

Full Length Research Paper

Enrichment of Pb, Hg and Cr in cultured carp otolith

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Fish otolith is a good recorder of water environment in which the fish lived. In order to identify the elements in otolith that are sensitive to environment and their enrichment rules, lab-culture experiments of carps were carried out. Pb, Hg and Cr were added separately into tanks in which the carps lived, and the tanks were named as Pb tank, Hg tank and Cr tank, respectively. Inductively coupled plasma mass spectrometer (ICP-MS) was used for measuring the concentrations of Pb, Hg and Cr in otolith and water. ICP-MS results of otoliths showed that the average, median, quartile and extreme values of Pb, Cr concentrations in otoliths obtained from Pb and Cr tanks are comparably higher than those obtained from other tanks. It showed that Pb and Cr in carp otoliths could respond to environment change as environment response sensitive elements can also be suitable for monitoring water pollution.

Key words: Otolith, inductively coupled plasma mass spectrometer (ICP-MS), heavy metal elements.

INTRODUCTION

Heavy metals are toxic substances that can induce water pollution. Even trace amount of heavy metals are very toxic, especially some substances with biological toxicity like Pb, Hg and Cr. They cannot be degraded by microbes, and can only be converted, dispersed or transferred among different forms. Heavy metals can be converted to certain organic metal compounds under microbial activity, and even be more toxic. The organic metal compounds are enriched by aquatic organisms, and enter human body through food chain. Therefore, heavy metal pollution has become an important research issue in recent years.

Physical-chemical analysis is an important method for monitoring the heavy metal pollution in water. However, water samples are polluted easily in the gathering process and the water monitoring data is often influenced by tide, season, rainfall, etc. Also, this monitoring method can not reflect the accumulation process of heavy metals because the concentration data of heavy metals just record the pollution condition at the sampling time. Recently, some international organizations and environmental protection organizations have cognized physical-chemical analysis cannot fully reflect the harmful that the

information and monitoring data simply based on degree of pollutants on organisms including human being. Thereby, the heavy metal monitoring method based on monitoring aquatic organisms is more and more attractive because it overcomes some limitations of traditional physical-chemical analysis.

Choosing a suitable aquatic organism as indicator is very important (Li et al., 2008). In these years, the chosen aquatic organisms are aquatic algae, zooplankton community and zoobenthos. Otolith is a new choice for this biological monitoring method (Thorrold, 1998; Anadon et al., 2002; Bacon et al., 2004; Gao et al., 2010; Li et al., 2011a; Li et al., 2011b; Du et al., 2011). Otolith is a kind of calcium carbonate biomineral that grows in the inner ear of teleost. There are three pairs of otoliths: asteriscus, lapillus and sagitta. Compared with conventional organism indicators mentioned above, otolith has advantages such as clear daily increments that can record some information accurate to year, season, month and even to single day (Pannella, 1971; Toshiyuki et al., 2003; Beatriz and Jacques, 2005). The elements deposited in the growth layers of otolith cannot be changed, absorbed or restructured (Campana, 1983; Campana and Neilson, 1985; Gao, 1997). The morphology and chemical composition of daily formed increments are very stable; they record the abundant biology-physics-chemistry information of water environment. The information from otolith reveals the living

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Table 1. The designed concentration of elements (ng/ml).

Container	*08-12	09-03	09-04	09-05	09-06
Pb	30	50	150	200	250
Hg	0.5	2	3	4	5
Cr	--	200	300	400	500
Blank	--	--	--	--	--

ND, Not detected. Date*

history of fish and the surrounding environment, the elements deposited in otolith can be considered as chemical indicator. Studies have proved that some elements in otolith can reveal certain characters of water environment (Campana, 1999; Hanson and Zdanowicz, 1999; Quinn et al., 1999; Weber et al., 2002; Wells et al., 2003 Gao et al., 2010; Li et al., 2011a; Du et al., 2011). There are only few studies on trace elements in otolith, and comparably, more on element such as Ca, Sr, K, Na and Ba than on heavy metal elements. Besides, most of the samples are gathered from nature, easily influenced by factors like location, water data, environment temperature, food resource, etc. Therefore, simple lab-research mode is meaningful: the factors above can be avoided by controlling experiment parameters, especially for enrichment elements, which are suitable for water environment evaluation.

