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EFFECTIVENESS OF USING RIVER INSECT LARVAE AS AN INDEX OF CU, ZN AND AS CONTAMINATIONS IN RIVERS, JAPAN

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ABSTRACT: Analysis of Dobsonfly (a kind of Megaloptera, *Protohermes grandis*) larvae for concentrations of Cu and Zn was found to be an effective method of determining levels of Cu and Zn contamination of rivers in metal mine areas and non-metal mine catchments. Metal concentration in Dobsonfly larvae was used as an index of metal contamination because the amount of metal concentration in Dobsonfly larvae decreased with the dry weight of the larvae and also on the degree of metal present in the river water. Dobsonfly makes an excellent tool for contamination evaluation because of their easy classification, wide distribution and commonness. Furthermore, due to their relatively lengthy 2-3 year lifespan, river contamination assessment over a long term could be performed. In this study, Cu, Zn and As concentrations in river insect larvae in metal mine areas were found to be higher than those in non-mine catchments.

Keywords: Mine waste, Dobsonfly, ecotoxicology, heavy metal, insect larvae

1. INTRODUCTION

Many papers concerning metal concentration in river insect larvae and the high concentration factor of heavy metal found in many kinds of insects have been published [1], [2], [3]. In particular, the influence of metal from waste water or mine tailings on river insects and the relation between metal concentrations in soil and water and metal concentration on insects have been studied [4], [5]. As a result, metal concentrations in river insect larvae were found to be very high relative to non-contaminated areas. The actual mechanism of metal accumulation was studied using nitrogen and carbon stable isotope [6]. Although monitoring water and soil is important for evaluating contamination, it requires taking numerous samples over a long term in order to determine average values of metal concentrations as water and soil metal concentrations vary with time and place. Some insect larvae live in rivers for several years and derive their food from points upstream to their living point therefore they can provide considerable information regarding metal contamination. Measuring metal concentrations in insect larvae is more useful than measuring contamination in water and soil in terms of time, area and concentration.

Dobsonfly (Megaloptera) is distributed widely in Japan, southern and eastern Asia, South and North America, South Africa, Madagascar, Australia, and New Zealand [7]. Dobsonfly is carnivorous with large jaws and lives in rivers for 2 to 3 years. Metal concentrations in Dobsonfly and Caddisfly (Trichoptera) are thus thought to provide information for wide areas over a long term. In Japan, metal concentrations in Caddisfly were measured and this metal concentration was used as an indicator of environmental pollution [8], [9]. However, Caddisfly has many kinds of species and classifying each species is difficult. Dobsonfly however, has just two main species, *Protohermes grandis* and *Parachauliodes continentalis* in Japan. *Protohermes grandis* is popular. Dobsonfly is thus thought to be more useful than Caddisfly for classification. Although metal concentrations of dobsonfly were measured [10], these values were not compared with other insects at the same river. Along the Kino River, Cu concentration of river insect and river plant were measured however sampled insect number and species was few [11]. The purpose of this study was therefore to evaluate the possibility of using Dobsonfly as an indicator of the degree of metal environmental pollution comparing other river insects.

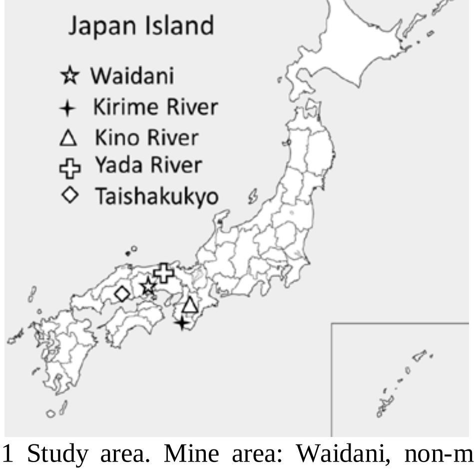

Fig.1 Study area. Mine area: Waidani, non-mine area: Taisyakukyo, Yada, Kino and Kirime Rivers

2. MATERIALS AND METHODS

River insect larvae were sampled at both mine and non-mine catchments and metal concentrations were measured. The Waidani area was selected for mine catchments in Okayama Prefecture as shown in Fig.1. The Waidani is a typical closed small scale copper mine area operated until 70 years ago and its tailings containing ores and slag were disposed of along the top of a local valley with no protection. Pyrite FeS_2, spharelite ZnS, chalcopyrite $CuFeS_2$ and arsenopyrite FeAsS were found in the tailings and Zn, Cu and As contamination was suspected. River water originated from the tailings seepage. River insect larvae were sampled along the downstream of the valley in May, June, November, December 2013 and January 2015. River water after filtration with 0.45 micrometer was sampled and flow rate was measured from December 2011 to January 2015. River insect was sampled using net and reserved in glass bottle with alcohol. 18 Dobsonfly samples in the Waidani were *Protohermes grandis*.

