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Full Length Research Paper

The thermoluminescence of carp otoliths: A fingerprint in identification of lake pollution

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This paper reports a pilot study on the thermoluminescence (TL) of carbonate minerals of carp otoliths from the heavily polluted Baiyangdian Lake (BYD) in Hebei Province and non-polluted Miyun Water Reservoir (MY) in Beijing Municipality of China. Analyses on trace elements of otoliths and water show that the heavy metal elements of BYD were much higher than those of MY, particularly on chromium (Cr), nickel (Ni), zinc (Zn) and rubidium (Rb) concentrations. These heavy metal elements could infiltrate into the otolith crystals as impurities or defeats, and were responsible for the different TL parameters and TL glow curves between BYD and MY samples. Such differences in carp otoliths would be applicable to other lakes or water reservoirs, and the TL technique could be used as "fingerprint" in identification of polluted and non-polluted lakes. Also, the TL characteristics of fish otoliths may provide a quick and qualitative means for environmental assessments in lake pollution.

Key words: Thermoluminescence, fish otolith, heavy-metal elements, lake, pollution.

INTRODUCTION

Fish otoliths are widely used as a proxy in determining the environmental temperature of the fish through its lifetime (Devereux, 1967; Ivany et al., 2000; Andrus et al., 2002) because the oxygen isotopic fractionation between otolith minerals and the ambient water is temperature-dependent (Kalish, 1991; Patterson et al., 1993; Gao, 2002). Similar principles and applications have been documented in trace elemental concentrations (Campana, 1999). However, a challenging presupposition of otolith analysis is to determine the historical water chemistry (either $^{18}O/^{16}O$ or elements) to which the fish was exposed. This problem has presented an obstacle to applications in fisheries science because water temperature is believed to affect migration and survival of many fish species, and climate regime shift could be one of the most important factors that should account for the declined fish stocks (Jensen, 1972; Beamish and Bouillon, 1993; Gao and Beamish, 2003). In northern China, many fishing lakes have been polluted by heavy metal elements due to fast urban development and industrial wastewater discharge. Using otoliths as a proxy might help to get a record of the lake pollution over the past one or two decades. However, we were concerned that we had no direct means to measure the chemical compositions of the lake water in the past. Thus some other measurements, such as the physical characteristics of otoliths, has been brought to our attention in recent

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Abbreviations: TL, Thermoluminescence; **BYD,** Baiyangdian Lake; **MY,** Miyun Water Reservoir; **NAA,** neutron activation analysis; **ICP-MS,** inductively coupled plasma mass spectroscopy.

years.

The thermoluminescence (TL) of minerals such as quartz, feldspar and calcite, is a popular technique for analyzing rock genesis, identifying structural processes and aiding in ore deposit prospecting (Li et al., 1996; Liu et al., 2002; Zhu et al., 2004). The TL technique has also been successfully used in the ageing of crystal samples (Miallier et al., 2006), photosynthesis (Rappaport and Lavergne, 2009), food and medical hygiene (Soika C, Delincee H (2000). Thus, if the TL signatures of otoliths can be identified between polluted and non-polluted lakes, this physical characteristic could provide a quick and qualita-tive discrimination on lake status and past environments.

The common carp (*Cyprinus carpio*) was used primarily due to its widely distribution in lakes and water reservoirs in northern China. The ecological spectrum of carp is broad, with an optimum growth in temperature between 21 to 30°C. Carp usually live in lower part or bottom of water layers and their diet includes macrophytes, detritus and algae, molluscs, aquatic insects and their larvae, minute crustaceans, and small fishes (Ye and Zhang, 2002). In general, male carp mature by the second year of life, but females may require an additional year for sexual maturation. Over carp's life history and growth, various elements, particularly heavy metal elements such as chromium (Cr), nickel (Ni), lead (Pb) and zinc (Zn), will incorporate into the crystal structures of calcium carbonate (Li et al., 2008). These impurities and structural defects in crystals, along with environmental temperature changes, will remain in otoliths of carp as a TL record.

