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Preliminary investigation of mercury in bone tissues of skua and penguin in Antarctica using AFS and Synchrotron Radiation X-ray Fluorescence (SR-XRF)

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Abstract Mercury (Hg) was investigated in bone tissues of skua (*Catharacta maccormick*) and penguin (*Pygoscelis adeliae*) collected in the maritime Antarctic using atomic fluorescence spectrometry (AFS) and synchrotron radiation X-ray fluorescence (SR-XRF) method. The total levels of mercury in bone tissues of penguin and skua are much lower than those in other organs (e. g., kidney, liver). The toxic effects of mercury in bone tissues of seabirds in polar region are not known. We have used SR-XRF method to map the distribution of trace levels of mercury in bones. The levels of mercury are found to be enriched somewhere near the periosteal surface and/or endosteal surface. The distribution of mercury shows strongly correlation with that of some essential elements and probably poses negative effect on the bone metabolism inferring from the relationship of mercury with the other elements. These studies represent a first step toward understanding the toxic effects of mercury on bone of polar animals by suggesting the possible microscopic investigation.

Key words mercury; bone; skua; penguin; Antarctic; microscopic; SR-XRF.

1 Introduction

Seabirds (e. g., penguin, skua, etc) living in the maritime Antarctic have served as important biomonitors or bioindicators of environmental pollution in this remote area (Sladen *et al.* 1966; Szeler *et al.* 1993; Sun and Xie 2001). During the last several decades, research has shown that the levels of heavy metals, especially mercury and cadmium may be high in many species living there (Bargagli *et al.* 1996; Bargagli *et al.* 1998). The findings have prompted further studies to examine the possible effects of contaminants on these birds. Possible deleterious effects include reduced survival, decreased annual reproduction and more subtle physiological and biochemical effects.

In seabirds, trace element concentrations have usually been determined in liver or kidney (Torgeir *et al.* 2001), a sample collection method that requires birds to be killed.

However, animal welfare concerns dictate that nondestructive sampling should take precedence over destructive sampling as long as scientific rigor is not compromised. Non-destructive sampling for trace elements in birds usually entails taking feather, blood samples and bone relic. Feathers and blood have been used with increasing frequency in recent years for studies of concentrations and effects of trace elements in wild birds (Wayland *et al.* 2001). Fewer studies have examined trace element concentrations in the bone relic of seabirds in polar region to date. In comparison with the other samples, bone relic can be preserved for a long time and is relatively easy to obtain in the maritime Antarctic or Arctic. Investigating the level and distribution of trace element in recent or historical bone relic will provide us an opportunity to assess toxic effects on seabirds.

In this study we preliminary report concentrations of mercury in bone tissues of penguin and skua, which are typical species of seabirds living in the maritime Antarctic. Moreover, we use synchrotron radiation X-ray fluorescence (SR-XRF) method, by which we can detect multiple elements at the same time with lower minimum detection limit (MDL) and excellent spatial resolution without damaging bone sample (Bockman *et al.* 1990; Gomez *et al.* 1999; Chen *et al.* 2000; Zhang *et al.* 2001; Xie *et al.* 2003), and qualitatively map the microscopic distribution of mercury in the transverse sections of skua and penguin bone to obtain baseline information of where within bone toxic element accumulates.

2 Materials and methods

We randomly selected a wing bone of skua (*Catharacta maccormick*, sample number zd-02) and a leg bone of penguin (*Pygoscelis adeliae*, sample number fd-03), which were collected on the Millor Peninsula (69°22′S, 76°24′E) during the fifteenth Chinese Antarctic Research Expedition (December 1998 – March 1999), for the analysis. Samples were placed and sealed in clean plastic bags, and kept at 4℃ until lab analysis (Xie *et al.* 2003). A plastic tool was used to clean samples of soft tissues and blood.

