Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Growth rate, catalase and superoxide dismutase activities in rock carp (*Procypris rabaudi* Tchang) exposed to supersaturated total dissolved gas*

Xiao-qing LIU[1], Ke-feng LI[†‡1], Jun DU[†‡2], Jia LI[1], Ran LI[1]

([1]*State Key Laboratory of Hydraulics and Mountain River Engineering, Sichuan University, Chengdu 610065, China*)

([2]*Fisheries Institute, Sichuan Academy of Agricultural Sciences, Chengdu 611731, China*)

[†]E-mail: kefengli@scu.edu.cn; dujun9100@yahoo.com.cn

Received Mar. 10, 2011; Revision accepted Aug. 30, 2011; Crosschecked Oct. 17, 2011

Abstract: Total dissolved gas supersaturation (TDGS) appears when the pressures of gases in a solution exceed the barometric pressures. TDGS is often caused by flood discharge at dams. It may lead to gas bubble disease (GBD) for fish and biochemical responses of selected fish and other aquatic organisms. The purpose of this study was to determine the impact of long-term TDGS levels on the growth and biochemical responses of rock carp (*Procypris rabaudi* Tchang) dwelling in the upper reaches of the Yangtze River. Three-year-old rock carp were exposed to TDGS levels at 100%, 104%, 108%, 112%, and 116% for 42 d. Samples were taken every 7 d after the start of the trial in order to determine catalase (CAT) and superoxide dismutase (SOD) activities in gill and muscle tissues. Samples were taken at Days 0 and 42 of exposure to determine growth rate. Little effect was found on growth rate in all treatment groups. SOD and CAT activities varied in different tissues, according to time of exposure and TDGS levels. The biochemical response of fish exposed to TDGS was more obvious in gill tissue than in muscle tissue. Surveys of SOD and CAT activities in different tissues offer important information about the effect of TDGS on the rare fish in the Yangtze River, and may help evaluate the risk to the aquatic eco-environment and aquatic ecosystem in the downstream of the Yangtze River.

Key words: Total dissolved gas supersaturation (TDGS), Rock carp (*Procypris rabaudi* Tchang), Growth rate, Catalase, Superoxide dismutase

doi:10.1631/jzus.B1100071 **Document code:** A **CLC number:** Q17

1 Introduction

Total dissolved gas supersaturation (TDGS) generated from flood discharge threatens the survival of aquatic organisms. Bouck (1980) indicated that TDGS results in a high mortality in fish at high concentrations, owing to gas bubble disease (GBD). GBD is an environmentally induced physiological condition that occurs among fish dwelling in the gas supersaturated water. TDGS can also result in biochemical responses of selected fish.

There are many studies on various fish species exposed to acute and chronic TDGS (Gunnarsli *et al*., 2008a). Medium lethal concentration and medium lethal time in rock carp exposed to TDGS have also been determined (Huang *et al*., 2010a). The acute lethality and avoidance responses have been studied in fish exposed to TDGS (Stevens *et al*., 1980; Cornacchia and Colt, 1984; Colt *et al*., 1985; Huang *et al*., 2010a). Some reports show that chronic effects of GBD are found at low TDGS levels (Alderdice and Jensen, 1985; Colt, 1986; Morris *et al*., 2003). However, few studies have focused on evaluating the

‡ Corresponding authors

* Project (No. 50979063) supported by the National Natural Science Foundation of China

biochemical responses of fish species exposed to TDGS. Studies of TDGS exposure in rare fish dwelling in the upper reaches of the Yangtze River are relatively scarce. Furthermore, although the chronic effect of TDGS plays an important role in environmental stress, the impact of TDGS on the growth rate and biochemical responses in rare species is unknown.

Therefore, the purpose of this study was to determine the impact of longer-term TDGS exposure on the growth rate and biochemistry responses of rock carp (*Procypris rabaudi* Tchang).

