Chin. Geogra. Sci. 2010 20(4) 330–336
DOI: 10.1007/s11769-010-0405-5

Distribution of Methyl Mercury in *Rana chensinensis* and Environmental Media in Gold-mining Areas of Upper Reaches of Songhua River, China

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Abstract: The distribution characteristics of methyl mercury in *Rana chensinensis* and water, sediment and soil in gold-mining areas of the upper reaches of the Songhua River, China were studied by field sampling and laboratory testing. The results show that the methyl mercury contents in water, sediment and soil in gold-mining areas are much higher than those in the control site, indicating that gold-mining activities intensify the methyl mercury pollution in the study area. Methyl mercury contents are in a descending order of sediment > soil > water in the environment, and in a descending order of brain > viscera > muscle > skin in *Rana chensinensis*. There are significant correlation between methyl mercury contents in *Rana chensinensis* and those in water and sediment. In particular, the methyl mercury content in the skin of *Rana chensinensis* is positively correlated with those in water and sediment in spring. Therefore, skin is one of main intake pathways for methyl mercury due to its high permeability.

Keywords: methyl mercury; *Rana chensinensis*; environmental pollution; gold-mining area; Songhua River

1 Introduction

The harmful effect of methyl mercury on human beings and aquatic organism has been a concern in academic researches since methyl mercury can be easily absorbed and concentrated by organisms due to its fat-solublility (Kannan *et al*., 1998). As a neurotoxic substance, methyl mercury represents tremendous threats towards humans and other organisms (Watras *et al.*, 1998; Sethajintanin *et al*., 2004; Hammerschmidt and Fitzgerald, 2006; Zhang *et al*., 2006; Pinheiroa *et al.*, 2008). For instance, the activation effect of mercury in water may lead to an increase of mercury content in fish (Lmura *et al*., 1971), which can cause the irreversible influence on and harm to human beings through food chain (Feng and Hong, 1997; Wu *et al*., 2007).

There are many mercury and methyl mercury sources in the environment. Recently, more attention has been paid to inorganic mercury release from non-ferrous metals-chlor-alkali industry combined pollution (Zheng Dongmei *et al*., 2007; Zheng Na *et al*., 2007), coal field development and non-ferrous metal metallurgy as well as methyl mercury pollution to the surroundings (Williams *et al*., 1999; Marszalek and Wasik, 2000; Mol *et al*., 2001; Suzana *et al*., 2007). Moreover, some studies have been conducted to investigate the influence of mercury ore development on the surrounding environment in Guizhou and Yunnan provinces of China (Qiu *et al*., 2006; Wang *et al*., 2006; Li *et al*., 2008).

The studies on mercury pollution in organism have mainly focused on fish and birds. In the 1980s, the long-term studies on the methyl mercury pollution in the Songhua River, China have been carried out (Wu *et al*., 1994; Liu *et al*., 1998). The results showed that the methyl mercury concentration in fish in the Songhua River increased significantly as a result of pollution. And the methyl mercury in fish mainly came from two ways. One was the transport of methyl mercury along the food chain, and the other was the transformation of inorganic mercury into methyl mercury in fish surface, which could be subsequently absorbed through skin and gills, *etc*. However, the mercury pollution in amphibians has been largely ignored. Amphibians have a close connection with land, water and atmosphere due to their

Received date: 2009-09-30; accepted date: 2010-01-05
Foundation item: Under the auspices of National Natural Science Foundation of China (No. 40673059), Testing Foundation of Northeast Normal University
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unique life habitat, therefore the methyl mercury content in them may indicate the local mercury pollution more clearly and accurately (Cristina *et al*., 2005).

Huadian City of Jilin Province, located in the upper reaches of the Songhua River, is a large gold-mining area in China with a long history and a good habitat for *Rana chensinensis*, a representative amphibian. In this area, because gold is extracted by amalgamation process, part of mercury leak into the environment in the forms of mercury vapor, tailing mine waste water as well as remained gold, thus causing pollution to the atmosphere, water, sediments and local organism. Some studies have been conducted on mercury pollution in *Rana chensinensis* in this area (Wang *et al*., 2005), however, the distribution characteristics and the sources of methyl mercury still need to be further clarified.

To understand the pollution level and the sources of methyl mercury in *Rana chensinensis* in accompanying with gold-mining, this paper studied the distribution and enrichment of the methyl mercury in various tissues and organs of *Rana chensinensis*, and analyzed the correlation between the methyl mercury contents in *Rana chensinensis* and those in environment media. The result can provide scientific basis for methyl mercury pollution evaluation and edible safety of *Rana chensinensis*.

