Environ Monit Assess (2007) 128:483–488
DOI 10.1007/s10661-006-9341-3

Contaminants-Induced Oxidative Damage on the Carp *Cyprinus carpio* Collected from the Upper Yellow River, China

D. J. Huang · Y. M. Zhang · G. Song · J. Long · J. H. Liu · W. H. Ji

Received: 13 November 2005 / Accepted: 14 June 2006 / Published online: 16 December 2006

Abstract The Yellow River, the second largest river in China, is the most important resource of water supply in North China. In the last 40 years, even in the upper Yellow River, with the development of industry and agriculture, more and more contaminants have been discharged into this river and greatly polluted the water. Although a routine chemical component analysis has been performed, little is known about the real toxic effects of the polluted water on organisms at environmental level. To explore whether the pollutants induced oxidative stress and damage to aquatic organisms, malondialdehyde (MDA) level and activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) in hepatopancreas, kidney and intestine of the field-collected carp *Cyprinus carpio* from a mixed polluted (Lanzhou Region, LZR) and a relatively unpolluted (Liujiaxia Region, LJXR) sites of the upper Yellow River were measured. The results showed that when the values of LZR compared with those of LJXR, SOD and GST activities increased and GPx activity decreased significantly in all the three organs ($P < 0.05$–0.01); CAT activity decreased but MDA level increased significantly ($P < 0.05$–0.01) only in kidney and intestine. In conclusion, the results of this study suggest that the pollutants can induce obvious oxidative damage in the carp, and the SOD, GST and GPx might be better indicators for the oxidative damage in aquatic organisms.

Keywords antioxidant enzyme · contamination · *Cyprinus carpio* · fish · MDA · oxidative damage · the Yellow River

D. J. Huang · Y. M. Zhang (✉) · G. Song · J. Long · J. H. Liu
School of Life Sciences, Lanzhou University, Lanzhou 730000, People's Republic of China
e-mail: ymzhang@lzu.edu.cn

W. H. Ji
Institute of Natural Resources, Albany Campus, Massey University, Albany, Auckland, New Zealand

1 Introduction

Many pollutants can result in some degree of oxidative damage by generating free radicals and/or altering antioxidant enzyme systems which scavenge reactive oxygen species (ROS). Antioxidant enzymes, such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.16), glutathione peroxidase (GPx; EC 1.11.1.9) and glutathione S-transferase (GST; 2.5.1.18), may protect the organisms from adverse effects of ROS (Ahmad, Hamid et al., 2000; Hayes & Strange, 1995). A number of reports have documented that the antioxidant enzyme activities can be affected by heavy metals (Berntssen, Aatland, & Handy, 2003; Shi, Sui, Wang, Luo, & Ji, 2005), pesticides (Oruc, Sevgiler, & Uner, 2004; Oruc & Uner, 2002; Sayeed et al., 2003), phenols (Zhang, Liu et al., 2005; Zhang,

Shen, Wang, Wu, & Xue, 2004) and oils (Zhang, Shen, Xu et al., 2003; Zhang, Wang, Guo, Wu, & Xue, 2004), finally resulting in some degree of oxidative damage in aquatic organisms. Therefore, the antioxidant enzymes might be biomarkers of contaminant-induced oxidative stress. Malondialdehyde (MDA) is one of the lipid peroxidation products deriving from oxidative attack on cell membrane phospholipids and circulating lipids, and its level directly reflects the degree of oxidative damage induced by contaminants (Banerjee, Seth, & Bhattacharya, 1999).