The objectives of this study were of two-fold. First, despite a great effort made in otolith studies in fish age determination, population structure, ecological and environmental investigations (Beamish and McFarlane, 1987; Hanson and Zdanowicz, 1999; Gao, et al., 2010; Li et al., 2011; Du et al., 2011), little is known about the TL characteristics of otoliths. This study will represent a pilot effort to measure the TL parameters of otoliths to see if these characteristics were detectable and useful in identi-fying different lakes or water reservoirs. Secondly, if distinct TL signatures of otoliths between polluted and non-polluted lakes did exist, we would be interested in examining if the TL parameters could be used for "fingerprinting" for water quality in lakes or water reservoirs. Since the TL characteristics of biominerals within human ureter concretion and bivalve shell have been reported in the literature (Song et al., 1994; Anderle et al., 1998), the TL technique should be applicable to carp otoliths.

MATERIALS AND METHODS

Experimental sites, Baiyangdian Lake (BYD) and Miyun Water Reservoir (MY)

The Baiyangdian Lake (BYD) is known as a polluted lake in Hebei Province of China. It is located at about 120 km southwest Beijing (Figure 1), and is composed of 143 ponds that cover an area of 366 km^2. It is the largest limnetic lake in northern China and historically named as the "bright pearl". At the end of the last century, unfortunately, a large amount of various metals were dumped into the lake due to the establishment of many small factories around the lake and the fast economic development of the region. According to investigations made in the late 1990s, nearly 80% of the water area is in fertile status, 13% in high fertile and 7% in super-fertile, respectively. Heavy metal element pollution of the water in BYD has been frequently reported (Mao et al., 1995; Wang et al., 1995; Cui, 1999).

The Miyun Water Reservoir (MY) is currently the major drinking water supplier for the people of Beijing City. It is located at about 80 km northeast Beijing and covers an area of about 200 km^2 (Figure 1). Although slightly fertilized, MY was considered as non-polluted and clean enough for human use.

Otolith and water sampling

42 otolith pairs of carp were collected from BYD and MY during the October 2004 and July 2005 sampling season, respectively (Table 1). Each carp has three pairs of otoliths: asterisci, saggitae and lapilli, which are composed of both aragonite and vaterite with 3.7 to 5.2% organic matter (Li et al., 2009). Based primarily on size (Figure 2), we chose asterisi as the main target in the present study. Since the carp otoliths were aged between two to six years (Table 1), this study may represent an average of elemental concentrations.

Otolith pairs were extracted from carp samples, cleaned with deionized water, and kept in ethanol for 24 h. After cleaning the organic matter on the surface, the otolith samples were air-dried at room temperature. In order to test the current lake conditions, a batch of water samples from both BYD and MY were collected in 2004. Water samples were taken with a special collector at 1.5 to 4.5 m from the surface and were then sealed in clean epoxy resin bottles for trace element analyses.

TL and elemental analyses

The TL measurement was performed in China University of Geosciences Beijing. Otolith samples were first ground into 0.25 ± 0.03 mm powders. The TL glow curves of otoliths were obtained by using a FJ-429A1 thermoluminescence instrument. At the first stage, the samples were warmed up from room temperature to 150°C for 5 s, while the second stage began from 150 to 490°C with the calefactive speed of 1°C /s.

The elemental analyses were conducted with two different instruments: using neutron activation analysis (NAA) to analyze otolith samples, and using inductively coupled plasma mass spectroscopy (ICP-MS) to analyze water samples. The NAA analyses were performed at the Institute of High-Energy Physics, Chinese Academy of Sciences. Otolith samples were weighed and wrapped in aluminum foil, and then received irradiation in the reactor. The analytical parameters were: 4.83 × 1013 $n \cdot cm^{-2} \cdot s^{-1}$ for neutron fluence rate; 8 h for irritation time; 8 Ortec HPGe γ for energy spectrum; and 1.80 keV for resolution.