2 Methodology

2.1 Study area

The basin area of the Weisha River, a tributary of the upper reaches of the Songhua River, is 533 km^2. There are many gold mines in the basin, among which Jiapigou is a national well-known large gold mine. It has been developed for 180 years by amalgamation process, and the annual production of ores can reach 5 700 t. Annually 40 kg of mercury is used to extract gold, of which 65%–87% enters water, soil and atmosphere through mercury vapor and other ways, thus harming human beings by means of ecological system and food chain (Akagi *et al*., 1995; Bai *et al*., 2006).

2.2 Sampling methods

Totally six sampling sites were selected in the Weisha River Basin, which are located in Jiapigou gold mine, Laoniugou gold mine, abandoned Laojinchang gold mine, Wudaogou Village, Shuanghe Village and Erdaogou Village, and one control site was selected in Ji′an City of Jilin Province, where no gold mine or other mine has been ever exploited (Fig. 1).

The samples in both polluted and non-polluted areas were collected in April (spring) and October (autumn), 2007, respectively. The samples of environmental media (water, soil and sediment) were collected from all six sampling sites in the polluted areas and one control site in the non-polluted area, and those of *Rana chensinensis* from the sampling sites in the polluted areas except for Laojinchang. However, *Rana chensinensis* was not found in Laoniugou in spring. Water was collected with 2.5 L polyethylene container, and adjusted by hydrochloric acid (HCl) to a pH of 3.0 after sampling, then the samples were taken to laboratory for testing immediately. The soil and sediment were collected in the shape of diagonal and sampled by quarter method. Then, they were purified to remove impurities, air-dried, passed

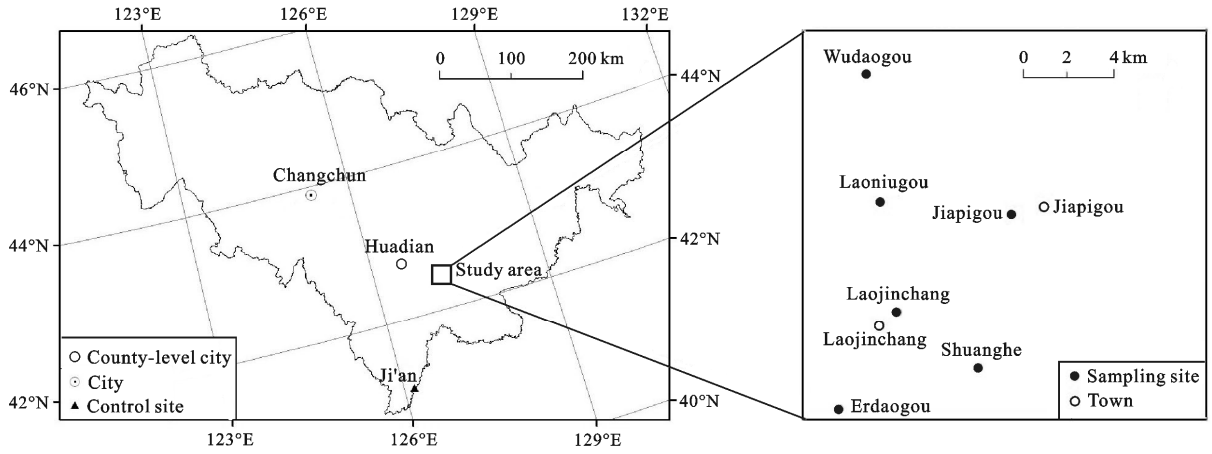

Fig. 1 Location sketch of sampling sites

through 80 mesh sieve, and put into polyethylene bags to be preserved. About 10–11 of *Rana chensinensis* were collected at every sampling site, which were dissected into four parts, i.e., skin, muscle, viscera and brain, and frozen in laboratory before test.

2.3 Analysis methods

Before test, 1 L water sample was first concentrated by using sulfhydryl cotton, then eluted with HCl, finally extracted by using benzene. For sediment or soil samples, we added 2 mL 2 mol/L HCl into 2 g sample and stirred, added 1 mL 0.01 g/L $CuSO_4$ into the samples, and extracted the samples for 10 min.

After sulfhydryl cotton concentrating, HCl eluting and benzene extracting, the *Rana chensinensis* samples were tested. We added 2 g NaCl into 2 g sample and grinded, then added 2 mL 2 mol/L HCl and continuously grinded into paste. After that the samples were transferred into a 25 mL color-comparison tubes and shaken for 10 min, filtered 1 h later, extracted by using benzene, and centrifuged at 2 500 r/min. Methyl mercury was determined with Gas Chromatography.