The Yellow River is the second greatest by length and basin area in China and its mainstream is 5,464 km long and drainage area is about 7.52×10^5 km^2. So, it becomes the most important source of water supply in North China. However, in the last 40 years, with the development of industry and agriculture, even in the upper Yellow River, a great number of contaminants have been discharged into this river and greatly polluted the water. In Lanzhou Region (LZR, 103°40′E, 35°54′N, alt. 1,524 m), a mixed polluted site in the upper Yellow River, the pollutants mainly include phenols, oils and unionized ammonia (NH_3) with concentrations of 0.004, 0.03 and 0.013 mg/l respectively, greatly exceeding those of the freshwater quality standard in China (Zhang, Ju et al., 2003). In contrast, Liujiaxia Region (LJXR, 103°27′E, 35°47′N, alt. 1,594 m), 75 km away from LZR, is a relatively unpolluted upstream site with the similar organism components and other natural characteristics to LZR. The quality of the water in LJXR is up to hygienic standard (for domestic drinking). Although a routine chemical component analysis has been performed (Chen, He, Zhang, & Cui, 2004; Zhang, Ju et al., 2003), no reports show the real toxic effects of the polluted water on organisms at environmental level. Therefore, it is necessary to use field animals inhabiting polluted sites as sentinel species for monitoring the environmental toxicity of the polluted water.

The carp *Cyprinus carpio* as a higher organism in the food chain of aquatic ecosystem and a foodstuff frequently consumed by human being has been considered a bioindicator species in waterways (Oruc & Uner, 2002; Sakamoto et al., 2003). The carp *C. carpio* chosen in our experiments is the most common fish in the Yellow River and is inevitably exposed to the pollutants in LZR. To explore whether the pollutants could induce oxidative stress and damage to aquatic organisms, SOD, CAT, GPx, GST activities and MDA level in the target organs of hepatopancreas, kidney and intestine of the carp collected from LZR and LJXR were analyzed.

2 Materials and Methods

2.1 Chemicals

Bovine serum albumin (BSA), reduced glutathione (GSH), 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB) were obtained

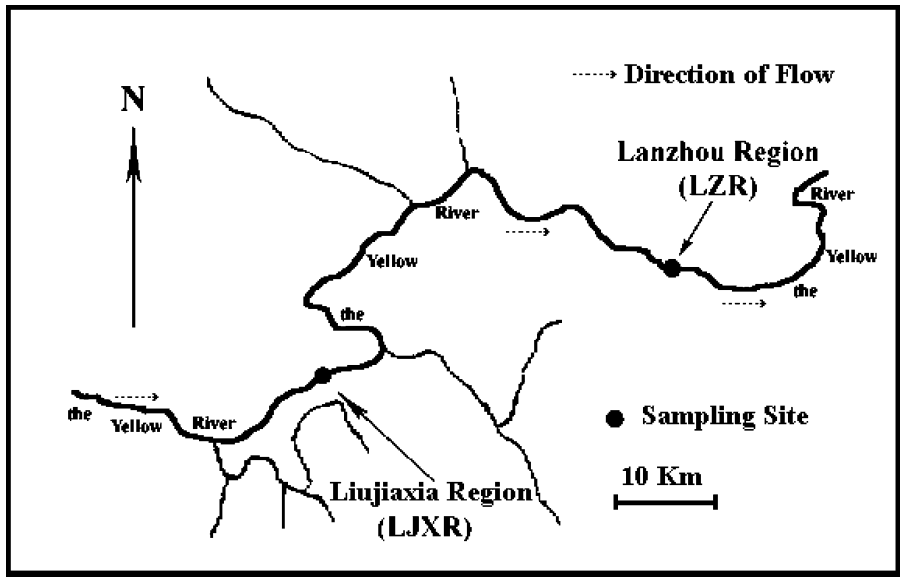

Figure 1 Sampling sites in the study area.

from Sigma (USA). All other chemicals and reagents were of analytical grade and procured from local chemical companies.

2.2 Sample collection and storage

The carp *C. carpio* was collected from LJXR and LZR (Figure 1), respectively, at the same time (during April to June 2004), and then transferred to the laboratory in a life-box. To eliminate the differences caused by sex and size, eight males and eight females with similar body size (495–650 g, 27.5–32.5 cm) at each sampling site were selected, and the average age of fish was about three years old determined by scale analysis. In the laboratory, after fish were euthanized (American Veterinary Medical Association, 2001), hepatopancreas, kidney and intestine samples were immediately removed, cleaned and frozen in liquid nitrogen until enzymatic activities were assayed.

2.3 Preparation of supernatant

Hepatopancreas, kidney and intestine samples were homogenized in chilled phosphate buffer (0.1 M, pH 7.4). The homogenates were centrifuged at 3,000 (for MDA level analysis) or 12,000 rpm (for enzyme assay) for 20 min at 4°C, and the supernatant was used for the subsequent assays.

