, and Fujinomiya (Basalt flow) were almost the same as the Cu mining areas (Ikuno, Tada, Naganobori, Rendaiji, and Iwami mines). Cu shell concentrations of the evening cicada and *Platypleura kaempferi* were very variable, from several to several 1,000 ppm. Their Cu concentrations at Cu mines (Ikuno, Tada, Naganobori, Rendaiji, and Iwami mines) were not higher than those at Cu non-producing. Therefore, the Cu concentrations in cicada shells at Cu metal mines were not always high.

The Cu concentration variations in cicada adults was very narrow relative to those of cicada shells when comparing Cu concentrations in cicada adults between Ikuno mine and Tokyo parks. Both variations were almost the same. Therefore, the Cu concentrations in cicada shells for Cu metal mines were not always high when comparing Cu concentrations between cicada shells and cicada adults at the same points and these results were consistent with past results [12]. Therefore, cicadas did not selectively release Cu into their shells during molting.

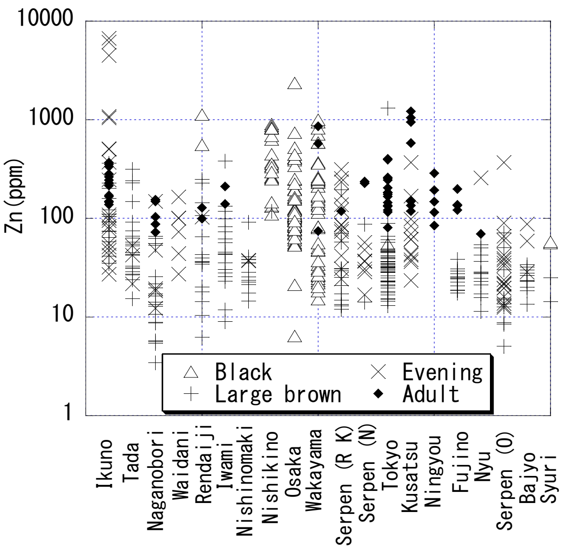

Fig. 12 Zn concentrations of cicada shell and cicada adult.

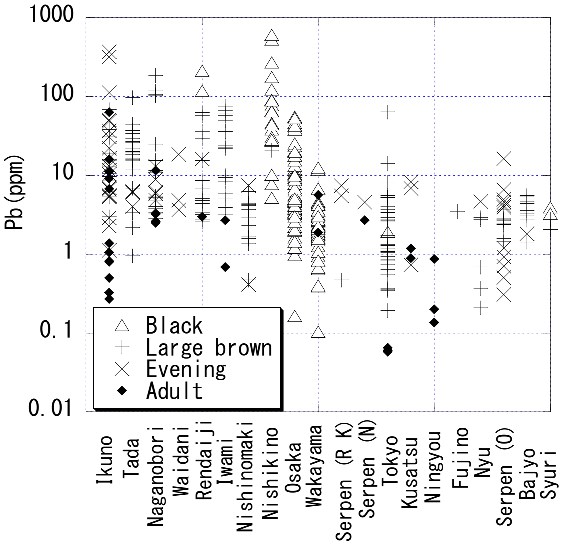

Fig. 13 Pb concentrations of cicada shell and cicada adult

Figure 12 shows Zn concentrations of cicada shells and cicada adults. The concentrations were from several ppm to 10,000 ppm for cicada shells and from 50 ppm to 1,200 ppm for cicada adults.

Zn concentrations in black cicada shells were from 10 to several 1,000 ppm, not lower than those of the other cicada shells and had a very wide variation unlike the Cu results. Zn shell concentrations in large brown cicadas and *Oncotympana maculaticollis* were from several 100 ppm and lower than those of the other cicada shells even for Zn mines such as Ikuno, Tada, Rendaiji and Iwami. Zn concentrations in the shells of large brown cicadas and *Oncotympana maculaticollis* at Zn metal mines were not always high relative to those for non-Zn contaminated areas, serpentinite, Tokyo parks, and Fujinomiya (Basalt flow).

Figure 13 shows Pb concentrations in cicada shells and cicada adults. The concentrations were from 0.1 ppm to several 100 ppm for cicada shells and from several 0.01 ppm to several 10 ppm for cicada adults.

Pb concentrations in black cicada shells had a wide variation, from 0.1 to several 100 ppm. However, these values were divided into three types. At Nishikinohama (ruins of wire factory), they were several ppm to several 100 ppm. At Osaka parks, they were from 1 to several 10 ppm. At Wakayama parks, they were from several 0.1 to 10 ppm. The differences were thought to depend on contamination because Osaka parks in the city were more polluted by air than Wakayama and the wire factory previously used Pb.

Pb concentrations in the shells of large brown cicadas and *Oncotympana maculaticollis* were from several ppm to several 100 ppm for Pb mines such as the Ikuno, Tada, Naganobori, Rendaiji and Iwami mines and from several 0.1 ppm to less than 10 ppm for non-Pb contaminated areas such as serpentinite. Similarly, Pb concentrations in the shells of evening cicadas and *Platypleura kaempferi* were from several ppm to several 100 ppm for Pb mines such as the Ikuno, Tada, Naganobor, and Waidani mines and from several 0.1 ppm to less than 10 ppm for non-Pb contaminated areas such as serpentinite. Therefore, Pb concentrations in cicada shells at Pb mines were higher than those for non-Pb contaminated areas.