2 Materials and methods

2.1 Treatment of experimental fish and monitoring instrument

This study was conducted by using three-year-old rock carp which dwell in the upper reaches of the Yangtze River. The endemic fish, with average (±standard deviation (SD)) weight of 211.78 g (±21.16 g), were obtained from the Sichuan Fisheries Research Institute, China. The three-year-old rock carp were transferred to the laboratory in November 2010 and the fish were kept for 10 d in tanks with a capacity of 720 L before the start of the trial (30 fish/tank). During the 10 d, de-chlorinated and aerated tap water was conveyed continuously to the tank by water pipe in order to allow the fish to acclimatize. Water temperature and pH values were kept constant during the entire experiment. The fish were fed on *Limnodrilus hoffmeisteri* once daily. The fish tanks were cleaned daily. The TDGS level and temperature were measured daily using the point four tracker (Point Four Systems Inc., Canada). pH value was monitored using the digital pH meter (JENCO Model 6010, China), and dissolved oxygen (DO) value was measured daily with the aid of dissolved oxygen meters (Oxi 3210 SET 3 Inc., Germany). The experimental conditions were as follows: temperature (24±0.5) °C, DO (7.5±0.6) mg/L, and pH 7.0±0.3.

2.2 Procedures of experiment

The experiment was carried out for 42 d in all tanks from Nov. 15 to Dec. 27, 2010. Thirty fish were held in each tank with a water depth of 0.35 m. The experimental system was used according to Huang *et al*., (2010b). TDG-supersaturated water and tap water were mixed together to create different levels of supersaturated water. The control group was provided with untreated water, while the treatment groups were given supersaturated water with levels of TDG being 104%, 108%, 112%, and 116%. The TDG values rose gradually in all treatment groups and were adjusted to the desired concentration. During the experiment, the test fish were kept in the TDG-supersaturated water and remained near the bottom of the tank. The TDG-supersaturated water was generated by adding pressure to the water, achieving desired TDG saturation levels by regulating the water inflow, water outflow, and air pressure of the pressure vessel (Huang *et al*., 2010b).

2.3 Growth rate measure

The total initial weights of the fish were calculated in each tank before the beginning of trial and the final weights of the fish were measured after the trial ended. Fish growth was determined by the total initial weight and the final weight of 15 fish per tank. The following equation was applied to measure the relative growth rate (RGR): $RGR=(m_t-m_0)/m_0\times100\%$, where m_0 is the initial weight at the start of the trial and m_t is the final weight at the end of the trial.

2.4 Sample treatment and biochemical assays

After exposure to TDGS, fish were sampled every other week. Fish samples were killed and their weights were measured. The fish were promptly cut open, the gills and muscles were excised, weighed, and homogenized using a chilled pestle and mortar under liquid nitrogen, and then were extracted following the method of Wang *et al.* (2010). The 1.5 ml reaction mixture consisted of 20 mmol/L sodium phosphate buffer (pH 7.8) and 0.5 mmol/L ethylene diamine tetraacetic acid (EDTA). A centrifuge was used to gain crude extract at 10000 r/min for 3 min at 4 °C. The supernatant was obtained and used for assays of antioxidant enzyme activity.

Superoxide dismutase (SOD) assay was determined according to the protocol of McCord and Fridovich (1969). During the process of measurement, the 3 ml reaction mixture consisted of sodium phosphate buffer (pH 7.8), methionine, nitro blue tetrazolium (NBT), riboflavin, and enzyme extract. Concentrations of the components were 50 mmol/L,

13 mmol/L, 75 μmol/L, 2 μmol/L, and 50 μl, respectively. The reaction mixture was incubated for 15 min in fluorescent light under condition of 25 °C. Meanwhile, a UV/vis spectrophotometer (Purkinje General Instrument Co., Ltd., Beijing, China) was used to measure the absorbance at 560 nm. Non-illuminated mixtures without enzyme extract were treated as control. The enzyme volume that corresponded with 50% inhibition of reaction was estimated. It was defined as an enzyme-activity unit and the activity was expressed in U/g fresh weight (FW).

Catalase (CAT) activity was determined by the method described by Montavon *et al.* (2007). The definition of the enzyme-activity unit of CAT was the amount that caused the decomposition of 1 μmol of H_2O_2 per min under the experimental conditions. The activity was expressed in U/g FW.

2.5 Statistical analysis

For anti-oxidative enzyme activities and growth rate, one-way analysis of variance (ANOVA) was used to analyze the difference between treatment groups and the control group. In all cases, the significance level was set at $P<0.05$.