2.4 MDA level assay

MDA level was measured after incubation at 95°C with thiobarbituric acid (TBA) in aerobic conditions (pH 3.4). The pink colour produced by these reactions was measured spectrophotometrically at 532 nm (Ohkawa, Ohishi, & Tagi, 1979). MDA level was expressed as μmol thiobarbituric acid reactive substance (TBARS) formed/h/mg protein.

2.5 Assays for antioxidant enzyme activities

SOD activity (unit/mg protein) was assayed by a modified pyrogallol auto-oxidation method (S. Marklund & G. Marklund, 1974). One unit of SOD activity was defined as the amount of protein that causes 50% inhibition of the pyrogallol auto-oxidation rate. CAT activity (μmol H_2O_2 consumed/min/mg protein) was assayed by the method of Claiborne (1985) with some modifications described by Ahmad, Hamid et al. (2000). GPx activity (μmol GSH consumed/min/mg protein) was assayed by the method of Sulochana, Biswas, and Ramakrishnan (1999), estimating the re-

Table I Antioxidant enzyme activities and MDA level in hepatopancreas, kidney and intestine of the carp *C. carpio*

Parameters (unit of expression)	Organs	Sampling sites	
		LJXR (n = 16)	LZR (n = 16)
SOD (unit/mg protein)	Hepatopancreas	819.78 ± 76.95	956.27 ± 87.24*
	Kidney	460.66 ± 51.85	567.38 ± 87.39*
	Intestine	588.39 ± 48.56	727.35 ± 28.42**
CAT (μmol H_2O_2 consumed/min/mg protein)	Hepatopancreas	15.97 ± 0.55	16.89 ± 1.31
	Kidney	7.45 ± 0.56	6.49 ± 0.94*
	Intestine	4.50 ± 0.03	3.99 ± 0.18*
GPx (μmol GSH consumed/min/mg protein)	Hepatopancreas	9.20 ± 4.96	5.20 ± 0.80**
	Kidney	6.85 ± 1.09	3.05 ± 0.33**
	Intestine	20.55 ± 2.89	12.63 ± 0.39**
GST (μmol CDNB conjugates/min/mg protein)	Hepatopancreas	0.0103 ± 0.0014	0.0418 ± 0.0033**
	Kidney	0.0145±0.0043	0.0878 ± 0.0252**
	Intestine	0.0208 ± 0.0031	0.1669 ± 0.0345**
MDA (μmol TBARS formed/h/mg protein)	Hepatopancreas	0.0467 ± 0.0044	0.0422 ± 0.0033
	Kidney	0.0206 ± 0.0020	0.0543 ± 0.0076**
	Intestine	0.0317 ± 0.0033	0.0693 ± 0.0058**

Values are expressed as mean ± SE.

*$P < 0.05$ when LZR values were compared with LJXR values at the same organ.

** $P < 0.01$ when LZR values were compared with LJXR values at the same organ.

duction in the level of GSH. GST activity (μmol CDNB conjugates/min/mg protein) was determined by the method of Habig, Pabst, and Jokoby (1974) with some modifications proposed by Ahmad, Hamid et al. (2000) using a molar extinction coefficient of 9.6 × 10^3 M^{-1} cm^{-1}.

2.6 Protein quantification

Protein contents in various samples were determined using the method of Bradford (1976) using BSA as standard.

2.7 Statistical analysis

The results were expressed as means ± standard errors (SEs), and statistical analyses were performed using a two-tailed Student's *t*-test. $P < 0.05$ accepted as statistically significant, $P < 0.01$ as greatly significant.

3 Results

Antioxidant enzyme activities and MDA level in hepatopancreas, kidney and intestine of the carp *C. carpio* from LJXR and LZR are summarized in Table I. Comparing the values of LZR with those of LJXR, SOD and GST activities increased but GPx activity decreased significantly in all the three target organs ($P < 0.05$–0.01), CAT activity decreased but MDA level increased significantly ($P < 0.05$–0.01) only in kidney and intestine. The increase of SOD activity in hepatopancreas, kidney and intestine was 16.65%, 23.17% and 23.62% , respectively, and that of GST was 305.83%, 505.52% and 702.40%; as concerning GPx activity, the decrease was 43.48%, 55.47% and 38.54%. The decrease of CAT activity was 12.89% and 11.33% in kidney and intestine, respectively, whereas the increase of MDA level was 163.59% and 118.61%.

