The Distribution of Trace Elements in Tissues of Fish Living in Acid Environments of Yangmingshan National Park, Taiwan

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The distributions of inorganic elements in the tissues of *Channa asiatica*, in lakes and rivers with different pH values and their influence affected by acidification of the aquatic environment were examined to search for the indicators for acidification in the environments. Waters and tissues of *C. asiatica* collected in Lengshuikeng (pH 3.5) and Chutzuhu (pH 6.9) in Yangmingshan National Park were analyzed. The dissected tissues of fish were decomposed using ultra-pure nitric acid in PTFE pressured decomposition vessels. The concentrations of inorganic elements in fish tissues were determined by ICP-MS using the standard addition method. The amounts of some elements in particular tissues of *C. asiatica* living in the acid environments were larger than those of fish living in the neutral environments and that the concentration of aluminium in the liver of fish was the most appropriate indicator of acidification.

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Recently, wide attention has been paid to the fact that acid deposition into the environment, mainly through various forms of combustion related to human activities, has caused an increase in the environmental mobility of acid soluble polluted substances (metals) in many aquatic and soil systems.[1, 2] These metals, especially aluminium, are suspected to pollute the ecosystem and inhibit the metabolism of living things. In particular, the behavior of aluminium in the soil has been of great interest to environmental scientists since the damage to trees caused by the toxicity of mobile aluminium such as Al^{3+} and its hydrolytic species in the soil solution has been suspected as being one of the possible pathways of the forest decline due to acidic deposition in some areas of Europe, *e.g.*, in southern Germany, where the soil has a very poor buffer capacity for acid.[3] In addition, the extinction of fish and living things in lakes and reservoirs in northern Europe, northern North America and China resulting from the acidification is one of the great problems. However, the effects of acidification of environments on the living body have not yet been clarified.

In contrast to lakes and rivers recently acidified by acid depositions, most of the lakes and rivers in volcanic areas in Japan and Taiwan Island are acidified by sulfuric and hydrochloric acids of volcanic origin.[4, 5] Naturally acidified lakes and rivers usually have a longer history of acidification than those which have recently been acidified by acid deposition. Therefore, they are suitable places to investigate the long-term influence of acid impact on the environment. In Taiwan, there are lots of lakes and reservoirs where acidification is now progressing.[6] However, in Yangmingshan National Park in the northern part of the island, there is a very rare area where fish are living in spite of acid environments. We got permission to get these extremely precious fish, and successfully collected them to clarify the distribution of elements in each tissue and organ of the fish. This is the first record on the fish living in such an acidic environment in Taiwan.

The investigation of the acid lakes will give us data relating to an extreme acidic environment and help us to elucidate the effects on fish with changing surrounding environments and the changes of uptakes of biological trace and major elements into the body. Now there are many areas where acidification is progressing, and the present study will provide important information on the effects of future acidification. To clarify their possibility as indicators that show the degree of acidification of aquatic environments at present and also the degree of the progressing acidification, major and trace elements of tissues of *C. asiatica* living in acid and neutral environments were analyzed.

Study areas and fish

The study area in Yangmingshan National Park (Lengshuikeng River and Pond, Chutzuhu River) is located in the northern part of Taipei City, Taiwan. The water quality formation of these areas was mentioned else where.[7] Lengshuikeng is a marsh where neutral hot spring waters, neutral surface and underground water and acid spring waters are mixed. A stream issues from the marsh. The pond was made by damming up one of the tributaries of the stream. In that pond, *C. asiatica* are living. The area is very rare in the world where fish live, in spite of the high acidity of the water. Chutzuhu is a small river where *C. asiatica* is alive.

Channa asiatica (Linnaeus) is a freshwater fish and carnivorous.[8] It lives in bottom of mud of closed water with many aquatic plants. It has a suprabranchial organ in the upper part of the suprabrancjoal chamber, which breathes the air.[9]

Experimental

Reagents: Working solutions of respective elements were prepared by diluting the respective 1000 mg dm^{-3} standard solutions (Wako, Osaka, Japan). Ultra-pure nitric acid (Cica-Merck Ultrapur, Kanto Chemical, Tokyo, Japan) was used. Water used throughout was Milli-Q high purity water. All containers and vessels were made of quartz, PTFE or polypropylene and to prevent the contamination of aluminium they were soaked in about 1 mol dm^{-3} nitric acid solution for at least 24 hrs, and rinsed with purified water before use.

Apparatus: Water temperature and pH, redox potential (*Eh*) and electric conductivity (*Ec*) were measured at the site with a HM-14P pH meter with a glass electrode, a RM-12P Eh meter, and a CM-1K Ec meter (TOA, Tokyo), respectively. Major components of cations (Na^+, Ca^{2+}, K^+, Mg^{2+}, and total-Fe) were determined with an atomic absorption spectrometer (Model AA8500, Nippon Jarrell-Ash, Kyoto). Major components of anions (NO_3^-, SO_4^{2-}, and Cl^-) were determined by ion chromatography (Pump: Sanuki DM2M-1024; Separation column: Toso TSK IC-Anion PW; Detector: Toso CM-8000). The concentrations of trace elements in samples were determined by ICP-MS with a spectrometer (Model PMS2000, YOKOGAWA, Tokyo).