Trace elemental concentrations of water samples were measured by the ICP-MS laboratory of China National Research Center of Geoanalysis at Beijing. At least 50 ml lake water solutions were collected during the sampling program. In order to ensure a representative sample, water samples were collected in pre-cleaned HDPE plastic bottles that have been rinsed with sample three times prior to use. Moreover, for metal elemental analysis the samples were acidified to pH <2 soon after field collection because of the problems of absorption or precipitation. The ICP-MS can

Figure 1. Location map of the Baiyangdian Lake and Miyun Water Reservoir in this study, showing the shape and size of the water bodies in the northern China (a). The inset map (b) illustrates the location of the area in of map China.

provide low detection limits needed to define background and anomalous levels. The major metal elements as analyzed [e.g., Cr, Ni, Pb, Zn, and copper (Cu)] were better than 0.5 ppb.

RESULTS

The crystal structure of carp otoliths (asterisci) showed an irregular and variable microstructure under the scanning electric microscope (Figures 3a to d). Analyses of water samples showed that the heavy metal elements of BYD were markedly higher than those of MY (Table 2), particularly on Cr, Ni, Zn and Rb concentrations. The MY water, however, had much higher silver (Ag) and gold (Au) concentrations than BYD samples, which was hard to explain at present. Although one-time sampling cannot represent the fluctuations of lake water over the period of carp growth, the high Cr, Ni, Zn, and Rb concentrations in BYD might be an indicator for metal pollution.

Results of NAA analysis show that the concentrations of most common elements such as strontium (Sr), barium (Ba), potassium (K) and sodium (Na), did not differ significantly between BYD and MY otoliths (Table 3). However, the heavy metal element concentrations [e.g., iron (Fe), Zn, Cr, Ni, and Rb] of BYD were three to seven times higher than MY otoliths, similar to those displayed in water sample analysis. If we define the initial 300°C as low temperature and the continuous heating to 490°C as high temperature, the TL parameters and TL glow curves of carp otoliths from BYD were different from those of MY (Figure 4; Table 4), particularly on W_1 (peak width for low temperature), W_2 (peak width for high temperature), and I_1 (peak intensity for low temperature) and I_2 (peak intensity for high temperature). Thus the W and I values could be potentially used as key index in the TL glow

Table 1. The background information about carp samples used in this study.

Sample	Length (cm)	Weight (g)	Gender (M/F)	Age (year)	
				Otolith	Scale
BYD-01	36.5	850	M		
BYD-02	41	1125	F		
BYD-03	39.5	900	F		
BYD-04	45	1050	F		
BYD-05	48.3	975	M	3	3
BYD-06	48.6	1000	F	2	2
BYD-07	35	850	M	2	2
BYD-08	31.5	650	F		
BYD-09	49.7	900	F		
BYD-10	40.1	900	F	3	3
BYD-11	38	800	M		2
BYD-12	35	650	F	2	2
BYD-13	36	700	F		
BYD-14	40.1	860	M		
BYD-15	39	800	F		
BYD-16	43.2	1180	F	2	2
BYD-17	41.4	1050	M	2	2
BYD-18	36.6	640	M		
MY-01		1150	M		
MY-02		3400			5
MY-03			M		
MY-04		2150	F		4
MY-05	114	16960	F	10	5
MY-06	67	5250	F		4
MY-07	75	6750	F		6
MY-08	64	4500	F		3
MY-09	68	4725	M	3	3
MY-10	43	1035	F	3	3
MY-11	46	1500	F		2
MY-12	46	1625	M		2
MY-13	49	1975	F		3
MY-14	43	1400	F		3
MY-15	41	1250	F		3
MY-16	42.5	1250	F		2
MY-17	44	1550	M	3	3
MY-18	49	1550	F		3
MY-19	76	7025	F	5	5
MY-20	76	7025	F		4
MY-21	67	4000	M	4	4
MY-22	67	5000	F	5	4
MY-23	50	2400	F		3
MY-24	70	4750	F	6	6

BYD, Baiyangdian Lake; MY, Miyun Water Reservoir; M, male; F, female.

curves of carp otoliths. In fact, the relationship of I, W, S (integral intensity of TL curve) and temperature show weak but clear separation between otoliths from BYD and MY (Figure 5), which is consistent with Figure 4. Overall, these TL parameters and glow curve patterns appeared to show consistence between heavy metal element concentrations and physical characteristics of otoliths in polluted and non-polluted water bodies.