River water and insect larvae in non-mine catchments were sampled along the Taishakukyo in a limestone valley in Hiroshima Prefecture, the Yada River with volcanic sediments in Hyogo Prefecture, the upstream of the Kino River with sedimentary rocks, basic and felsic metamorphic rock and limestone in Nara Prefecture and the Kirime River with sedimentary rocks in Wakayama prefecture as shown in Fig.1. The number of Dobsonfly sample in the Yada, Taishakukyo, Kino and Kirime were 15, 0, 2 and 3. Just one *Parachauliodes continentalis* was found in the Kino and Kirime, respectively. Sampling dates for river water and river insect larvae was in August 2013 at the Taishaku Valley, in July and December 2012 at the Yada River composed of three branch rivers, in December 2013 and December 2014 at the upstream of Kino River with three sampling points and in October 2014 at the Kirime River. Sampled insect larvae were dried then dissolved with concentrated nitric acid solution. The solution after filtration with 0.45 micrometer and river water sample were analyzed for metal concentration by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy, SPS1700HVR ; Seiko Instruments Inc.).

3. RESULTS

Table 1 shows pH, EC, Cu, Zn, and As concentrations of river water along the valley for the Waidani area. At the top of the valley, there are large tailing places from which the south and north rivers originated. The upstream, middle and downstream of the south river are SR U, SR M and SR D and the length was 3 km. The sampling points from the upstream to downstream of the north river are NR UP, NR UM, NR MD and NR D and the length was 3 km. The sampling points, SR U, NR UP and NR UM were beside the tailing place. Although most mine waste water contains high concentrations of H^+ and SO_4^{2-}, EC values were low and pH values were high indicating that the Waidani area was not heavily contaminated by waste water. Then Zn, Cu and As concentrations in upstream river water were under the Japanese Effluent Standard but high relative to the downstream subsequently mine tailings were thought to be metal source along the rivers. Cu, Zn and As loads reached about 20, 150 and 10 kg per year calculated from flow rates and metal concentrations. Metal load was not always small for these small catchments of only 3 km in length.

Table 1 pH, EC, Cu, Zn, and As concentrations of river water

	pH	**EC (ms/m)**	**Cu (mg/l)**	**Zn (mg/l)**	**As (mg/l)**
SRU	**6.7 ~ 7.6**	**15.7~ 18.8**	**0.010~ 0.143**	**0.247~ 0.543**	**0.127~ 0.178**
SRM	**6.9 ~ 8.0**	**8.6~ 9.8**	**0.007~ 0.086**	**0.031~ 0.273**	**0.022~ 0.046**
SRD	**7.0 ~ 7.5**	**8.0~ 12.2**	**0.008~ 0.028**	**0.026~ 0.051**	**0.017~ 0.053**
NRUP	**6.5 ~ 7.5**	**9.8~ 13.0**	**0.063~ 0.198**	**1.047~ 1.914**	**0.011~ 0.039**
NRUM	**6.2 ~ 7.7**	**9.2~ 15.3**	**0.052~ 0.182**	**0.831~ 1.429**	**0.002~ 0.073**
NRMD	**6.6 ~ 7.5**	**8.2~ 12.1**	**0.011~ 0.037**	**0.308~ 0.637**	**N.D.~ 0.045**
NRD	**6.6 ~ 7.7**	**9.0~ 11.8**	**N.D.~ 0.014**	**0.125~ 0.205**	**N.D.~ 0.026**

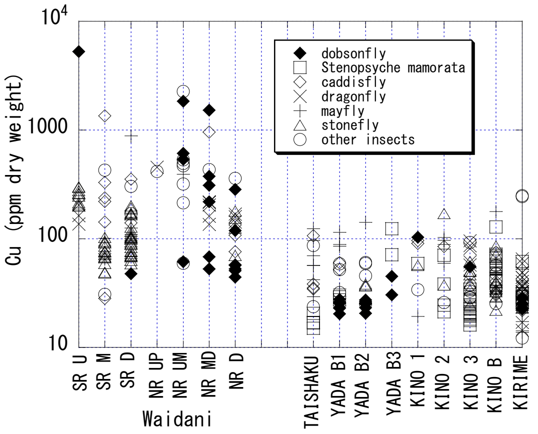

Fig. 2 Cu concentrations under dry weight samples for all sampled river insect larvae.