Sampling methods and analytical procedures

Field survey was done in August, and September 1999 and March 2000. After measured water temperature, pH, redox potential (*Eh*) and electric conductivity (*Ec*) at the site, water were collected in polypropylene and PTFE bottles after filtered through 0.20- and 0.45-μm membrane filters (Millipore, Bedford, USA).

C. asiatica were dissected under asphyxiation. Twenty parts (gills, livers, kidneys, intestines, muscles, stomach, heart, lung, eyes, pectoral fins, anal fins, gall bladder, air bladder, spleen, brain, ovary/testis, blood, bones (front), bones (back) and skins) were extracted immediately after collection from the lakes and the river.[9] Three fish from each site were analyzed. The dissected organs were dissolved in PTFE pressured decomposition vessels using 2 cm^3 of nitric acid in a microwave oven or a hot plate, in order to minimize any contamination. In the case of using a hot plate, the vessels were heated for more than ten days. After the decomposition, the samples were finally diluted to 25 cm^3 with purified water, and preserved at 5 ℃ until analyzed.

The concentrations of 14 trace elements were determined by ICP-MS. The measurements were done using standard addition methods.

Table 1(a) Contents of five elements in the tissues of fish (μg g^{-1}, wet weight; mean±sd)

		Al	Cu	Fe	Mn	Zn
C. asiatica						
Lengshuikeng	gill	12 ± 2.9	0.58 ± 0.055	48 ± 16	7.1 ± 3.4	19 ± 0.63
	liver	83 ± 20	22 ± 7.6	3324 ± 552	4.6 ± 2.1	33 ± 3.2
	intestine	1.6 ± 0.77	2.8 ± 0.85	675 ± 351	8.2 ± 3.3	21 ± 1.3
	muscle	1.3 ± 1.7	0.20 ± 0.11	5.9 ± 2.8	0.36 ± 0.27	5.6 ± 0.19
	bone	9.3 ± 3.7	0.25 ± 0.10	29 ± 19	23 ± 1.9	29 ± 0.49
	skin	6.1 ± 4.7	2.4 ± 1.8	23 ± 13	9.6 ± 0.80	61 ± 20
Chutzuhu	gill	2.6 ± 1.0	1.4 ± 0.55	144 ± 80	7.2 ± 3.8	32 ± 8.9
	liver	20 ± 4.4	11 ± 3.2	1810 ± 660	2.5 ± 1.2	31 ± 1.7
	intestine	0.85 ± 0.53	1.2 ± 0.69	44 ± 17	3.4 ± 3.0	19 ± 3.1
	muscle	0.21 ± 0.078	0.19 ± 0.047	5.4 ± 1.7	0.45 ± 0.45	6.8 ± 0.90
	bone	1.5 ± 0.10	0.23 ± 0.014	11 ± 2.5	25 ± 0.25	36 ± 2.9
	skin	2.8 ± 1.5	0.65 ± 0.057	22 ± 3.4	6.9 ± 1.7	37 ± 7.3
T. hakonensis						
Usoriko	gill	42 ± 23	1.2 ± 0.16	42 ± 16	4.4 ± 2.1	25 ± 2.9
	liver	13 ± 5.8	37 ± 28	412 ± 180	2.0 ± 0.35	28 ± 7.0
	intestine	6.0 ± 1.2	2.5 ± 0.52	128 ± 69	2.2 ± 0.58	26 ± 3.9
	muscle	4.2 ± 2.6	0.63 ± 0.11	5.5 ± 1.6	0.31 ± 0.13	5.0 ± 0.60
	bone	6.9 ± 2.7	0.44 ± 0.090	7.0 ± 3.6	5.2 ± 2.4	31 ± 5.6
	skin	9.1 ± 2.8	1.1 ± 0.18	8.1 ± 1.3	2.1 ± 0.68	138 ± 34
Tenryu River	gill	1.1 ± 2.4	0.83 ± 0.11	33 ± 5.2	3.4 ± 0.95	22 ± 1.8
	liver	6.2 ± 3.6	28 ± 29	112 ± 61	2.1 ± 0.26	35 ± 5.0
	intestine	0.77 ± 1.4	2.5 ± 0.69	16 ± 6.2	2.2 ± 0.47	28 ± 3.6
	muscle	0.22 ± 0.087	0.84 ± 0.29	7.4 ± 2.6	0.34 ± 0.040	11 ± 2.7
	bone	0.44 ± 0.43	0.42 ± 0.17	4.1 ± 1.3	8.7 ± 2.5	42 ± 4.7
	skin	0.64 ± 0.34	1.0 ± 0.18	7.7 ± 2.1	4.6 ± 1.6	155 ± 38

Results and Discussion

The concentration of calcium, sodium, potassium, and magnesium, which are biological major elements and other 15 trace elements of each tissue of *C. asiatica*, were analyzed. Among trace elements, the concentrations of five elements, showing high content in tissues of *C. asiatica*, were compared with those of *T. hakonensis*[10] (Table 1). Usoriko is an acid lake and Tenryu river is a neural river in Japan. The concentrations of major elements in each tissue of *C. asiatica* were almost same. This result indicates that the concentrations of major components in *C. asiatica* are not affected by those in the living environment and their concentrations are regulated based on the homeostatic mechanism.