In order to reveal the process by which trace elements enter otolith from water, and the relationship between element concentration in water and otolith, Pb, Hg and Cr were chosen as marker elements, lab-cultured carp experiment was carried out with controlled Pb, Hg and Cr concentration, while trying to identify whether these elements responded to water variation, and their deposition modes in otolith. Carp is a widely distributed fish in fresh water, which makes comparison and application easy to spread.

MATERIALS AND METHODS

Carp samples were bought from pet carp cultivation center in Beijing, China. Four tanks with size of 90 × 80 × 60 cm and filtration systems were set up in the laboratory. Pb, Hg and Cr were added into 3 tanks, respectively and a tank without element was used as the control group.

Hg(NO₃)₂, Pb(NO₃)₂ and Cr(NO₃)₃ solutions were added to water respectively to obtain solution with certain concentrations of Hg²⁺, Pb²⁺ and Cr³⁺. The concentrations of different ions were adjusted periodically. Thirty carps were cultured in each tank for 7 months; food (frozen crism worm), temperature and light were controlled the same way in all tanks. The carps were then dissected, lapillus and asteriscus were gathered, then dipped in 75% alcohol solution and distilled water for 24 h each, and dried in air.

Table 1 shows the designed concentration of element in each tank, the elements concentration were designed according to the water quality national standards, and adjusted by fish adaptive capacity.

The element concentration in otolith and corresponding water was tested by X-series inductively coupled plasma mass

spectrometer (ICP-MS). The test parameters included: In 20000 CPS/ppb, precision: 1.5%RSD, background noise: <0.5 cps (220 amu), SNR: >120000000, short-term stability: <1.5% RSD, long-term stability: <3% RSD.

RESULTS

Analysis results of elements in tank water

Water samples were gathered from tanks every 15 to 40 days and the element concentrations were analyzed. Table 2 lists the water element concentrations in tanks at different experimental stage. Tank type and sampling time are given in sample names. Figure 1 also shows the comparison between the actual concentrations of each tank and the designed ones: the Pb element was the best controlled one, the Pb concentration in Pb tank water showed a good increasing tendency. For Cr element, the control was not as good as Pb, Cr concentration did not match well as the designed one, but Cr concentration in the Cr tank was significantly higher than the other three tanks, which provided a sharp contrast for Cr element concentration analysis. For Hg element, the element concentration was controlled well for the first three months, but then suddenly dropped in the data of 090429Hg, but Hg concentration was comparably much lower than Cr and Pb elements, the point of discontinuity might be measurement error but more likely caused by purification cotton absorption.

According to Table 2, the real element concentrations did not match well with the designed ones, the possible reason is that inorganic ions (Cl⁻, CO₃²⁻ and OH⁻) and organic radicals (corrosions) could react with heavy metal ions and create insoluble complex or chelates, and then be adsorbed by purification cotton in tanks, so the element concentrations are unable to be controlled precisely as designed. Even though, the element concentrations in tanks with corresponding element addition were still significantly higher than the other tanks, Pb concentration in Pb tank was 1 to 2 times higher than the others. Hg concentration in Hg tank was 1 to 7 times higher than the others, and Cr concentration in Cr tank was higher than the others for two orders of magnitude. The differences in element concentration could meet the requirements for studying the deposition rules of elements in otolith under the artificial control of

Table 2. ICP-MS results of the element concentrations in tanks (Unit: ng/ml).

Water sample	Pb	Hg	Cr	Water sample	Pb	Hg	Cr
Blank-081216	ND	0.49	2.786	Pb-081216	ND	0.326	2.747
Blank-090311	0.411	0.783	2.686	Pb-090311	32.35	0.333	3.19
Blank-090327	0.461	ND	0.854	Pb-090327	51.64	ND	0.875
Blank-090429	1.095	ND	0.954	Pb-090429	176.8	ND	1.155
Blank-090528	1.055	ND	1.128	Pb-090528	270.6	ND	1.103
Blank-090622	0.534	0.35	0.875	Pb-090622	200.3	0.354	0.869
Hg-081216	ND	0.314	2.886	Cr-090311	0.73	0.268	220.1
Hg-090311	0.779	2.263	3.81	Cr-090327	0.598	ND	205.4
Hg-090327	1.115	2.865	1.027	Cr-090429	0.598	ND	157.7
Hg-090429	6.589	0.715	1.487	Cr-090528	0.562	ND	523.4
Hg-090528	1.871	1.213	1.572	Cr-090622	0.133	0.429	249.1
Hg-090622	2.054	1.343	1.578				

ND, Not detected.

water environments.