3 Results

3.1 Growth rate

During the execution of experiments, all rock carp survived. Moreover, there were no significant differences in the growth rate of rock carp exposed to TDGS for 42 d and the controls ($P>0.05$) (Table 1).

Table 1 Changes in the weight of rock carp exposed to various TDGS levels for 42 d

TDGS (%)	Weight (g)		Relative growth rate (%)
	Day 0	Day 42	
100	218.6±18.9	222.6±19.5	1.9
104	200.3±24.2	203.6±25.0	1.6
108	220.5±19.3	223.8±22.0	1.5
112	199.5±20.8	202.1±21.2	1.3
116	216.3±18.2	217.7±18.8	0.6

Data are expressed as mean±SD (n=30). No significant difference was observed between groups

3.2 Activities of antioxidant enzymes

The activities of antioxidant enzymes changed according to TDGS levels, time of exposure, and rock carp tissues (Figs. 1–2). As shown in Fig. 1, significant differences in CAT activity of the rock carp were not observed after 7 d exposure ($P>0.05$). Fig. 1a shows that CAT activity in the muscle tissue was significantly increased ($P<0.05$) in rock carp exposed to the 112% and 116% concentrations at 21 d. Over the exposure duration, the TDG 104% and 108% concentrations increased considerably the CAT activity compared to the control by the end of 28 d ($P<0.05$). However, after 35 d exposure, CAT activity reduced and there were no significant differences in the muscle tissue of rock carp amongst treatment groups and the control group ($P>0.05$). In Fig. 1b, CAT activity in the gill tissue had a significant increase ($P<0.05$) in rock carp exposed to the 112% concentration at 14 d. However, after 21 d exposure, CAT activity increased and significant variations were found at the 108%, 112%, and 116% concentrations ($P<0.05$). In the following 7 d, the gill tissue of rock carp exposed at the 112% concentration had no significant CAT activity response ($P>0.05$). Significant differences existed between the 116% and the control group to the end of 42 d exposure ($P<0.05$).

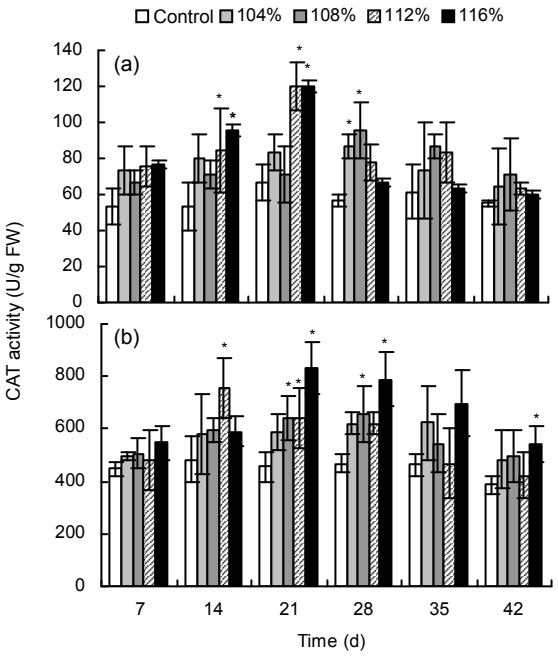

Fig. 1 Effects of different levels of TDGS on CAT activities in the muscle (a) and gill (b) tissues of the three-year-old rock carp

* indicates significant difference amongst treatment groups and the control group ($P<0.05$). Data are expressed as mean±SD (n=3)

In addition, the change of SOD activity was similar to the CAT activity in the tissues of the three-year-old rock carp, but significant differences were not observed in the muscle tissue in all treatment groups after 7, 14, and 42 d exposure (P>0.05) (Fig. 2). The highest values were observed in the groups (TDG 112% and 116%) at 21 d and in the 108% group at 28 d. Both TDG 104% and 108% groups were significantly different from the control at 35 d (P<0.05). The significant differences were not observed in the gill tissue in all the treatment groups after 7 d and 42 d (P>0.05). However, SOD activity in the gill tissue showed a significant increase (P<0.05) in rock carp exposed to the 116% concentration at 14 d. SOD activity in the gill tissue showed a significant increase (P<0.05) in rock carp exposed to the 112% and 116% concentrations over time. After 35 d exposure, SOD activity decreased gradually, but other treatment groups (TDG 108%, 112%, 116%) showed significant differences from the controls (P<0.05).