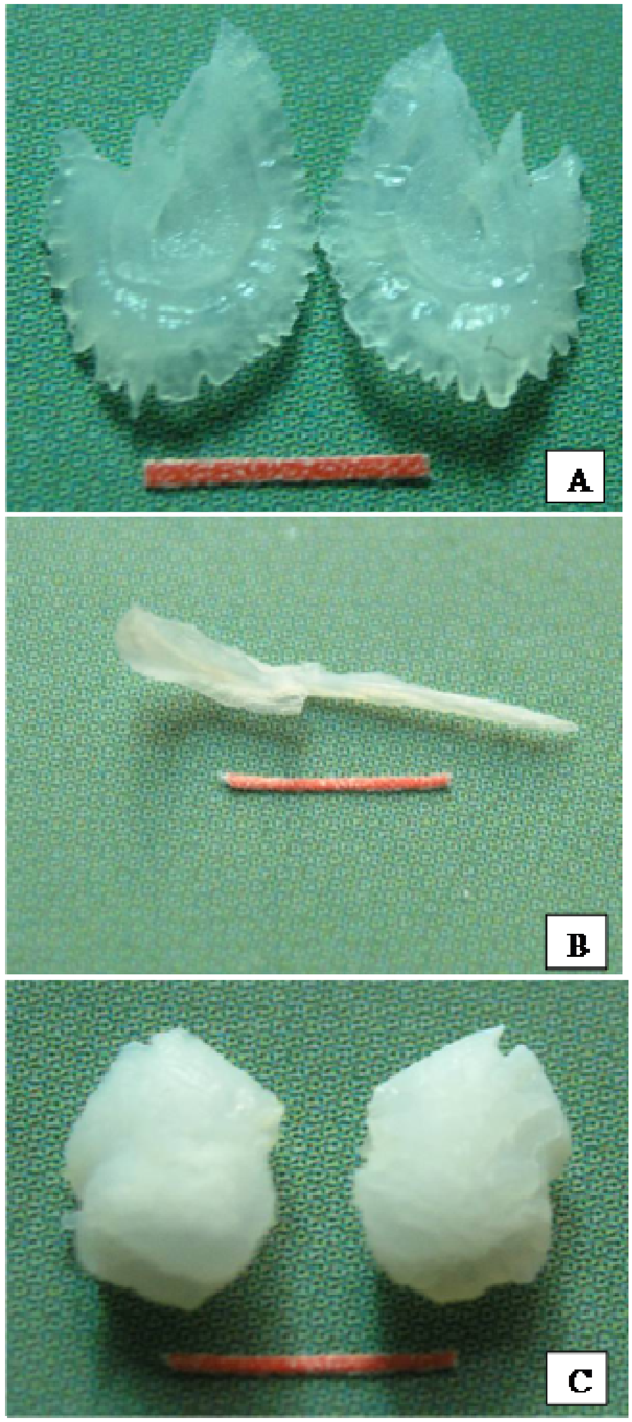

Figure 2. Photos of three pairs of otoliths of carp. (a) Asterisi; (b) Sagittae; and (c) Lapilli. Scale = 5 mm.

DISCUSSION

TL is a physical feature in light release when a dielectric crystal is heated. The phenomenon only occurs during the first heating, and has been successfully applied in geology and antique assessment (Gong et al., 2003; Zheng et al., 2006). In fisheries, Hoff and Fuima (1995) found that the environment can induce variations in elemental composition of red drum (*Sciaenops ocellatus*) otoliths. David and Simon (2001) measured the trace metals in otoliths of juvenile barramundi (*Lates calcarifer*) and suggested that these metals were uptaken from