2.1 Total Mercury Analysis

Samples were air-dried and weighed into glass beaker. The sample mass varied from about 100 to 250 mg. 5.0mL HNO_3 (70%, *V/V*) and iron catalyst were added and kept for about 12 h. After that, 5mL H_2O_2 (30%, *V/V*) was added and the samples were heated to slightly boil for 1hr. After cooling, the volumes were adjusted to 10mL. Mercury was analyzed by atomic fluorescence spectrometry (920-AFS, Beijing XiaoTian'E Co.). The detection limit is 0.02ppb. Standard reference material (GSW07601) was used to control the analysis accuracy.

2.2 SR-XRF Targeting and Analysis

Since measurement of concentration requires a flat surface, zd-02 and fd-03 were cut with a diamond saw, washed in deionized water in order to remove the sawdust, and dried at 80°C for about 12h. The newly formed surface was sufficiently smooth and did not need further polishing. Samples were measured for two series (S1 and S2, crossing perpendicu-

larly) for fd-03 and one series for zd-02 depending on the condition of facility, from the exterior to the interior of the bone. Depth profiles along the bone sections were scanned in front of the beam in precisely recorded steps of about 0.2mm.

Samples were analyzed at the 4W1A hard X-ray microprobe at the synchrotron radiation experimental station of Beijing Synchrotron Radiation Facility (BSRF). Electron energy is 2.2 GeV; intensity is 40 – 100mA; the energy of radiation is 3.5 – 27keV. The reflector is not used. The X-ray irradiation area touching the samples was set by adjustable slits, which were fixed on 50 μm × 60 μm. The Si (Li) detector worked under liquid nitrogen and was placed 10 cm away from the samples with energy resolution about 150 – 350 eV HWFM. The dead time rate of the detector was between 20% and 25%. A 2048 multichannel analyzed (MCA) was used to record and analyze the XRF spectra. The continuous spectrum X-ray in this experiment was adapted to multi-element detection from sodium to uranium with rapid velocity favorable for qualitative analysis of samples. Samples were immobilized on the sample platform about 1 m away from the adjustable slits. The angles between the incident X-ray beam, the sample plane and the detector were 45° and 90°, respectively. The optical microscope was used to adjust the position of the samples. The effective time of X-ray irradiation was 200 s at room temperature. The detail of experiment was also described in the previous reference(Xie *et al.* 2003).

The experiment data were processed by software AXIL. In this study, the absolute concentrations of the element were not evaluated. The relative concentrations of element were shown by the specific emitted fluorescence intensity that is proportional to the concentration of the element of interest. To eliminate errors due to variations in sample thickness or the amount of bone mineral under the beam, the data were normalized to calcium concentration. Hence, the data were expressed as the intensity ratio of the trace element to calcium. One-dimensional map of mercury was obtained by simply plotting the intensity ratio of mercury to calcium.

3 Results and Discussion

3.1 Total level of mercury

The mercury levels in bone tissues were very low. Penguin bone contained only 0.004 $\mu g \cdot g^{-1}$ (Fig. 1). Skua bone had relatively higher levels (0.066 $\mu g \cdot g^{-1}$), approximately 15 times higher than those in penguin bone. The other organs of adult skua had higher levels of mercury than the bone tissue; by a factor of between about 20 times and 80 times depending on the organ (Fig. 1, muscle, 1.01 $\mu g \cdot g^{-1}$; kidney, 3.83 $\mu g \cdot g^{-1}$; liver, 5.08 $\mu g \cdot g^{-1}$) (Torgeir *et al.* 2001).

The levels of mercury in the tissues of seabirds vary considerably in relation to their positions in the food chain, age, gender, species and geographical location(Torgeir *et al.* 2001). For example, it was found that in the skuas, the eggs, nestlings, and adults had levels well above those found in the same organs of petrels since they feed almost exclusively on petrels (Torgeir *et al.* 2001). For age-dependent difference, it is a general trait among birds that adults have higher levels of mercury than young ones (Torgeir *et al*.