Table 1(b) Contents of five elements in water ($\mu g\ g^{-1}$)

	pH	Al	Cu	Fe	Mn	Zn
Lengshuikeng	3.5	1.7	0.010	2.3	0.16	0.031
Chutzuhu	6.9	0.013	0.00037	0.23	0.20	0.0024
Usoriko	3.2	1.6	0.01>	1.4	0.23	0.01>
Tenryu River	7.7	0.017	0.01>	0.02	0.01>	0.01>

Most of the amounts of trace elements accumulated in tissues were higher than their living environments and fish living in an acid and neutral environments both showed biological accumulation.

Moreover, most of the elements tended to accumulate in fish in the acid environments. However, the concentrations of manganese and zinc did not show remarkable differences between both fishes that lived in the acid and neutral environments, and as for copper, the concentration was rather small. Therefore, as an indication of acidification of environment, it is appropriate to select aluminium and iron. However, even if the concentration of iron was higher due to the effect of acidification, iron is one of biological essential elements, so the ascending range may not be apparent. Originally, the concentration of iron in the living body is high especially in the blood. Hence, the concentration in organs that gather blood, such as the liver and intestines, tends to be high and does not clearly reflect the changes of environments.

The solubility of aluminium-containing minerals in acid environments is large and the concentration of dissolved aluminium in water is high with the acidification of the water. Recently many surveys have been studying the effect of aluminium in water on the ecosystem. The concentration of aluminium clearly reflects the effects on living things, so it is an appropriate indicator for acidification of the environment.

The concentration of aluminium in each tissue of *C. asiatica* living in the acid environment was higher than that in the neutral environments. Especially, the concentration in the liver was higher than that in the neutral environment. The accumulation pattern of aluminium in *T. hakonensis* living in Usoriko was different from that of *C. asiatica* living in Lengshuikeng. The tissue with the greatest accumulation was the gill. *T. hakonensis* is known to respire only by gills and incorporates waters from gills, so the increase in the frequency of respiration should result in the accumulation of the amounts of aluminium in gills. On the other hand, *C. asiatica* respires not only by gills but also by a kind of lungs and the incorporation of water from gills is considered to be less than that for *T. hakonensis*. Therefore, the amount of aluminium accumulated in gills of *C. asiatica* is smaller than that of *T. hakonensis*.

In general, gill sections were considered to reflect the surrounding water quality accompanied by acidification. The results of local analysis of aluminium showed that the gills of *C. asiatica* living in acid environment were not suitable for an indicator of acidification, as opposed to *T. hakonensis*. The concentration of aluminium in liver may be an appropriate indicator of acidification.

The bioaccumulation factors (BAFs) of aluminium in various fish tissues, calculated by dividing the wet tissue concentration by the water concentration, are shown in Table 2.

Table 2 Bio accumulation factors of aluminium in various tissues of fish

C. asiatica	Lengshuikeng	Chutzuhu
gill	6.8	207
liver	48	1594
intestine	0.93	68
muscles	0.73	17
bones	5.3	117
skins	3.5	224
T. hakonensis	Usoriko	Tenryu River
gill	29	64
liver	8.7	363
intestine	4.1	45
muscles	2.9	13
bones	4.7	26
skins	6.2	37

BAFs of organs of fish in Lengshuikeng were smaller than those in Chutzuhu. This means that stronger bioaccumulation occurred in a neutral environment than in an acid environment. For example, the concentration of aluminium in fish living in a neutral environment was higher than that in an acid environment, suggesting that dissolved chemical species of aluminium is related to their toxicities. The inorganic monomeric aluminium appears to be the most toxic species[11], and more than 90% of the aluminium in water from Usoriko was present as Al^{3+}.[10] *T. hakonensis* in Usoriko did not accumulate aluminium as much as in a neutral environment. This suggested that *T. hakonensis* in Usoriko could exclude aluminium by a self-protective mechanism of living things or that Al-accumulated *T. hakonensis* could not survive. In Taiwan, BAFs were smaller in the acid environment. The speciation of aluminium in those waters has not been done, but probably the situation may be the same as that in Japan. The variations of aluminium concentrations in the organs of *C. asiatica* in Lengshuikeng and Chutzuhu were not much larger than those in the waters. This means that the aluminium concentration in the body of the fish does not directly reflect the concentration in the water and the accumulation of aluminium in the fish is somehow controlled by chemical and biological factors in these acidified lakes as in the case of *T. hakonensis* in Japan.

Conclusion

The indicator that enables us to estimate the degree of acidification in the environment was clarified. To know the effects of acidification to living things, it is very important to analyze the trace elements of each tissue of fish. Among trace elements, the concentration of aluminium in the liver of fish was the most appropriate indicator of acidification.

There are many areas where acidification is now progressing worldwide. The present study could afford important information for monitoring the present situation of acidification of the environments and for the prediction of the effect in the future.

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