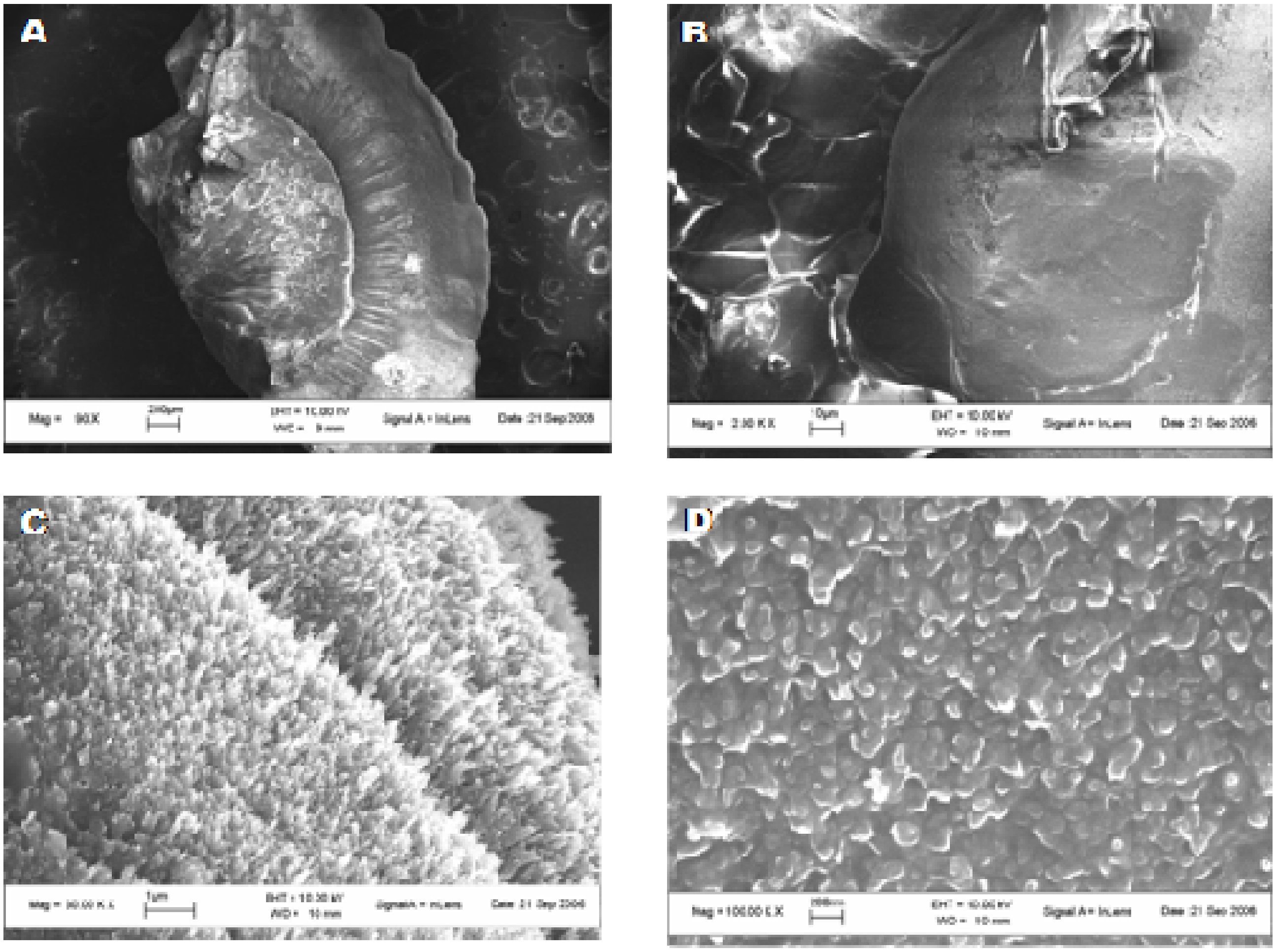

Figure 3. The crystal structure of carp otoliths through scanning electric microscope analysis. (a) The reverse side of asteriscus otoliths showing the microstructure of annual growth increments. (b) The original growth of asterisi, showing irregular and variable microstructure. (c) The surface structure of asterisi after EDTA etching. (d) The original surface structure of asterisi without EDTA etching. EDTA, Ethylene diamine tetra-acetic acid.

water. Norman et al. (2000) investigated the oscillatory zinc distribution in otoliths of Arctic char (*Salvelinus alpinus*) and used it for tracing the environmental origin. These studies indicate that when inhabiting a lake, the fish will consume food along with some polluted matter that includes harmful heavy metal ions such as Zn^{2+}, Pb^{2+} and Cr^{6+}. The heavy metal ions cannot be digested by the fish itself, and will be finally inserted into the otolith crystals as impurities or crystal defeats. The various crystal defects in otoliths form different levels of energy, which will be released in light as heating and the electron jumping from one energy level to another. Therefore, TL is the emission of light from a dielectric crystal when heated, and it is the transformation of the energy levels in crystal from substable state to ground state. In most cases, presence of impurities is considered essential for TL to occur (McKeever et al., 1985). The trap levels and their particle systems with the TL function are called centers of thermoluminescence. The types and concentration of centers in certain mineral crystal depend mainly upon the geological condition during its crystallization process, and the TL characters of minerals are controlled by the characters of the centers and therefore bear important significance of typomorphism (Chen et al., 1996).