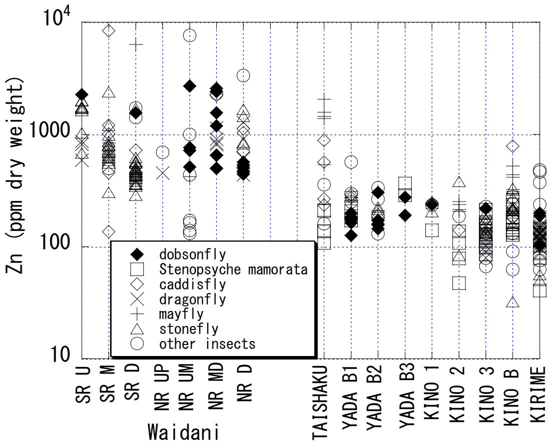

Fig. 3 Zn concentrations under dry weight samples for all sampled river insect larvae.

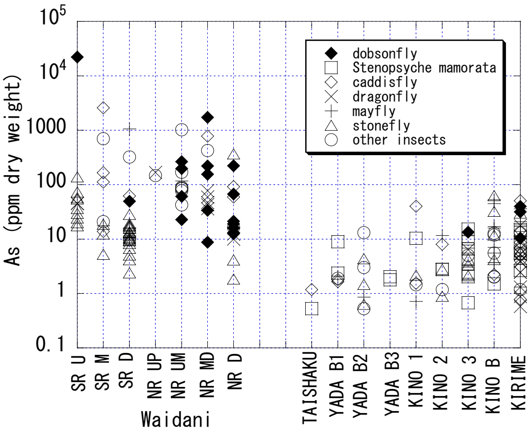

Fig. 4 As concentrations under dry weight samples for all sampled river insect larvae

Figs. 2, 3, and 4 show Cu, Zn, and As concentrations under dry weight samples for all sampled river insect larvae. Although Cu concentrations in river insect larvae in the Waidani north and south areas were variable values, several 10 to several 1000 ppm, they decreased from upstream to downstream. Cu concentration in river insect larvae was thought to depend on river Cu concentration. Cu concentrations in river insect larvae in the Taisyakukyo, Yada, Kino and Kirime Rivers, were uniform, 10 to 100 ppm but Cu concentrations in river insect larvae in the Waidani area were 10 times higher than those in non-mine catchments. Cu concentrations for river insect larvae in the Waidani area even 3 km downstream were higher than those in non-mine catchments. Thus, the high Cu concentration in river water in the Waidani area was thought to increase Cu concentration in river insect larvae. Therefore, the Cu concentration value of the Japanese Effluent Standard was thought to be too high judging from the high Cu concentration in river insect larvae. Cu concentration in river insect larvae depended on the environment’s Cu concentration. Then, river insects were thought to be effective as Cu contamination indicators. Dobsonfly was expected to be useful as a metal index because Dobsonfly was found widely, however Cu concentrations in Dobsonfly larvae in the Waidani area were very variable, several 10 to several 1000 ppm. *Stenopsyche mamorata* is a kind of Caddisfly and very common in Japan. However, *Stenopsyche mamorata* was not found in the Waidani area although it was very abundant in non-mine catchments. Dragonfly (Anisoptera), Caddisfly and Stonefly (Plecoptera) were found in the Waidani area and were higher than those in non-mine catchments although each concentration had a wide range. In this study, there were 18 species of Stonefly, 13 species of Mayfly (Ephemeroptera) and 13 species of Caddisfly and excluding Dobsonfly, many species were thought to bring out variable concentration values because each species was thought to have each concentration character.