2.4 Calculation method of enrichment factor

Rana chensinensis lives in water and sediment for almost half a year. Therefore, the enrichment factor (EF), i.e. the proportion of methyl mercury concentration of biological tissue (dry weight) to that in water or sediment, can be used to analyze the methyl mercury enrichment in *Rana chensinensis.* EF can be expressed as:

$$EF = C_i / C_0$$

where *EF* is the enrichment factor of methyl mercury; C_i is the methyl mercury content in *Rana chensinensis* (μg/kg); C_0 is the methyl mercury content in water or sediment (μg/L or μg/kg).

3 Results

3.1 Methyl mercury contents in environment media

The methyl mercury contents in the samples from polluted areas are significantly higher than those from non-polluted area (Table 1). The methyl mercury contents are in a descending order of sediment > soil > water, and in both sediment and soil they are higher in autumn than in spring, but no obvious changes are observed for water samples.

3.2 Methyl mercury contents in *Rana chensinensis*

The methyl mercury contents in *Rana chensinensis* in spring and autumn are shown in Fig. 2. The methyl mercury contents in most samples of *Rana chensinensis* are in a descending order of brain > viscera > muscle > skin. Particularly, the contents in brain in Shuanghe and Jiapigou in spring as well as in Jiapigou and Laoniugou in autumn are 1.0–7.1 times of those in other organs. In addition, the contents in *Rana chensinensis* collected from Wudaogou are the lowest among all the sites.

3.3 Enrichment of methyl mercury in *Rana chensinensis*

From Table 2, the enrichment factors of methyl mercury in *Rana chensinensis* from water change in the range of 5 176–116 770, and they are higher in autumn (6 240–116 770) than in spring (5 176–67 560). From Table 3, the enrichment factors of methyl mercury in *Rana chensinensis* from sediment range from 0.03 to 5.33. In addition, the enrichment factors of methyl mercury in brain are higher than those in other organs, particularly in the females, indicating that the methyl mercury can harm the nervous centralis of organisms by reaching brain stem rapidly after entering organism.

Table 1 Methyl mercury contents in various environmental media (Mean ± S.D.)

Sampling site	Water (μg/L)		Soil (μg/kg)		Sediment (μg/kg)	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
Ji'an	–	0.04±0.028	–	0.58±0.007	–	5.90±0.011
Wudaogou	3.40±0.053	3.67±0.066	51.10±0.051	43.90±0.031	26.00±0.112	56.30±0.048
Shuanghe	2.50±0.061	3.57±0.110	ND	58.00±0.064	25.80±0.069	59.90±0.039
Erdaogou	2.60±0.121	1.61±0.009	13.70±0.104	37.00±0.055	34.00±0.054	55.50±0.008
Laojinchang	3.30±0.048	1.74±0.033	14.50±0.069	35.20±0.105	25.80±0.051	54.10±0.102
Jiapigou	4.10±0.145	2.10±0.058	13.70±0.025	35.80±0.045	25.90±0.035	52.50±0.052
Laoniugou	–	1.61±0.105	–	37.30±0.049	–	53.20±0.036