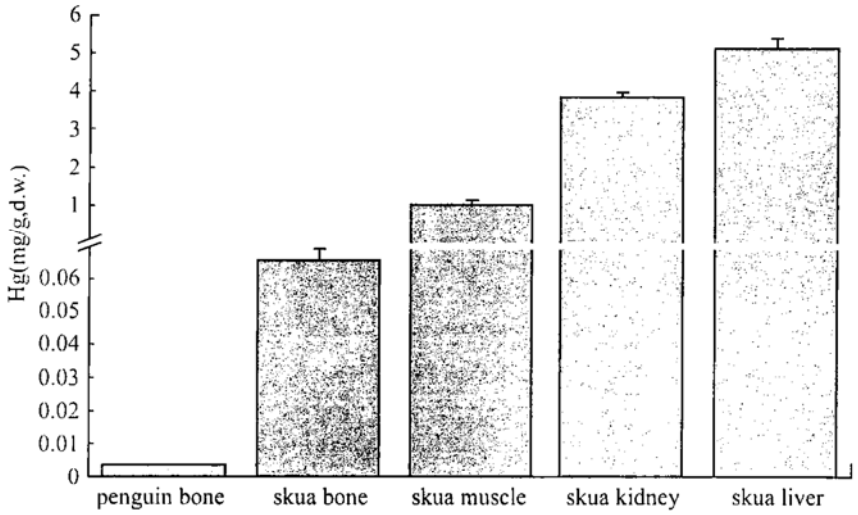

Fig. 1 The total levels of mercury in the tissues of polar seabirds. The data of muscle, kidney and liver of skua were cited from the reference (Torgeir *et al.* 2001).

2001). Both the south polar skua and penguin are long living species, bone tissues may therefore accumulate mercury over a long period of time.

Out of the tissues of seabird, liver and kidney are favorable for mercury storage. During the process of metabolism, mercury commonly transports from the body to plumage and binds to the sulphydryl groups in feather keratin (Torgeir *et al.* 2001). Thus, bone tissues contain markedly low level of mercury in comparison with other organs.

3.2 Microscopic distribution of mercury

Many variables influence the precision, and hence, the detection limits of the SR-XRF technique; these factors include the beam conditions (focused photon flux and incident energy), detector placement, overall background fluorescence from the sample chamber, and target analysis parameters (step size, dwell time) (Twining *et al.* 2003). The precision of SR-XRF technique was previously assessed with repeated analyses of sample (Twining *et al.* 2003). The coefficient of variations (CVs) for detected elements were lower than 10%, indicating that the SR-XRF method has the precision needed for environmental analyses (Twining *et al.* 2003). As with the minimum detection limits (MDLs), the precision can be increased by increasing detector dwell time (Twining *et al.* 2003). The MDLs can be calculated as $3 \times SD$ (standard deviation) of the element concentrations in the blank[13]. In this study, the MDLs of mercury is estimated at approximately 29 ppb, which will be described elsewhere, and the intensities of element lower than $3 \times SD$ were deleted.

The presence of mercury in skua and penguin bone were confirmed directly by SR-XRF analysis. In Table 1 the relative concentrations of detected elements expressed as the intensity ratio of trace element to calcium were listed. The average ratios of mercury over calcium were 0.017, 0.004, 0.002 for samples of skua bone, penguin bone (S1 and S2), respectively. The ratio for skua bone was higher than penguin bone, which was in agreement with the total level of mercury analyzed by AFS. The corresponding CVs were 92%, 39% and

25% , all of which were obviously higher than 10% , suggesting that mercury distribution in bone tissues was unhomogeneous, especially in skua bone. The total level of mercury for penguin bone obtained by AFS is only 4ppb lower than the SR-XRF MDLs. Mercury detectable in the miscroscale with an area of 50 μm × 60 μm indicated that this element be diluted in the whole bone while enrich in small region. The results demonstrated that SR-XRF analysis enables us to find out mercury distribution in microscopic scale despite its low level in total. In addition, in skua and penguin bone, Ca, P, S, K, Fe, Mn, Cu, Zn, Sr, Zr, Br, and As were also detected at the same time.