4 Discussion

Under the laboratory conditions, it was well known that organic or metallic contaminants alone could stimulate ROS production, decrease antioxidant enzyme activities and result in oxidative damage in aquatic organisms in acute experiments (Ahmad, Oliveira, Pacheco, & Santos, 2005; Shi et al., 2005; Zhang, Shen, Wang et al., 2004; Zhang, Shen, Xu et al., 2003), and the toxicity effects of ROS could be prevented or decreased by supplement of specific antioxidant enzymes such as SOD, CAT and GPx (Pinto et al., 2003); however, in chronic experiments, antioxidant enzyme activities were usually increased in aquatic organisms (Banerjee et al., 1999; Company et al., 2004; Zhang, Liu et al., 2005). In field conditions, antioxidant enzymes have been used as biomarkers to various aquatic pollutants, but their changes are very different according to the aquatic species, enzymes and contaminants (Ahmad, Hamid et al., 2000; Gadagbui & James, 2000; Oruc et al., 2004).

SOD which catalyses the conversion of the superoxide anion radical to molecular oxygen and hydrogen peroxide (H_2O_2) has been called the cell's first defense line against ROS and could protect against superoxide-induced oxidative damage (Fridovich, 1989). Previous reports showed that many contaminants such as insecticides (Sayeed et al., 2003), oils and phenols (Zhang, Shen, Wang et al., 2004; Zhang, Wang et al., 2004) could affect SOD activity in fish, and which was dependent on the organs (Oruc et al., 2004). However, in the present study, regardless of organs, SOD activity in the carp collected from LZR was significantly higher than that from LJXR, which indicated that the carp could protect itself against the toxic effect of superoxide anion radical by increasing its activity. CAT catalyses the conversion of H_2O_2 to water and molecular oxygen; GPx converts H_2O_2 to water and lipid peroxides to unreactive hydroxyl fatty acids. It was reported that organic pollutants such as pesticides (Oruc et al., 2004; Uner, Oruc, Canli, & Sevgiler, 2001), oils (Zhang, Shen, Xu et al., 2003; Zhang, Wang et al., 2004) and phenols (Zhang, Liu et al., 2005; Zhang, Shen, Wang et al., 2004) could alter CAT and GPx activities in fish according the organs. In this study, CAT and GPx activities in the carp from LZR decreased significantly, which indicated that the abilities to protect against hydrogen peroxide were reduced (Mcfarland et al., 1999). The different tendency on SOD and CAT/GPx activities could be due to their characteristics and physiological functions. The difference of CAT activity in hepatopancreas of the carp between LZR and LJXR was not significant, which might be because it is a detoxification organ with a high ability to moderate adverse pressures or influences. GST catalyzes conjugation of many xenobiotics to the tripeptide glutathione and plays an important role in protecting organs from oxidative stress (Hayes & Strange, 1995). The study by

Armknecht, Kaattari, and Vanveld (1998) showed that the elevated GST activity in mummichog (*Fundulus heteroclitus*) following exposure to creosote played a critical role in toxicity resistance. In the present study, GST activity increased significantly in all the three organs of the carp from LZR, which was consistent with most investigations (Oruc et al., 2004; Sayeed et al., 2003; Shailaja & Silva, 2003), suggesting that GST activity could be induced to resist the pollutants' toxicity (Gadagbui & James, 2000). These results may suggest that the carp in LZR were undergoing oxidative stress.