Notes: – represents not sampled; ND: not detected at detection limit

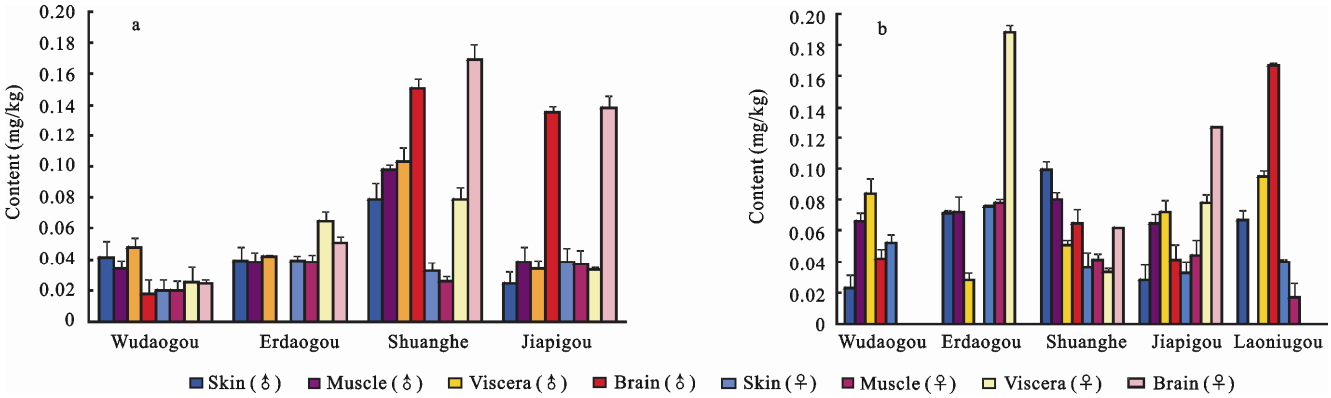

Fig. 2 Methyl mercury contents in *Rana chensinensis* in spring (a) and autumn (b)

Table 2 Enrichment factor of methyl mercury in *Rana chensinensis* from water

Sampling site	Sampling time	Male				Female			
		Skin	Muscle	Viscera	Brain	Skin	Muscle	Viscera	Brain
Wudaogou	Spring	12029	10029	13971	5176	5971	5853	7324	7147
	Autumn	6240	17847	22752	11499	25014	ND	ND	ND
Shuanghe	Spring	31480	39040	41240	60320	12760	10240	31440	67560
	Autumn	27843	22465	14314	18067	10056	11513	9356	17227
Erdaogou	Spring	14846	14962	16269	ND	14846	14692	25154	19769
	Autumn	43851	44969	17453	ND	47019	48634	116770	ND
Jiapigou	Spring	5878	9220	8195	32902	9220	9073	7976	33634
	Autumn	13286	31000	34429	19381	15000	21143	36952	60286
Laoniugou	Autumn	41801	ND	58696	103416	24534	10435	ND	ND

Note: ND means that there was no enrichment factor because methyl mercury was not detected

Table 3 Enrichment factor of methyl mercury in *Rana chensinensis* from sediment

Sampling site	Sampling time	Male				Female			
		Skin	Muscle	Viscera	Brain	Skin	Muscle	Viscera	Brain
Wudaogou	Spring	1.57	1.31	1.83	0.68	0.78	0.77	1.08	0.93
	Autumn	0.41	1.16	0.15	0.08	0.16	ND	ND	ND
Erdaogou	Spring	0.11	1.10	0.13	ND	0.11	0.11	0.19	0.15
	Autumn	0.13	1.30	0.51	ND	0.14	0.14	0.52	ND
Shuanghe	Spring	0.31	0.38	0.40	1.97	1.23	0.99	0.01	2.67
	Autumn	1.66	1.34	0.85	1.08	0.60	0.69	0.56	1.03
Jiapigou	Spring	0.93	1.46	1.30	5.22	1.46	1.44	1.26	5.33
	Autumn	0.05	0.12	0.14	0.08	0.06	0.09	0.53	0.24
Laoniugou	Autumn	0.13	ND	0.37	0.31	0.07	0.03	ND	ND

Note: ND means that there was no enrichment factor because methyl mercury was not detected

3.4 Correlation of methyl mercury contents between *Rana chensinensis* and environmental media

Table 4 shows the correlation of methyl mercury contents in water, sediment, soil and inner organs of *Rana chensinensis* in spring and autumn. In spring, the methyl mercury contents in most organs of *Rana chensinensis* are correlated or significantly correlated with those in water and sediment, however, only the methyl mercury content in muscle of female *Rana chensinensis* is correlated with that in soil. This is because that *Rana chensinensis* hibernate in stone cracks or sediment in both sides of rivers in winter, and female *Rana chensinensis* live in sediment for a long time for laying eggs in spring. In autumn, the methyl mercury contents in most organs of *Rana chensinensis* are correlated or significantly correlated with that in water, however only male skin, female skin and female

Table 4 Correlation between methyl mercury contents in *Rana chensinensis* and environment media

Sampling time	Environment media	Male				Female			
		Skin	Muscle	Viscera	Brain	Skin	Muscle	Viscera	Brain
Spring	Water	0.7564**	0.5651*	0.6560*	0.2345	0.5374*	0.7304**	0.7818**	0.3026
	Soil	0.0269	0.0376	0.0345	0.1042	0.0124	0.6620*	0.0977	0.1631
	Sediment	0.5257*	0.4898	0.5146*	0.5468*	0.8767**	0.8384**	0.5746*	0.4674
Autumn	Water	0.3220	0.9377**	0.7524**	0.8167**	0.4000	0.6456*	0.2654	0.9641**
	Soil	0.5906*	0.1958	0.1791	0.0043	0.7038**	0.6156*	0.2760	0.3659
	Sediment	0.4459	0.3304	0.2198	0.1950	0.9491**	0.9476**	0.0859	0.6230*