Our data in carp otoliths show that some heavy metal element (e.g., Fe, Zn, Cr, Ni and Rb) compositions of BYD were different from those of MY samples. Because the BYD water was heavily polluted with inhomogeneity from pond to pond by the drainage from the surrounding local factories, the higher heavy metal contents are not surprising to the local and scientific communities. The MY water has been strictly protected for many years by the Beijing government, so it is not difficult to understand why the MY samples had lower heavy metal element concentrations. However, our data reveals a connection not only in the relationship between carp otolith and water chemistry, but also in the response between otolith

Table 2. Major heavy metal element concentrations between Baiyangdian (BYD) and Miyun (MY) water samples.

Element	Mean concentration		(Element/Ca) x10^6	
	BYD	MY	BYD	MY
Cr	58.71	1.14	1.077	0.039
Co	0.43	1.4	0.008	0.047
Ni	3	0.02	0.055	0.001
Cu	1.94	2.02	0.036	0.068
Zn	1.47	0.15	0.027	0.005
Rb	6.44	0.01	0.118	0
As	0.69	0.4	0.013	0.014
Ag	0.01	53	0	1.795
Au	0.02	22	0	0.745

Cr, chromium; Co, cobalt; Ni, nickel; Cu, copper; Zn, zinc; Rb, rubidium; As, Ag, silver; Au, gold.

Table 3. NAA analyses of elemental concentrations between Baiyangdian (BYD) and Miyun (MY) otoliths of carp.

Element	Mean concentration		(Element/Ca) x10^3	
	BYD	MY	BYD	MY
Sr	480	159.4	10.87	3.66
Ba	11.65	6.05	0.26	0.14
K	346	352.44	7.84	8.09
Na	1174	901.2	26.6	20.69
Fe	73.57	16.07	1.67	0.37
Zn	40.3	5.72	0.91	0.13
*Cr	291.93	131.5	0.007	0.003
*Co	25.71	8.84	0.001	0
*Ni	3431	1801	0.078	0.041
*Rb	695.14	263.8	0.016	0.006
*Mo	85.85	63.62	0.002	0.001
*As	122	32.31	0.003	0.001
*Ag	82.62	49.72	0.002	0.001
*Au	1.14	0.41	0	0

The elements with mark * are in ppb, whereas elements without marks are in ppm. Sr, strontium; Ba, barium; K, potassium; Na, sodium; Fe, iron; Zn, zinc; *Cr, chromium; *Co, cobalt; *Ni, nickel; *Rb, rubidium; *Mo, molybdenum; *As, arsenic; *Ag, silver; *Au, gold.

chemistry and TL characteristics. From Table 4, the TL parameters of BYD otoliths are distinctly different from those of MY, and the correlation of these parameters could isolate the samples between BYD and MY (Figure 5). Previous studies reported that TL measurements are highly sensitive to trace impurities within dielectric crystals (McKeever, 1985), and our results made it clear that the trace elements (especially heavy metal elements Cr, Ni, Zn, and Rb) in the carp otoliths play an important role in affecting the TL parameters. The high and inhomogeneous concentrations of trace elements in BYD otoliths yielded high and more variable TL parameters, while the low and homogeneous concentrations of trace elements in MY samples yielded small and less variable TL parameters. If such identification was applicable to other lakes or water reservoirs, the TL technique in carp otoliths would therefore be used for "fingerprinting" as a quick and qualitative means in identification of polluted and non-polluted lakes in northern China. Due to the life history of common carp (Table 1), the TL of this study only represents a short period (two to six years) for BYD and MY; however, the technique could be extended to longer term if we had older fish samples.