Analysis results of elements in otoliths

Table 3 gives the ICP-MS analysis results of Pb, Hg and Cr in otolith. Tank type and sampling time are given as sample names. Seven, seven, seven and five samples from the blank, Pb, Hg and Cr tanks were tested, respectively. Figure 2 shows the variation trend of the three elements in the otoliths from the four tanks.

For Pb element, two samples from blank tank, four from Cr tank and six from Hg tank were not detected. For Hg element, only four samples from Hg tank and two from Pb tank were higher than the limit of detection. The Cr concentrations in almost all otolith samples from all tanks were higher than the ICP-MS detect limitation, only one sample from Hg tank was not detected (lower than the ICP-MS detect limitation).

Table 4 shows the mean and standard deviation of Pb, Hg and Cr concentration of otolith samples from each tank. And it reveals that concentration of Cr from the Cr tank is significantly higher than those from other tanks, and so does Pb element. But for otolith from the blank tank and the Cr tank, Hg was not detected, and three samples from the Hg tank and five samples from the Pb tank were also ND (not detected). The few samples of Hg element cannot show that Hg concentration in otolith samples from Hg tank was higher than those from other tanks.

Figure 3 shows box diagrams of the concentration distribution of Pb, Hg and Cr element in the blank, Cr, Pb and Hg tanks, the data is gotten from the statistics with SPSS10.0 on ICP-MS results of otolith elements, and represent the minimum (the lowest line), the 25% (the

bottom of the box), the median (the middle line in the box), the 75% (the top line of the box) and the maximum (the highest line). The Cr enrichment in each tank, the minimum, quartile, median and maximum of Cr concentration in otolith samples from the Cr tank are significantly higher than those from other tanks, Pb element share the same feature (Figure 1b). This mean Cr and Pb elements were enriched in otoliths.

DISCUSSION

Multiple experiment results show that many kinds of aquatic animals could accumulate pollutants to a certain extent, and such extent is different for different animals, not all kinds of animals could be used as indicating animal. Oyster and shell are the most widely used organisms for indicating heavy metal pollute in ocean. But, normally, soft tissues in organisms are used for monitoring heavy metal content in sea water, and heavy metal accumulation in soft tissues is influenced by physiological processes and outside environment. Heavy metal accumulation data from monitoring could vary markedly with time and space. In fresh water monitoring, types and contents of fresh water organisms are usually used for deciding water pollution and extent (such as alga and crustacean, normally for organic pollution).

The basic requirement for an indicating animal is that such animal should be able to accumulate heavy metal from the environment, and such accumulation is relevant to the heavy metal concentration from environment. The ideal indicates that animal should also: (1) be easy to be recognized, rich in quantity and widely distributed, (2) live long, easy to be gathered and cultured and studied in laboratory, (3) have large body, enough tissue for