Lipid peroxidation is acknowledged as being highly deleterious and deriving from attacks by hydroxide free radical, resulting in oxidative damage to organs or tissues (Shi et al., 2005). Hydroxide free radical is produced from H_2O_2 via the Fenton reaction (Evans & Halliwell, 1994). CAT is the primary enzyme in scavenging H_2O_2, so when CAT activity is inhibited, more H_2O_2 is available for production of hydroxide free radical. In this study, the decrease of CAT activity both in kidney and intestine of the carp collected from LZR, together with the increase of SOD activity, could explain the increased MDA level in these two organs. The elevated MDA level directly reflected that the pollutants may cause lipid peroxidation due to toxic chemical accumulation in kidney and intestine. Respecting to LJXR, no peroxidation was observed in hepatopancreas of the carp from LZR, which implies that hepatopancreas has a stronger antioxidant capacity than kidney or intestine.

5 Conclusions

In conclusion, the results of this study suggest that the pollutants can induce obvious oxidative damage in the carp, and the SOD, GST and GPx might be better indicators for the oxidative damage in aquatic organisms.

Acknowledgments This work was supported by National Natural Science Foundation of China (No. 30470320) and Natural Science Foundation of Gansu Province, China (No. 3ZS051-A25-084).

References

Ahmad, I., Hamid, T., Fatima, M., Chand, H. S., Jain, S. K., Athar, M., et al. (2000). Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure. *Biochimica et Biophysica Acta, 1523,* 37–48.

Ahmad, I., Oliveira, M., Pacheco, M., & Santos, M. A. (2005). *Anguilla anguilla* L. oxidative stress biomarkers responses to copper exposure with or without beta-naphthoflavone pre-exposure. *Chemosphere, 61,* 267–275.

American Veterinary Medical Association (2001). 2000 report of the AVMA panel on euthanasia. *Journal of the American Veterinary Medical Association, 218,* 669–696.

Armknecht, S. L., Kaattari, S. L., & Vanveld, P. A. (1998). An elevated glutathione S-transferase in creosote-resistant mummichog (*Fundulus heteroclitus*). *Aquatic Toxicology, 41,* 1–16.

Banerjee, B. D., Seth, V., & Bhattacharya, A. (1999). Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. *Toxicology Letters, 107,* 33–47.

Berntssen, M. H., Aatland, A., & Handy, R. D. (2003). Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (*Salmo salar*) parr. *Aquatic Toxicology, 65*(1), 55–72.

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry, 72,* 248–254.

Chen, J. S., He, D. W., Zhang, N., & Cui, S. B. (2004). Characteristics of and human influences on nitrogen contamination in Yellow River system, China. *Environmental Monitoring and Assessment, 93,* 125–138.

Claiborne, A. (1985). Catalase activity. In R. A. Greenwald (Ed.), *CRC handbook of methods in oxygen radical research* (pp. 283–284). Boca Raton, Florida: CRC Press Inc.

Company, R., Serafim, A., Bebianno, M. J., Cosson, R., Shillito, B., & Fiala-Medioni, A. (2004). Effect of cadmium, copper and mercury on antioxidant enzyme activities and lipid peroxidation in the gills of the hydrothermal vent mussel *Bathymodiolus azoricus*. *Marine Environmental Research, 58,* 377–381.

Evans, P. J., & Halliwell, B. (1994). Measurement of iron and copper in biological systems: Bleomycin and copper–phenanthroline assays. *Methods in Enzymology, 233,* 82-92.

Fridovich, I. (1989). Superoxide dismutases. An adaptation to a paramagnetic gas. *Journal of Biological Chemistry, 264,* 7761–7764.

Gadagbui, B. K., & James, M. O. (2000). Activities of affinity-isolated glutathione S-transferase (GST) from channel catfish whole intestine. *Aquatic Toxicology, 49,* 27–37.

Habig, W. H., Pabst, M. J., & Jokoby, W. B. (1974). Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry, 249,* 7130–7139.

Hayes, J. D., & Strange, R. C. (1995). Potential contribution of the glutathione S-transferase supergene family to resistance to oxidative stress. *Free Radical Research, 22,* 193–207.

Marklund, S., & Marklund G. (1974). Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry, 47,* 469–474.

Mcfarland, V. A., Inouye, L. S., Lutz, C. H., Jarvis, A. S., Clarke, J. U., & McCant, D. D. (1999). Biomarkers of oxidative stress and genotoxicity in livers of field-collected

brown bullhead, *Ameiurus nebulosus*. *Archives of Environmental Contamination and Toxicology, 37,* 236–241.