Table 1. SR-XRF intensity ratio of element to calcium for skua bone and penguin bone (two series S1 and S2). CV represents the coefficient of variance. The dash line in the table represents the elements are not detected

		P/Ca	S/Ca	K/Ca	Fe/Ca	Mn/Ca	Cu/Ca	Zn/Ca	Sr/Ca
	Mean	0.033	0.026	0.021	0.292	–	0.025	0.318	0.665
	CV(%)	111	136	42	170	–	128	90	136
skua(zd-02)		Co/Ca	Ni/Ca	Ti/Ca	Rb/Ca	Zr/Ca	Br/Ca	As/Ca	Hg/Ca
	Mean	–	–	–	–	0.113	0.061	0.04	0.017
	CV(%)	–	–	–	–	145	107	113	92
		P/Ca	S/Ca	K/Ca	Fe/Ca	Mn/Ca	Cu/Ca	Zn/Ca	Sr/Ca
	Mean	0.007	0.002	0.010	0.234	0.001	0.001	0.035	0.123
	CV(%)	6	20	23	109	103	129	54	44
Penguin(fd/S1)		Co/Ca	Ni/Ca	Ti/Ca	Rb/Ca	Zr/Ca	Br/Ca	As/Ca	Hg/Ca
	Mean	0.003	0.002	0.001	0.002	0.008	0.001	0.002	0.004
	CV(%)	88	44	89	50	52	169	37	39
		P/Ca	S/Ca	K/Ca	Fe/Ca	Mn/Ca	Cu/Ca	Zn/Ca	Sr/Ca
	Mean	0.006	0.001	0.010	0.011	0.000	0.001	0.032	0.117
	CV(%)	6	17	33	107	91	138	15	37
Penguin(fd/S2)		Co/Ca	Ni/Ca	Ti/Ca	Rb/Ca	Zr/Ca	Br/Ca	As/Ca	Hg/Ca
	Mean	0.001	0.003	–	0.002	0.006	0.001	0.002	0.002
	CV(%)	85	64	–	39	61	137	33	25

Table 2. Pearson's correlation matrix of mercury with the other detected elements in skua bone and penguin bone

Sample									
Skua (Zd-02,	Hg	0.806*	0.815*	0.931**	0.542	–	0.696	0.883**	0.834*
$n=7$)		Cr	Co	Ni	Ti	Rb	Zr	Br	As
	Hg	–	–	–	–	–	0.824*	0.881*	0.847*
		P	S	K	Fe	Mn	Cu	Zn	Sr
	Hg	0.368	−0.548	−0.031	0.950**	0.821**	−0.044	−0.163	−0.079
Penguin (fd/S1,		Cr	Co	Ni	Ti	Rb	Zr	Br	As
$n=10$)	Hg	0.815**	0.853**	−0.224	0.500	−0.027	0.038	0.493	−0.240
		P	S	K	Fe	Mn	Cu	Zn	Sr
Penguin (fd/S2,	Hg	−0.134	0.575	0.654*	0.706*	−0.238	0.807**	0.452	0.847**
$n=10$)		Cr	Co	Ni	Ti	Rb	Zr	Br	As
	Hg	−0.575	−0.922**	−0.856**	−0.849**	0.868**	0.800**	0.056	0.790**

*: Correlation is significant at the 0.05 level (2-tailed).

**: Correlation is significant at the 0.01 level (2-tailed).

Fig. 2 exhibited the typical patterns of Hg distribution in skua and penguin bone. Fig. 2a showed one-dimensional map for mercury distribution from the site near the periosteal surface to endosteal surface for skua bone. The levels of mercury presented an approximately U-shaped variation, peaking at the site near the periosteum and somewhere near the endosteal surface. Fig. 2b displayed the mercury distribution in two series for penguin bone, which cross perpendicularly. Although U-shaped variation of mercury in penguin bone was not obvious in comparison with the one for skua bone, mercury also enriched somewhere near the periosteal surface and endosteal surface. No general extrapolation or reasoning can be done for this phenomenon now. More works are expected to answer this question.