It should be pointed out that the TL parameters of carp otoliths originated from the different conditions of lake water: the heavy metal polluted BYD versus non-polluted MY water, thus resulting in impurities and crystal defeats. Geffen (1998) discussed two main ways of incorporation of trace metal elements in otoliths: either as a substitute for calcium (Ca^{2+}) or by being entrapped within the crystal

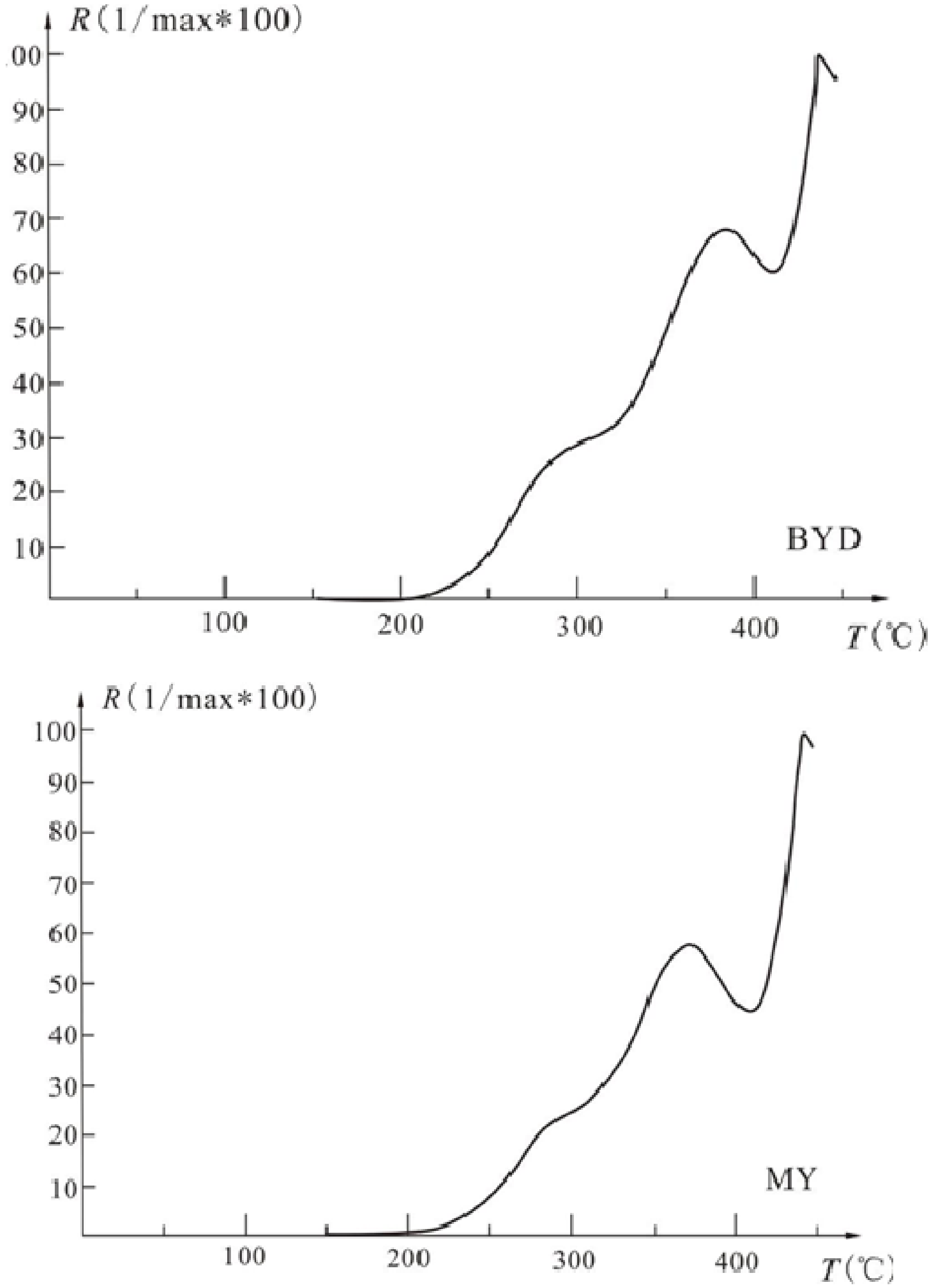

Figure 4. A typical TL glow curve from Baiyangdian (BYD) and Miyun (MY) otolith samples, respectively.