Notes: * $P < 0.05$; ** $P < 0.01$

muscle are correlated or significantly correlated with that in soil, and only female skin and female muscle are significantly correlated with that in sediment. *Rana chensinensis* enter forest in summer and autumn, and live both in river and on land. Therefore, the methyl mercury contents in *Rana chensinensis* are highly dependant on water and soil in autumn. Moreover, correlativity between the methyl mercury contents in skin and muscle and environmental media is generally high. Therefore, skin is one of main intake pathways for methyl mercury due to its high permeability.

4 Discussion

The variations of methyl mercury contents in *Rana chensinensis* may be related to the biological methylation rate of mercury in various environment media. Methylation rate depends on the microbial species in anaerobic environment, pH, temperature, oxidation-reduction potential and other media (Eckley and Hintelmaun, 2006). Particularly, the anaerobic environment of sediment is probably the most favorable condition for transformation of inorganic mercury into organic mercury. Results of previous research showed that the methyl-cobalt ammonia in sediment contain u-methane producing bacteria that can methylate inorganic mercury (Wang, 1997). In contrast, the anaerobic environments in water and soil are poor, so the possibility of methylation may be relatively little.

In addition, the climatic factors can also exert impacts on methylation process. The low temperature in winter and spring has a certain influence on enzyme and micro-organism activity, while the high temperature, moist climate and high biological activity in summer and autumn are conducive to the biological methylation of mercury. Eckley and Hintelmaun (2006) found that inorganic mercury was methylated on the junction of sand and water in sediment, and then diluted into water. The methylation activity of microorganisms in sediment can be affected by water temperature. Furthermore, the abundant precipitation in summer and autumn leached mercury into soil and rivers. The rise in rivers′ water temperature and the disturbance of current enhanced the translated rate from total mercury to methyl mercury and accelerated the released process to water, which was also one of the reasons why methyl mercury content in sediment was higher in summer and autumn.

The methyl mercury content in *Rana chensinensis* in Shuanghe Village is the highest. Although Shuanghe Village is not a mining area, there are many small private mining sites around it, which makes the methyl mercury content in *Rana chensinensis* be the highest. Jiapigou is the greatest gold mining area in the study area, but the methyl mercury contents in the environment are not the highest, which can be ascribed to the migration of mercury and methyl mercury for a long time. Moreover, the methyl mercury content in brain stem of *Rana chensinensis* is the lowest in Wudaogou, which is very likely due to the long distance from the mining area, deep gutters, dense forest and small population, *etc*.

Previous reasearch showed that the enrichment factors of methyl mercury in fish in a branch of the Songhua River in 1990–1991 were in a range of 69 756–120 243 during spring and 122 926–173 800 during autumn (Liu *et al*., 1998), which were averagely 1.6 times (spring) and 1.4 times (autumn) higher than those in *Rana chensinensis*. Some studies also indicated that the methyl mercury in organism could come from either methylation by organism or transporting from external environment (Lin *et al*., 1994), and the methylation by fish was very little (Yu *et al*., 1992). Therefore, water

and sediment may be the major sources of methyl mercury in *Rana chensinensis*. From the EF of methyl mercury reported in this paper, it can be found that methyl mercury in *Rana chensinensis* is very likely from water. It is generally agreed that the organic mercury produced by methylation of inorganic mercury in sediment and released into land or water can be absorbed by and concentrated in organism.

Furthermore, skin is one of main intake pathways for methyl mercury into *Rana chensinensis* bodies due to its high permeability**,** and more intensive studies on the detailed mechanism need to be carried out in the future.

5 Conclusions

In the gold-mining areas of Huadian City in the upper reaches of the Songhua River, methyl mercury contents are high not only in environmental media but also in the tissues and organs of *Rana chensinensis*, especially the brain, indicating that gold mining activities can definitely contribute to the accumulation of methyl mercury in *Rana chensinensis*. The enrichment factor of methyl mercury in *Rana chensinensis* from water is the highest and the correlation between the methyl mercury contents in skin and environmental media including water and sediment are remarkable in spring. It is reasonable to propose that the methyl mercury in water and sediment can be accumulated inside the bodies of *Rana chensinensis* by osmosis, which is obviously different from that in fish. Osmosis through the skin is an important way of methyl mercury accumulation inside *Rana chensinensis*. More intensive studies on the detailed mechanism need to be carried out in the future.

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