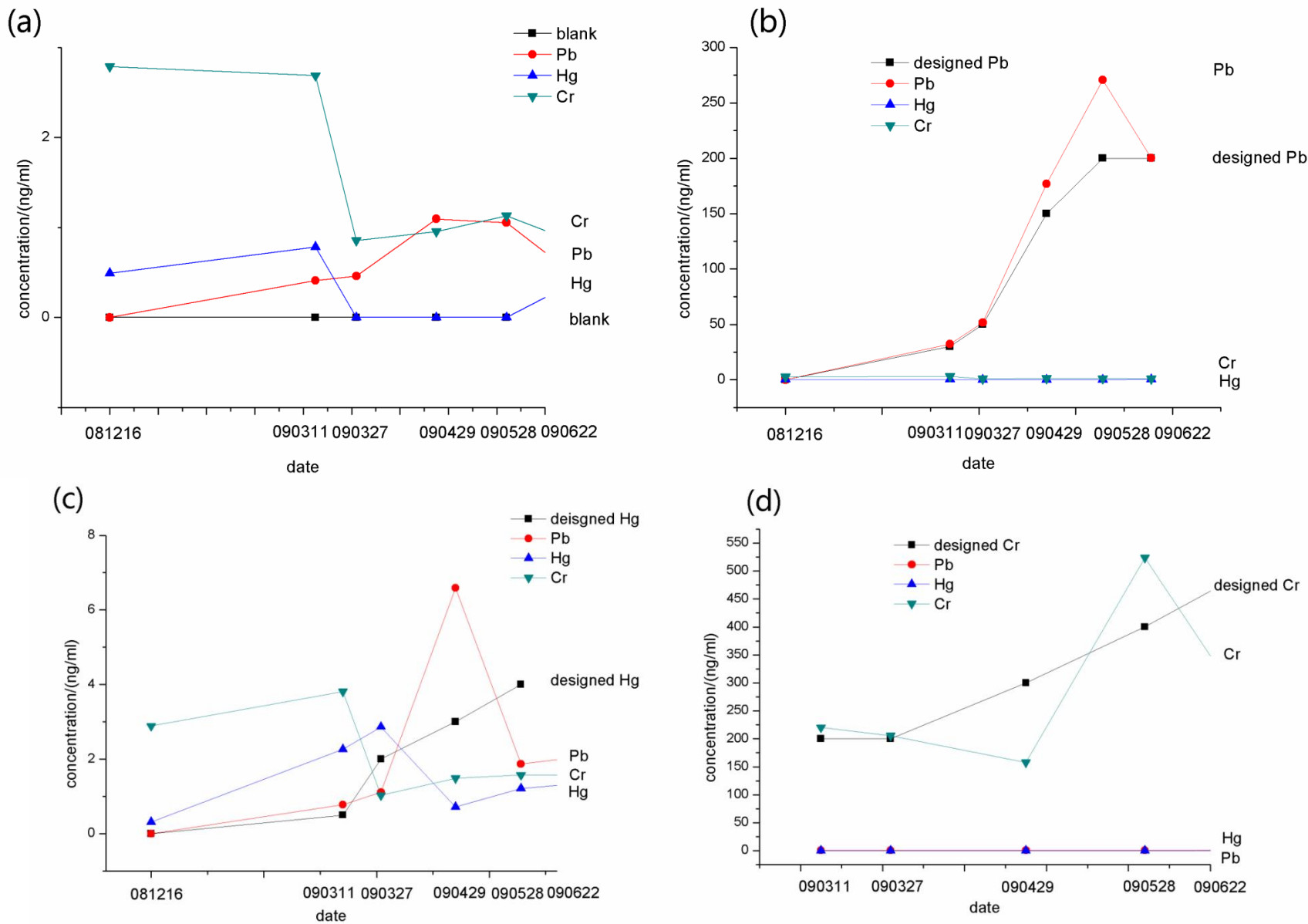


Figure 1. The element variation in water of the four tanks: a) Blank tank; b) Pb tank; c) Hg tank; d) Cr tank.

Table 3. ICP-MS results of the element concentrations in otolith (Unit: ng/mg).

Container	Pb	Hg	Cr
090421-blank	0.355422	ND	0.759036
090526-blank	0.158252	ND	0.278641
090601-blank	0.256818	ND	0.201136
090603-blank	0.238095	ND	0.327381
090618-blank	0.110204	ND	0.329592
090619-blank	0.365625	ND	0.341667
090620-blank	ND	ND	0.270093
090517-Pb	0.352778	ND	0.505556
090521-Pb	0.385263	0.964211	0.927368
090526-Pb	0.260526	ND	0.475
090618-Pb	0.48866	ND	0.145361
090619-Pb	0.554878	0.95	0.270732
090621-Pb	0.698246	ND	0.325439
090622-Pb	0.538554	ND	0.485542
090504-Hg	ND	ND	0.396203
090511-Hg	ND	ND	0.368235
090517-Hg	0.52069	0.504598	0.503448
090531-Hg	0.121212	0.432323	0.166667
090609-Hg	ND	0.931002	1.189524
090612-Hg	ND	0.3248	ND
090621-Hg	ND	ND	0.3008
090407-Cr	ND	ND	0.536066
090510-Cr	0.134211	ND	0.511842
090514-Cr	ND	ND	0.632727
090604-Cr	ND	ND	0.523944
090622-Cr	ND	ND	0.592188

ND, Not detected.

separation and analysis, (4) have strong vitality, high durability to high heavy metal concentration and a wide heavy metal concentration in environment. From the reasons mentioned above, otolith as a new water chemical "indicator" can fulfill almost all the acquirements. The most highlighted character of otolith is its delicate growth ring structure, which is convenient for ring (time) separation analysis. The results show that carp otolith can response to Cr and Pb sensitively, which makes it possible for the use of otolith to evaluate water chemical conditions. And one of the most important function is to identify the relevance of sensitive element in water and otolith. With the solving of this problem, a new evaluation method could be put together to evaluate chemical characteristics of close lakes for a period of time.