The high Pb concentrations in cicada adults sampled at Pb mines were higher than those at non-Pb contaminated areas although they had wide variations. When comparing the Pb concentrations in cicada shells and adults, the Pb concentrations in cicada shells were higher than those in cicada adults even though both concentrations had wide variations. As a result, these results were consistent with past results [12]. Therefore, cicadas were thought to release Pb into their shells during molting and keep high Pb concentrations in their shells in Pb contaminated areas. Pb concentrations in cicada shells was thought to be contamination index.

Figure 14 shows As concentrations in cicada shells and cicada adults and their concentrations were from 0.1 ppm to several 1,000 ppm for cicada shells and several 0.1 ppm to several ppm for cicada adults.

Generally, As concentrations in black cicada shells had a wide variation, from 0.1 to10 ppm. As The concentrations in the shells of large brown cicadas, *Oncotympana maculaticollis.* evening cicadas and *Platypleura kaempferi* at the Ikuno, Naganobori, Nishinomaki and Bajyo mines and serpentinite (Nakase mine), of As minerals were higher than those of non-As contaminated areas (mines without As mineral and serpentinite (R, K, O). Therefore, As concentrations in cicada shells at As mines were higher than those for non-As contaminated areas.

The As concentrations in cicada adults were from several 0.1 to several 1 ppm at the Ikuno mine The As produced was almost the same as other areas. Therefore, As concentrations in cicada adults for As contaminated areas was not always high. When comparing the As concentrations in cicada shells and adults, the As concentrations in cicada shells were over 10 times, higher than those of cicada adults and in particular, for the Ikuno mine and Tokyo parks

Therefore, cicadas were thought to release As into their shells during molting and keep a high As concentration in their shells. As concentration in cicada shells was thought to be contamination index.

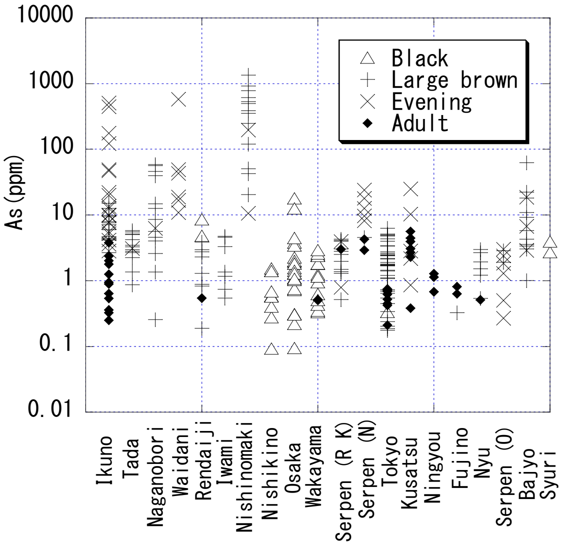

Figure 14 As concentrations of cicada shell and cicada adult

5. CONCLUSION

In this study, effective indicators of metal contamination need a wide variation of metal concentrations depending on the contamination. Therefore, metal concentrations in bryophytes, plants, cicada shells and cicada adults sampled at metal-contaminated and non-contaminated areas were measured.

The metal concentrations in bryophytes varied from several 0.1 ppm to several 10,000 ppm for Cu, from several ppm to a few 10,000 ppm for Zn, from several 0.1 ppm to about 100,000 ppm for Pb, from several 0.1 ppm to several 1,000 ppm for As and from several ppm to a few 100 ppm for W and increased depending on the metal contamination conditions. Therefore, it is considered to be effective as an index of Cu, Zn, Pb, As, and W contaminations. However, the Zn concentrations in bryophytes was not always the highest among the plants in each of the metal-contaminated areas. The highest Zn concentrations in a kind of cruciferous plants were several 10,000 ppm, which was the highest among the sampled plants. In addition, where there is a large amount of W, such as the Kiwada W mine, the heavy metal concentrations in bryophytes were not high, and the heavy metal concentrations in other plants showed a higher concentration than that of the bryophytes. At the W mine, the alders had the highest Cu, Zn, Pb, and As concentrations. The presence of W is thought to be the cause of the decrease in the heavy metal concentration in bryophytes, which prevents the adsorption of metals into the cell walls of the bryophytes. The other possibility is that only bryophytes with a low metal concentration were growing.

The metal concentrations in cicada shells from black cicadas, large brown cicadas, *Oncotympana maculaticollis*, evening cicadas and *Platypleura kaempferi* varied from several 0.1 ppm to several 1,000 ppm for Cu, and several ppm to 10,000 ppm for Zn. The metal concentration variations in cicada adults was from several 10 ppm to 300 ppm for Cu, and 50 ppm to 1,200 ppm for Zn. The Cu and Zn concentrations in both cicada shells and adults were not always high for the metal mines and cicada shell concentrations were not always higher than those of cicada adults. Therefore, the cicadas did not selectively release Cu and Zn into their shells during molting and as a result the Cu and Zn concentrations in cicada shells sampled from Cu metal mines (Cu and Zn contaminated areas) were not always high. As a result, it is unsuitable for a Cu and Zn contamination index.

The metal concentrations in cicada shells and cicada adults were from 0.1 ppm to several 100 ppm and from several 0.01 ppm to several 10 ppm for Pb and from 0.1 ppm to several 1,000 ppm and from several 0.1 ppm to several ppm for As. Pb and As concentrations in cicada shells at Pb and As mines were higher than those for non-Pb As contaminated areas. Pb and As concentrations in cicada shells were higher than those of cicada adults. Therefore, cicadas were thought to release Pb and As into their shells during molting and keep Pb and As high concentration in cicada shells in Pb and As contaminated areas. Pb and As concentrations in cicada shells were thought to be a good contamination index.

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