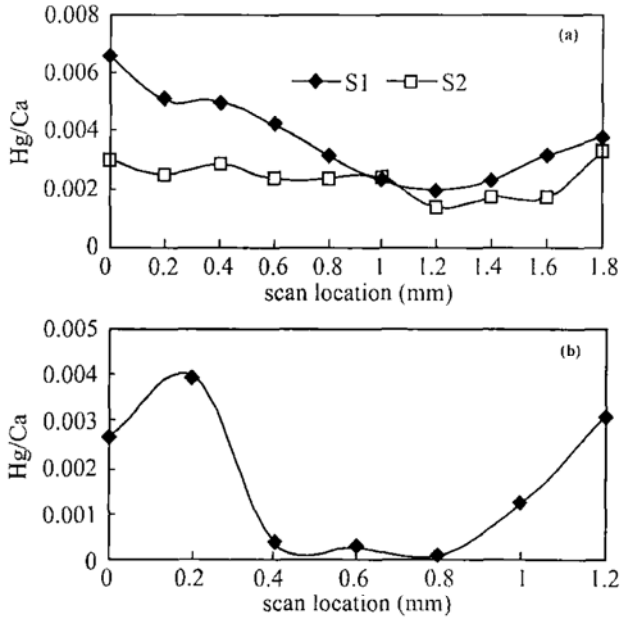

Fig. 2 Relative concentration profile of mercury in the transverse bone of skua bone (a) and penguin bone (b) from the periosteum to the medullary channel.

3.3 The relationship between mercury and other elements

To understand the role of mercury in bone, we thus calculated the relationship between mercury and the other elements and expected to find out some preliminary information. The results were listed in table 2. In skua bone, the level of mercury showed strongest correlation with the ones of Zn, As, P, S, K, Sr, Zr, and Br, indicating that the distribution of these elements in space is similar to mercury, namely presenting U-shape. It is well known that Sr has chemistry similar to that of Ca, which is dominant composition of mineral part of bone, namely biological apatite ($[Ca_{10}(PO_4)_6(OH)_2]$ for normal bone). Inferring from the U-shape distribution, the course of Sr substituting Ca and incorporating into mineral materials seems to notably occur somewhere near the periosteal surface and/or endosteal surface, corresponding to the relative high level of mercury in these area. This might result in the osteoporosis due to the Ca being replaced by Sr. Another notable characteristics is that mercury shows distinctive correlation with S, indicating that mercury might probably bind to sulfhydryl (SH) in bone. This is in agreement with the current understanding of the mechanisms of mercury-induced development toxicity basing on the assumption that mercury acts

by binding to sulfhydryl (SH) groups(Silbergeld *et al.* 2000). In comparison with a simple type of relationship in the skua bone, the relationship of mercury with the other elements in penguin bone was somewhat complex. In series 1, the concentration of mercury only significant correlated with trace element of Fe, Mn, Cr and Co, while in series 2, the one of mercury showed notably correlation with K, Sr, Zr, Rb, Fe, Cu, Co, Ni, Ti and As. In the two series, the relationship between S and Mercury is not observed, differently from the one in the skua bone. It is obvious that the occurrence of mercury poses complicated role in the major and trace elements in bone. More work should be done to obtain direct evidence of the role of mercury in bone metabolism.

4 Summary and Outlook

The total levels of mercury in bone tissues of penguin and skua were somewhat lower than the ones in the other organs. However, SR-XRF method enabled us to find out microscopic distribution of mercury in seabirds in polar region. The levels of mercury are found to enrich somewhere near the periosteal surface and/or endosteal surface. Inferring from the relationship of mercury with the other elements, mercury might probably bind to sulfhydryl (SH) in bone. The role of mercury in bone metabolism is very complicated. The information inferred from the relationship of mercury with the other elements is limited. More work should be done to give a direct evidence for the toxic behavior of mercury in bone tissues (e.g., using SR-XRF to directly determine the sub-species of mercury in osteon).

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