lattice as crystal inclusions. Most common elements such as Sr^{2+} and Ba^{2+}, should be incorporated in otoliths as the substitutes for Ca^{2+}; however, this is not the focus of TL technique in the present study. Instead, our data suggest that only heavy metal elements (mostly Cr^{6+}, Ni^{2+}, Zn^{2+} and Rb^{+}) were responsible for the differences in TL parameters, TL glow curves and energy gap of carp otoliths, which agrees well with the biological investigations on contamination and eutrophication between BYD and MY.

In summary, the TL parameters and glow curves of carp otoliths showed a clear difference and separation between BYD and MY samples, suggesting that the TL technique can be potentially used as a fingerprint in identification of polluted and non-polluted lakes. The heavy metal element pollution of lake water could be

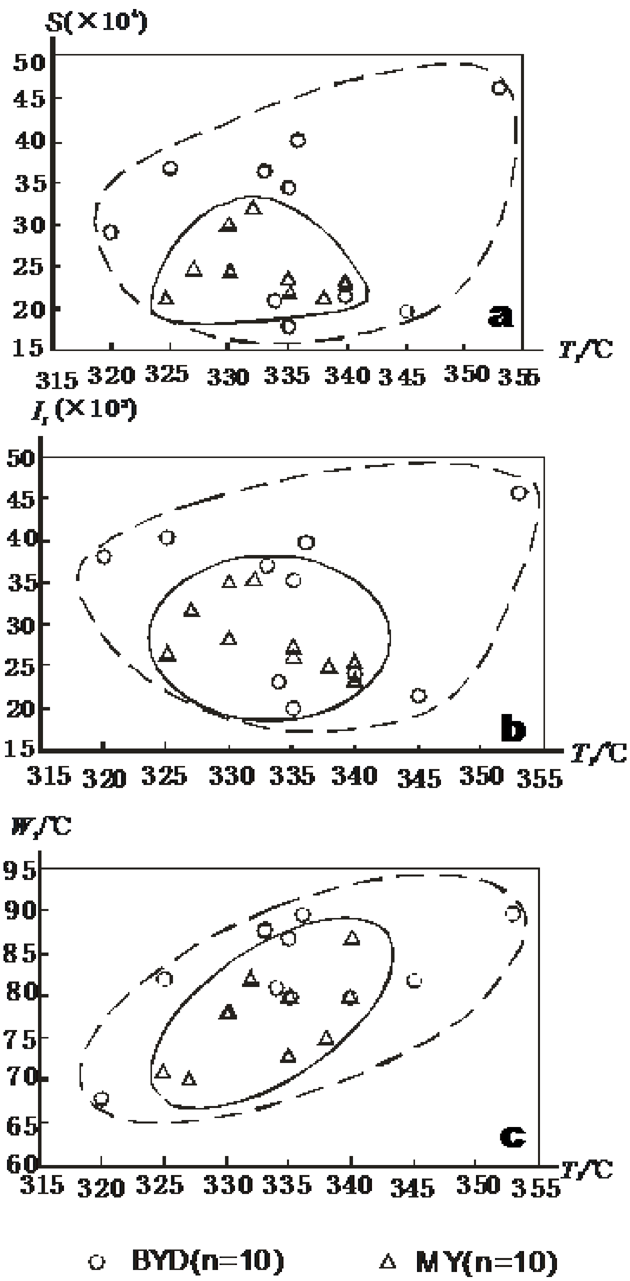

Figure 5. Major TL parameters of carp otoliths between Baiyangdian (BYD) and Miyun (MY) samples. (a) I vs. T; (b) W vs. T; and (c) S vs. T. TL, Thermoluminescence; W, peak width for temperature range; I, peak intensity for temperature range ; S, integral intensity of TL curve. T, temperature.

responsible for the trace elemental concentrations in otoliths of carp, which resulted in the emission of light from impurity or defeated otolith crystals.

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