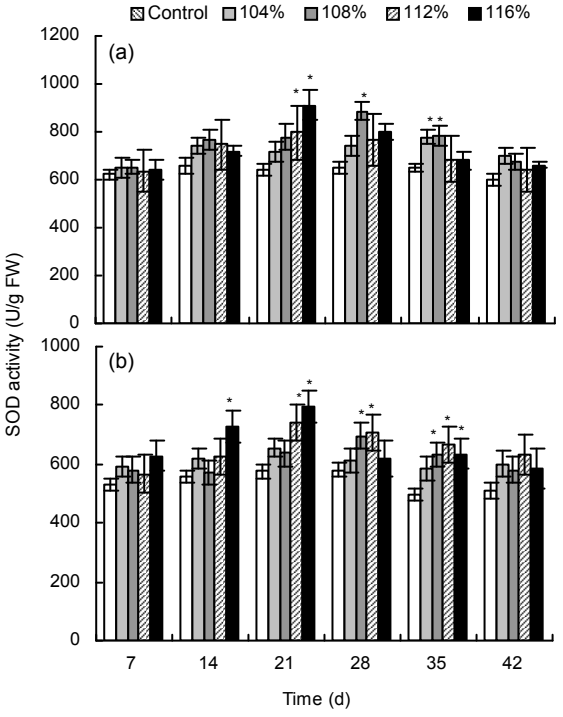

Fig. 2 Effects of different levels of TDGS on SOD activities in the muscle (a) and gill (b) tissues of the three-year-old rock carp

* indicates significant difference amongst treatment groups and the control group (P<0.05). Data are expressed as mean±SD (n=3)

4 Discussion

4.1 Growth rate

In our study, the results showed that the growth was not significantly different when the rock carp were reared for six weeks. However, with respect to the effects of TDGS on growth in rock carp, further experiments of longer duration will be taken into consideration.

There have been reports that gas supersaturation adversely affects survival and growth of fish (Krise *et al*., 1990). Gunnarsli *et al*. (2008b) indicated, however, that no effects of nitrogen gas supersaturation were found on the growth of Atlantic cod (*Gadus morhua* L.) reared for seven weeks. The report discribed by Person-Le Ruyet *et al*. (2002) also demonstrated that no substantial increase in growth of turbot occurred when the turbot were reared for 30 d in O_2-supersaturated and saturated water. However, in the study by Krise (1993), growth rate reductions were obvious when lake trout were exposed to gas supersaturation for more than 252 d with a gas pressure ΔP=17 mmHg. In addition, sublethal exposures to different concentrations of dissolved gas significantly affected the growth of chinook salmon and steelhead trout (Dawley and Ebel, 1975).

4.2 Activities of antioxidant enzymes

In the present study, changes of biochemistry were found clearly in the muscle and gill of rock carp after exposure to TDGS. CAT and SOD activities were shown to change according to the tissue type, time of exposure, and TDGS levels. Significant differences were not observed in the enzymatic activities in the muscle and gill tissues after 7-d exposure. In addition, significant variations were found at the 108%, 112%, and 116% concentrations. With time, CAT activity deceased gradually, but remained higher than that of the control. After the end of the experiment, the CAT activity indicated that this antioxidant enzyme had the capability to deal with TDGS stress, but this might be inhibited owing possibly to dysfunction or apoptosis in the cell with more exposure (Sweet *et al*., 1999).

Comparatively, the tissues of rock carp displayed a higher variation in SOD activity than in CAT activity. Change of SOD activity in the gill of rock carp was more obvious than that in the muscle, and an

abundant protective effect can be exerted by the SOD activity under the synergetic effects of the CAT activity. Namely, an increase in the activity of this antioxidant enzyme arose under conditions of TDGS, as an adaptive response.