Although Zn concentrations in river insect larvae in the Waidani south and north areas were variable values, 100 to 10,000 ppm, they decreased from upstream to downstream. Zn concentration in river insect larvae was thought to depend on river Zn concentration. Although Zn concentrations in the Taishakukyo were variable values, 100 to several 1000 pm as well as mine areas, Zn concentrations in the Kino and Kirime River were several 10 to several 100 ppm and Zn concentrations in the Yada River were several 100 ppm and then Zn concentrations in river insect larvae in mine areas were higher than those in non-mine catchments. Thus, the high Zn concentration in river water in the Waidani area was thought to increase Zn concentration in river insect larvae. Therefore, Zn concentration in the Japanese Effluent Standard was thought to be too high judging from high Zn concentration in river insect larvae. Zn concentration in river insect larvae depended on the environment Zn concentration because Zn concentration in river insect larvae in the Waidani area decreased down the stream and

Zn concentration in river insect larvae in the Waidani area was higher than those in non-mine catchments. Therefore river insect larvae were thought to be effective as a Zn contamination indicator. Zn concentration in Dobsonfly larvae in the Waidani area was 10 times higher than those in non-mine catchments as well as Stonefly although Zn concentrations in Dobsonfly larvae were variable.

Although As concentrations in river insect larvae in the Waidani south and north areas were variable values, several to several 1000 ppm, they decreased from upstream to downstream. As concentration in river insect larvae was thought to depend on river As concentration. As concentrations in insect larvae in non-mine catchments were several 0.1 to several 10 ppm and As concentrations in river insect larvae in the Waidani area were 10 to 100 times higher than those in non-mine catchments. The high As concentration in river in the Waidani area was thought to increase As concentration in river insect larvae. As concentration in river insect larvae depended on the environmental As concentration because As concentration in river insect larvae in the Waidani area decreased going downstream and was higher than those in non-mine catchments. Therefore river insect larvae was thought to be effective as an As contamination indicator. As concentration in Dobsonfly larvae in the Waidani area was 10 times higher than those in non-mine catchments as well as Stonefly although As concentrations in Dobsonfly larvae had a wide range.

Figs.5, 6, and 7 show the relation between Cu, Zn, and As concentrations in river insect larvae and dry weight in river insect larvae because metal concentrations in insect larvae were thought to change with insect growth. Cu concentration in river insect larvae in both the Waidani area and non-mine catchments decreased with dry weight in insect larvae. Although Cu concentration in river insect larvae had a wide range, Cu concentration for each weight had a narrow range. In particular, Dobsonfly was found to show a good relation between dry weight and concentration while other insects such as Stonefly were not as clear. Other insects had many species and each species was thought to have its own character. Decrease (log(Cu)/log(weight)) in Cu concentration with dry weight for Dobsonfly was about -0.5 log(ppm) /log(g) in the Waidani area and about -1/3 log(ppm) /log(g) in non-mine catchments. At the condition in 0.01 g for dry weight (a common dry weight in river insect larvae), Cu concentration in Dobsonfly larvae in the Waidani area and non-mine catchments were several 100 and several 10 ppm and then Cu concentration in Dobsonfly larvae in the Waidani area was 10 times higher than those in non-mine catchments. Therefore, Cu concentrations in Dobsonfly under the uniform dry weight condition could be useful as an index of Cu contamination.

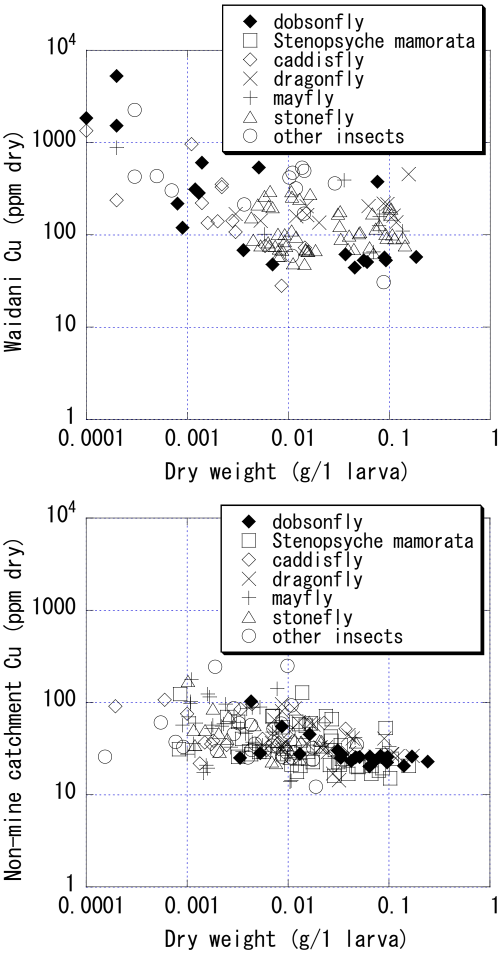

Fig. 5 the relation between Cu concentrations in river insect larvae and dry weight in river insect larvae, upper: Waidani, lower: non-mine catchments