One of the authors (Gao et al., 2008) and other researchers (Gao et al., 2010; Li et al., 2011a) successfully tested 33 elements in otolith of wild carp in

environment factor change, (5) the accumulation in organisms should be simple and relevant to average Heilongjiang, China with the neutron activation method, and they were Ag, As, Au, Ba, Ca, Ce, Co, Cr, Cs, Eu, Fe, Hf, K, La, Lu, Mo, Na, Nd, Ni, Rb, Sb, Sc, Se, Sm, Sr, Ta, Tb, Th, U, W, Yb, Zn and Zr (Pb and Hg could not be tested by neutron activation), and this mean many elements were involved in otolith formation and growth. For freshwater fish, only a few elements were gotten from food resources, most of the other elements were gotten from fish gill, which means most of the elements may possibly be adsorbed from water directly, and then transferred by cells into endolymph, and finally deposited in otoliths.

Element balance between fish and water was gotten with a long process, but such balance might be heavily broken by water pollution. Fish could isolate or block unnecessary elements in abnormal metabolism and deposit them in otolith, to avoid them damaging the

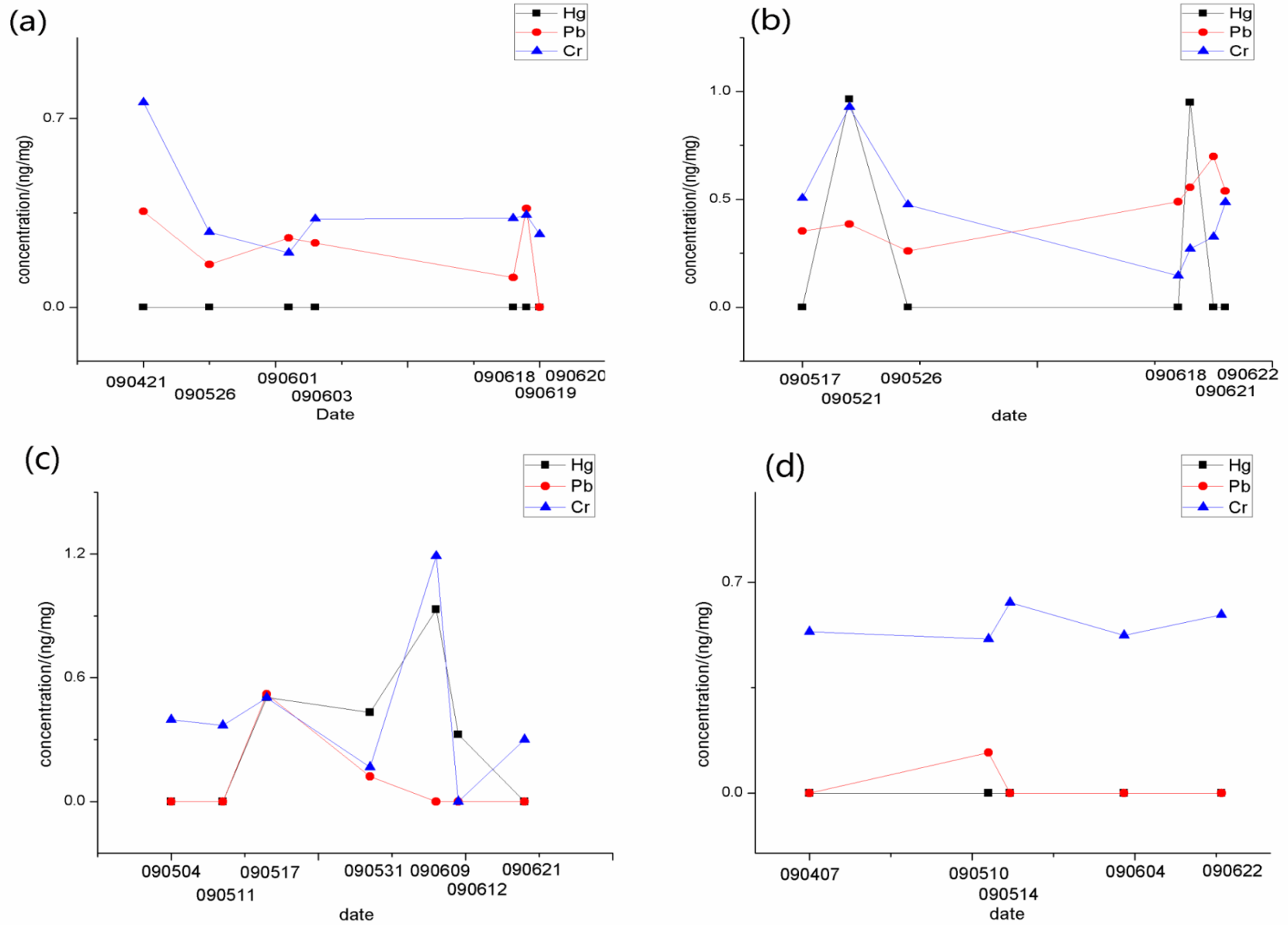


Figure 2. The element variation of the otoliths from the four tanks: a) Blank tank; b) Pb tank; c) Hg tank; d) Cr tank.

Table 4. Statistic data of element concentration of otolith samples in each tank.

Container	Pb (Mean)	Hg (Mean)	Cr (Mean)
Blank Container	0.247403	--	0.364311
Cr Container	0.134211	--	0.559353
Hg Container	0.264801	0.548181	0.452165
Pb Container	0.456908	0.957106	0.415062

ND, Not detected.

normal biophysical processes (Luo et al., 2008). The process of element entering otolith from water could be influenced by many factors: water temperature, salinity, food resources, physiological change, and there are also synergism and antagonism among these factors, which leads to a very complicated micro-biological environment. Among the factors above, element concentration in water is the most significant one.

The ways by which elements enter otolith could be different; the lattice space between Ca ions decides the ion replacement types: ion replacement or co-precipitation. Element can stay in three states in the otolith: (1) replace Ca ions by isomorph, and the radius of these elements are always smaller than Ca^{2+} , (2) stay in interstitial voids as inclusions and (3) combine with polypeptide chains of protein (Campana, 1999).