In 1970, TDGS had been treated as a water quality parameter because fish survival was threatened in TDG supersaturated water, similar to other contaminants (Dawley and Ebel, 1975; Gunnarsli *et al*., 2008a). As we know, reactive oxygen species (ROS) can lead to an oxidative stress in fish exposed. ROS may be increased by TDGS. Antioxidant enzymes play an important role in eliminating ROS. The enzymatic antioxidants mainly include SOD, CAT, and peroxidase (POD), etc.

Considerable attention has been paid to oxidative stress in relation to ecotoxicology (Gül *et al*., 2004; Sanchez *et al*., 2005). Consequently, CAT and SOD activities are treated as important and reliable biomarkers for oxidative stress. In addition, most studies on antioxidant enzyme activity focus on fish exposure to pesticides and heavy metal (Atli *et al*., 2006; Campa-Córdova *et al*., 2009). Few reports deal with antioxidant enzyme activities in fish exposed to TDGS. Therefore, the purpose of this research is to determine the growth rate and the responses of CAT and SOD activities to various levels of the TDGS in the tissues of rock carp. The data may provide important information for future research of contaminant effects on the antioxidant system.

5 Conclusions

The experiments conducted show that three-old-year rock carp exposed to a higher TDGS level (116%) has no mortality after 42 d exposure and the fish species show a high tolerance to lengthy exposure of TDGS. Meanwhile, no significant effects of TDGS were found on the growth of rock carp. Furthermore, the changes in the antioxidant enzyme (CAT and SOD) activities in the different tissues of rock carp exposed to different levels of TDGS indicate a long-term biochemical response. The sensitivity in rock carp for TDGS is dependent on time of exposure and TDGS levels. Moreover, the surveys of biochemical responses in rock carp exposed to TDGS can offer important information concerning the effects of TDGS on the endemic fish in the Yangtze River. Further work concerning the acute stress of TDGS should be taken into consideration in order to fully investigate the effect of TDGS on the endemic fish in the Yangtze River.

Acknowledgements

The authors thank Wen-min YI, Bo LI, Shi-chao CHEN, Wen JIANG, Qiu-cheng DU, Chun-ling LI, and Luo SUN (Sichuan University, China), for their help during the experiments.

References

Alderdice, D.F., Jensen, J.O.T., 1985. An explanation for the high resistance of incubating salmonid eggs to atmospheric gas supersaturation of water. *Aquaculture*, **49**(1): 85-88. [doi:10.1016/0044-8486(85)90192-9]

Atli, G., Alptekin, O., Tükel, S., Canli, M., 2006. Response of catalase activity to Ag^{+}, Cd^{2+}, Cr^{6+}, Cu^{2+} and Zn^{2+} in five tissues of freshwater fish *Oreochromis niloticus*. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, **143**(2): 218-224. [doi:10.1016/j.cbpc.2006.02.003]

Bouck, G.R., 1980. Etiology of gas bubble disease. *Trans. Am. Fish. Soc*., **109**(6):703-707. [doi:10.1577/1548-8659(1980)109<703:EOGBD>2.0.CO;2]

Campa-Córdova, A.I., Núñez-Vázquez, E.J., Luna-González, A., Romero-Geraldo, M.J., Ascencio, F., 2009. Superoxide dismutase activity in juvenile *Litopenaeus vannamei* and *Nodipecten subnodosus* exposed to the toxic dinoflagellate *Prorocentrum lima*. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.*, **149**(3):317-322. [doi:10.1016/j.cbpc.2008.08.006]

Colt, J., 1986. Gas supersaturation—impact on the design and operation of aquatic systems. *Aquac. Eng.*, **5**(1):49-85. [doi:10.1016/0144-8609(86)90005-1]

Colt, J., Orwicz, K., Brooks, D., 1985. The effect of gas supersaturation on the growth of juvenile channel catfish, *Ictalurus punctatus*. *Aquaculture*, **50**(1-2):153-160. [doi:10.1016/0044-8486(85)90161-9]

Cornacchia, J.W., Colt, J.E., 1984. The effects of dissolved gas supersaturation on larval striped bass, *Morone saxatilis* (Walbaum). *J. Fish Dis*., **7**(1):15-27. [doi:10.1111/j.1365-2761.1984.tb00903.x]

Dawley, E.M., Ebel, W.J., 1975. Effects of various concentrations of dissolved atmospheric gas on juvenile Chinook salmon and steelhead trout. *Fish Bull.*, **73**(4):787-796.