Zn, as well as Cu concentrations in river insect larvae in both the Waidani area and non-mine catchments decreased with dry weight in river insect larvae. Although Zn concentrations in river insect larvae had a wide range, Zn concentrations for each weight had a narrow range. In particular, Dobsonfly was found to have a good correlation between the dry weight and Zn and Cu concentrations although other insects such as Stonefly were not clear. Decrease (log(Zn)/log(weight)) in Zn concentration with weight for Dobsonfly was about -1/3 log(ppm) /log(g) both in the Waidani area and non-mine catchments however Zn concentration in river insect larvae in the Waidani and non-mine catchments were different. At the condition in 0.01 g for dry weight, Zn concentration in Dobsonfly

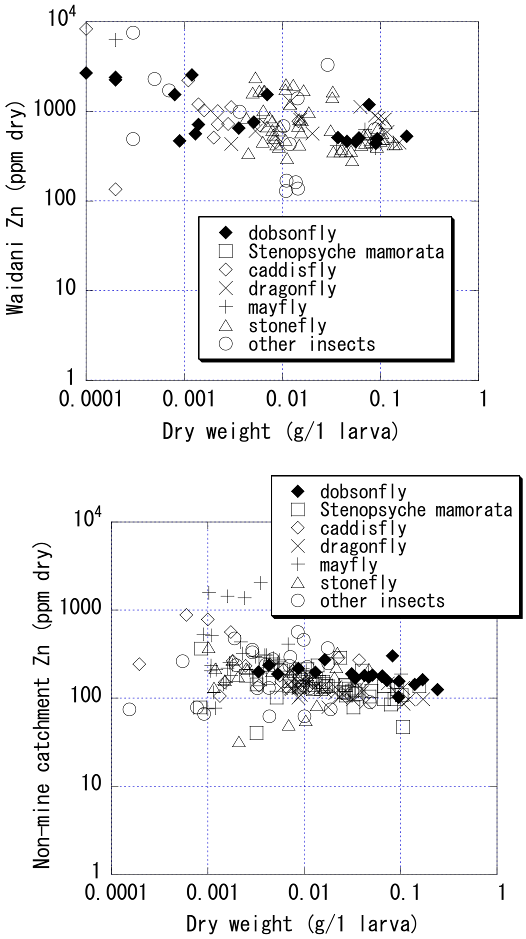

Fig. 6 the relation between Zn concentrations in river insect larvae and dry weight in river insect larvae, upper: Waidani, lower: non-mine catchments

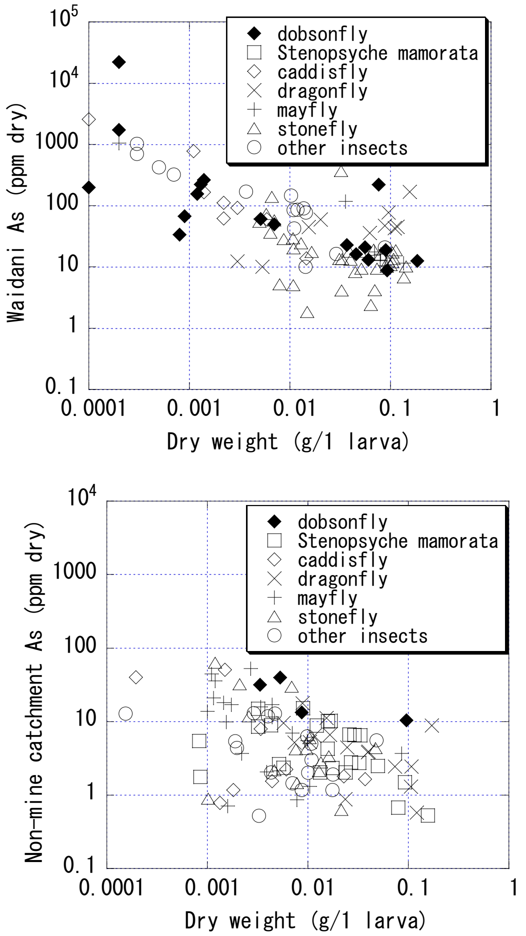

Fig. 7 the relation between As concentrations in river insect larvae and dry weight in river insect larvae, upper: Waidani, lower: non-mine catchments

larvae in Waidani area and non-mine catchments were 1000 ppm and several 100 ppm and then Zn concentration in Dobsonfly larvae in Waidani area was about 10 times higher than those in non-mine catchments. Therefore, Zn concentration in Dobsonfly under the uniform dry weight condition could be useful as an index in Zn contamination.