The distribution of impurity elements could be explained as the dispersion of solute in solvent. The impurity defect concentration depends on the content of impurity ions, and not related to temperature. Normally, when the characteristics of chemical bonds are similar, and $(R_{\text{big}}-R_{\text{small}})/R_{\text{big}} < 15\%$ (R_{big} and R_{small} represent the replace ions with big and small radius), ion replacement is more likely to happen. When the radius of replacement ions and host ions are almost the same, the resultant is more stable. While when $(R_{\text{big}}-R_{\text{small}})/R_{\text{big}} > 30\%$, ion replacement is unlikely to happen, and impurity ions are more likely to stay in the interstitial voids. The radius of Ca^{2+} is 1.00, 0.62 for Cr^{3+} , 1.19 for Pb^{2+} , 1.02 for Hg^{2+} . $(R_{\text{Ca}}-R_{\text{Cr}})/R_{\text{Ca}}=38\% > 15\%$, $(R_{\text{Pb}}-R_{\text{Ca}})/R_{\text{Pb}}=15.96\% > 15\%$, $(R_{\text{Hg}}-R_{\text{Ca}})/R_{\text{Hg}}=1.96\% < 15\%$. Since the electrovalences of Hg^{2+} and Ca^{2+} are the same, and their ion radius are similar, it seems that Hg^{2+} could replace Ca^{2+} in calcium carbonate crystals theoretically. The electrovalences of Cr^{3+} and Ca^{2+} are different, and their radiuses are quite different, Cr^{3+} would normally stay in interstitial voids. And when the electrovalences of Pb^{2+} and Ca^{2+} are the same, and their radius are very similar, Pb^{2+} might replace Ca^{2+} in CaCO_3 crystals, and form stable resultant.

Researchers also found that metal binding proteins (peptides) with metal affinities exist in many kinds of microbes, cells of animals and plants, and they could react with metal ions and form composites that would

enrich, reduce or eliminate metal ion toxicity for cells (Xu et al., 2004). In 1957, Margoshes and Vallee studied natural structure proteins in horse kidney and found that MTs (metallothioneins) could bind metal ions and transfer to other proteins, control trace element concentration in cells, protect cells from heavy metal damage, etc. (Chan et al., 2002). There is 5% wt organic matrix in otolith, and such MTs might exist in the matrix, and bind certain heavy metals, making it easier for them to enter into the otolith.