Ohkawa, H., Ohishi, N., & Tagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95,* 351–358.

Oruc, E. O., Sevgiler, Y., & Uner, N. (2004). Tissue-specific oxidative stress responses in fish exposed to 2,4-D and azinphosmethyl. *Comparative Biochemistry and Physiology, 137C,* 43–51.

Oruc, E. O., & Uner, N. (2002). Marker enzyme assessment in the liver of *Cyprinus carpio* (L.) exposed to 2,4-D and azinphosmethyl. *Journal of Biochemical and Molecular Toxicology, 16,* 182–188.

Pinto, E., Sigaud-kutner, T. C. S., Leitao, M. A. S., Okamoto, O. K., Morse, D., & Colepicolo. P. (2003). Heavy metal-induced oxidative stress in algae. *Journal of Phycology, 39,* 1008–1018.

Sakamoto, K. Q., Nakai, K., Aoto, T., Yokoyama, A., Ushikoshi, R., Hirose, H., et al. (2003). Cytochrome P450 induction and gonadal status alteration in common carp (*Cyprinus carpio*) associated with the discharge of dioxin contaminated effluent to the Hikiji River, Kanagawa Prefecture, Japan. *Chemosphere, 51,* 491–500.

Sayeed, I., Parvez, S., Pandey, S., Hafeez, B. B., Haque, R., & Raisuddin, S. (2003). Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. *Ecotoxicology and Environmental Safety, 56,* 295–301.

Shailaja, M. S., & Silva, C. D. (2003). Evaluation of impact of PAH on a tropical fish, *Oreochromis mossambicus* using multiple biomarkers. *Chemosphere, 53,* 835–841.

Shi, H., Sui, Y., Wang, X., Luo, Y., & Ji, L. (2005). Hydroxyl radical production and oxidative damage induced by cadmium and naphthalene in liver of *Carassius auratus*. *Comparative Biochemistry and Physiology, 140C,* 115–121.

Sulochana, K. N., Biswas, J., & Ramakrishnan, S. (1999). Eales' disease: Increased oxidation and peroxidation products of membrane constituents chiefly lipids and decreased antioxidant enzymes and reduced glutathione in vitreous. *Current Eye Research, 19,* 254–259.

Uner, N., Oruc, E. O., Canli, M., & Sevgiler, Y. (2001). Effects of cypermethrin on antioxidant enzyme activities and lipid peroxidation in liver and kidney of the freshwater fish, *Oreochromis niloticus* and *Cyprinus carpio* (L.). *Bulletin of Environmental Contamination and Toxicology, 67,* 657–664.

Zhang, J. F., Liu, H., Sun, Y. Y., Wang, X. R., Wu, J. C., & Xue, Y. Q. (2005). Responses of the antioxidant defenses of the Goldfish *Carassius auratus*, exposed to 2,4-dichlorophenol. *Environmental Toxicology and Pharmacology, 19,* 185–190.

Zhang, J. F., Shen, H., Wang, X. R., Wu, J. C., & Xue, Y. Q. (2004). Effects of chronic exposure of 2,4-dichlorophenol on the antioxidant system in liver of freshwater fish *Carassius auratus*. *Chemosphere, 55,* 167–174.

Zhang, J. F., Shen, H., Xu, T. L., Wang, X. R., Li, W. M., & Gu, Y. F. (2003). Effects of long-term exposure of low-level diesel oil on the antioxidant defense system of fish. *Bulletin of Environmental Contamination and Toxicology, 71,* 234–239.

Zhang, J. F., Wang, X. R., Guo, H. Y., Wu, J. C., & Xue, Y. Q. (2004). Effects of water-soluble fractions of diesel oil on the antioxidant defenses of the goldfish, *Carassius auratus*. *Ecotoxicology and Environmental Safety, 58,* 110–116.

Zhang, J. H., Ju, T. Z., Ren, Z. W., Tian, Y. J., Suo, A. N., & Wen, F. (2003). An analysis of water pollution along Lanzhou reaches of Yellow River. *Science and Technology Management of Land Resources, 20*(4), 47–50 (in Chinese).