As concentration in river insect larvae in both the Waidani area and non-mine catchments decreased with dry weight in insect larvae as well as Cu and Zn.

Although As concentration in river insect larvae had a wide range, As concentration for each weight had a narrow range. Dobsonfly was only found to have a good relation between the dry weight and concentration in the Waidani area. Most in As analysis for river insect larvae in the non-mine catchment was under the detection limit in ICP and there were only 4 samples for Dobsonfly. The relation between concentration and dry weight in both the Waidani area and non-mine catchments was on the same line, decrease (log(As)/log(weight))in As concentration with weight for Dobsonfly was -0.5 log(ppm) /log(g). At the condition in 0.01 g for dry weight, As concentration in Dobsonfly larvae in the Waidani area and non-mine catchments were several 10 ppm and then As concentration in Dobsonfly larvae in the Waidani area and non-mine catchments was the same. On the other hand, As concentration in other river insect larvae in the Waidani area were higher than those in non-mine catchments. Therefore, As concentration in Dobsonfly under the uniform dry weight condition was not thought to be satisfactory as an index in As contamination probably because of a lack of data for Dobsonfly.

4. DISCUSSION

Dobsonfly larvae are being considered as an index in metal contamination because they are common, live 2 or 3 years in easy to classify, have a wide distribution, are very rivers, and are carnivorous (top of the insect food chain). Metal concentrations in river insect larvae in mine areas and non-mine catchments were measured. Metal concentrations in insect larvae in mine areas were higher than those in non-mine catchments although they were very variable, by as much as two or three orders. However Cu, Zn, and As concentrations in river insects were still variable values, one or two orders at the same area, mine area or non-mine catchments, because the concentrations decreased with weight and depended on species. Then, at the same weight condition, the concentrations for one species, Dobsonfly larvae indicated uniform values, one order, however other insect larvae maintained wide ranges, over one order because these insects had many species and each species had its own concentration character. Concentration in Dobsonfly decreased with dry weight in Dobsonfly under the same river metal condition and the relations, decreases in metal concentration with weight for Cu, Zn, and As were different values and Cu and Zn concentration in Dobsonfly was thought to depend on river metal condition. However, As concentration in Dobsonfly was not thought to depend on river metal condition because As concentration in Dobsonfly under the same dry weight was the same in the Waidani area and non-mine catchments although As concentration in river insect larvae in the Waidani area was lower than those in non-mine catchments in spite of scarce data on As concentration in Dobsonfly in non-mine catchments. Therefore, metal contamination was estimated from the relations for Dobsonfly excluding As. For example, metal concentration in Dobsonfly at the same dry weight was thought to become an index in metal contamination. Dobsonfly is distributed on a world scale and is very common but limited in its number of species in Japan. Generally, metal is accumulated in body over time with growth. However, the relation between metal concentration and weight showed reverse results. Elimination ability for metal was thought to increase with growth or during ecdysis, the insect was thought to release metal. Decrease in metal concentration with weight was low for Zn and Cu. It was also thought to depend on toxicity in metal. Main river water resource at the upstream in the Waidani area, mine area was seepage from the tailings. Therefore, river water was partly mine effluent water. River insect larvae in the upstream were assumed to have grown up in effluent water. From Cu, Zn and As concentration and weight for Dobsonfly, Cu, Zn and As concentration in Dobsonfly in the Waidani, mine area had high concentrations relative to the non-mine catchments.

5. CONCLUSION

Comparing metal concentrations in river water and river insect larvae in mine areas and non-mine catchments, Cu, Zn and As concentrations in river insect larvae were higher than those in non-mine catchments. However, the metal concentrations had wide ranges and river water metal concentration, insect species and larvae weight made wide ranges. Then, using one species, the Dobsonfly larvae which have a wide distribution, are very common, have long lives and are carnivorous, river metal concentration was evaluated from the relation between metal concentration and weight for Dobsonfly because the relation between metal concentration and dry weight for Dobsonfly was very clear. The metal concentration decrease with weight for Cu, and Zn was different and also depended on river water metal concentration.

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