In the water evaluation system, chemical assessment only provides the types and concentrations of the pollutants, without their hazard extent, and cannot reveal the joint effect of multiple pollutants, while biologic assessment can reveal the comprehensive pollution situation. Heavy metal pollution is durable, easy to be enriched and accumulated in marine animals. In natural water, the heavy metal content is low, and often fluctuates and influenced by bioavailability; the heavy metal enrichment is a more suitable indication for heavy metal pollution than the direct concentration of heavy metal in the environment (Roditi, 2000).

Conclusion

The element analysis of carp otolith cultured in designed water shows that the median, quartile, minimum and maximum concentration of Cr and Pb in otolith from the Cr and Pb tanks are significantly higher than the other tanks, which proves that Cr and Pb in otolith could respond to water environment and is enriched in otoliths. Cr and Pb are sensitive elements to environment in the deposition process, and can be used to rebuild water condition. Testing Cr and Pb concentration in otoliths could support water environment monitoring.

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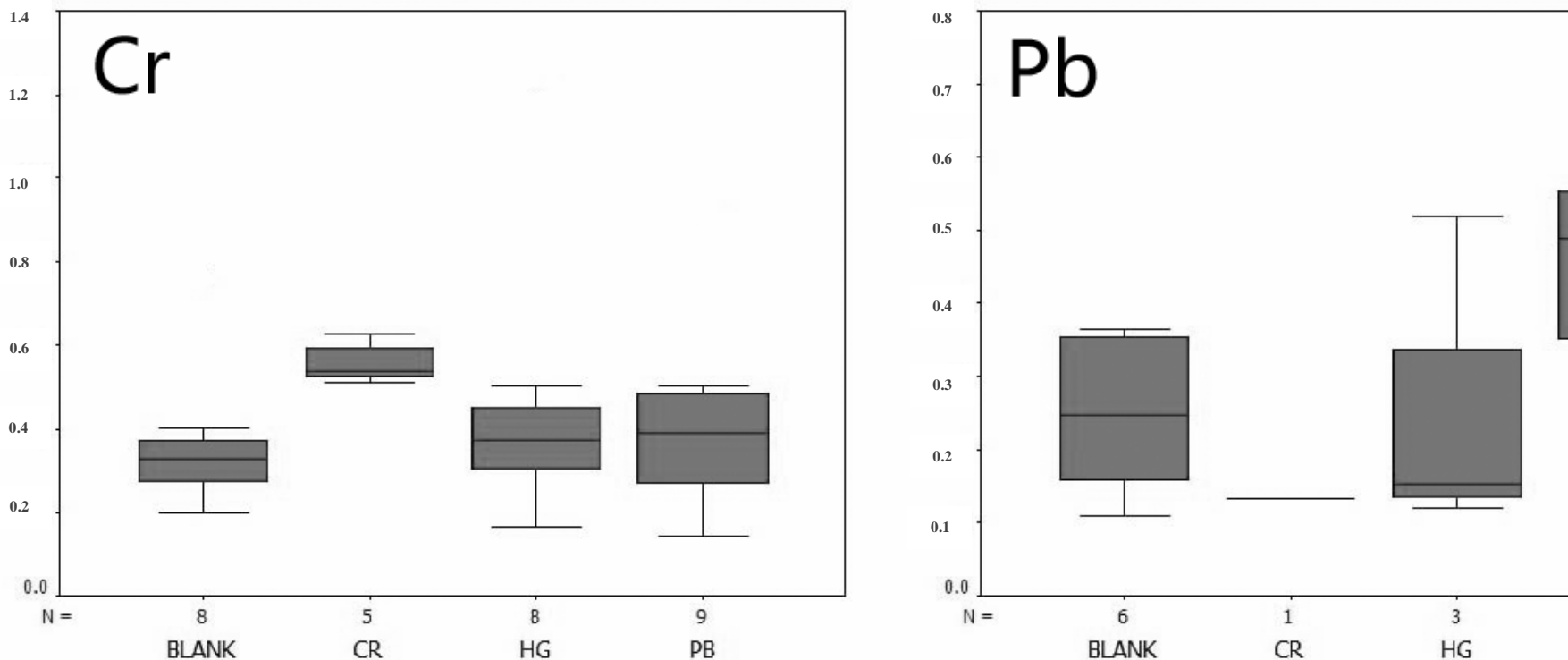


Figure 3. The box plot of Cr and Pb element concentration distribution of otolith in each tank.

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