Gül, Ş., Belge-Kurutaş, E., Şahan, A., Doran, F., 2004. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (*Cyprinidae*) living in Seyhan Dam Lake, Turkey. *Environ. Int*., **30**(5):605-609. [doi:10.1016/S0160-4120(03)00059-X]

Gunnarsli, K.S., Toften, H., Mortensen, A., 2008a. Effects of nitrogen gas supersaturation on growth and survival in

juvenile Atlantic cod (*Gadus morhua* L.). *Aquaculture*, **283**(1-4):175-179. [doi:10.1016/j.aquaculture.2008.06.029]

Gunnarsli, K.S., Toften, H., Mortensen, A., 2008b. Effects of nitrogen gas supersaturation on growth and survival in larval cod (*Gadus morhua* L.). *Aquaculture*, **288**(3-4): 344-348. [doi:10.1016/j.aquaculture.2008.11.039]

Huang, X., Li, K.F., Du, J., Li, R., 2010a. Effects of gas supersaturation on lethality and avoidance responses in juvenile rock carp (*Procypris rabaudi* Tchang). *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, **11**(10):806-811. [doi:10.1631/jzus.B1000006]

Huang, X., Li, K.F., Li, R., Li, J., Du, J., 2010b. Experimental system for the simulation of total dissolved gas supersaturated water of high dams. *J. Sichuan Univ. (Eng. Sci. Ed.)*, **42**(4):25-28 (in Chinese).

Krise, W.F., 1993. Effects of one-year exposures to gas supersaturation on lake trout. *Prog. Fish-Cult*. **55**(3):169-176. [doi:10.1577/1548-8640(1993)055<0169:EOOYET>2.3.CO;2]

Krise, W.F., Meade, J.W., Smith, R.A., 1990. Effect of feeding rate and gas supersaturation on survival and growth of lake trout. *Prog. Fish-Cult.*, **52**(1):45-50. [doi:10.1577/1548-8640(1990)052<0045:EOFRAG>2.3.CO;2]

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.*, **244**(22):6049-6055.

Montavon, P., Kukic, K.R., Bortlik, K., 2007. A simple method to measure effective catalase activities: optimization, validation, and application in green coffee. *Anal. Bichem.*, **360**(2):207-215. [doi:10.1016/j.ab.2006.10.035]

Morris, R.G., Beeman, J.W., VanderKooi, S.P., Maule, A.G., 2003. Lateral line pore diameters correlate with the development of gas bubble trauma signs in several Columbia River fishes. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, **135**(2):309-320. [doi:10.1016/S1095-6433(03)00072-2]

Person-Le Ruyet, J., Pichavant, K., Vacher, C., le Bayon, N., Severe, A., Boeuf, G., 2002. Effects of O_2 supersaturation on metabolism and growth in juvenile turbot (*Scophthalmus maximus* L.). *Aquaculture*, **205**(3-4): 373-383. [doi:10.1016/S0044-8486(01)00689-5]

Sanchez, W., Palluel, O., Meunier, L., Coquery, M., Porcher, J.M., Ait-Aissa, S., 2005. Copper-induced oxidative stress in three-spined stickleback: relationship with hepatic met-al levels. *Environ. Toxicol. Pharmacol*., **19**(1): 177-183. [doi:10.1016/j.etap.2004.07.003]

Stevens, D.G., Nebeker, A.V., Baker, R.J., 1980. Avoidance responses of salmon and trout to air-supersaturated water. *Trans. Am. Fish. Soc.*, **109**(6):751-754. [doi:10.1577/1548-8659(1980)109<751:AROSAT>2.0.CO;2]

Sweet, L.I., Passino-Reader, D.R., Meier, P.G., Omann, G.M., 1999. Xenobiotic induced apoptosis: significance and potential application as a general biomarker of response. *Biomarkers*, **4**(4):237-253. [doi:10.1080/135475099230778]

Wang, S.T., He, X.J., An, R.D., 2010. Responses of growth and antioxidant metabolism to nickel toxicity in *Luffa cylindrica* seedlings. *J. Anim. Plant Sci.*, **7**(2):810-821.