Effect of Sewage on Plasma Cortisol and Element Concentrations in Goldfish, Carassius auratus

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Raw sewage contains high levels of BOD, ammonia, nutritive salts and other pollutants. Ammonia, in particular, is a serious toxicant to aquatic animals[1-3]. High BOD also causes the decrease in dissolved oxygen (DO), while low DO lowers the lethal concentrations for various toxicants[4-6]. Raw sewage thus give harmful effects on the health of fish in and around the inflow area.

It is well known that plasma cortisol increases under stress conditions[7,8]. Elemental contents of plasma may provide a good indication of the physiological conditions of fish, as it has been known in mammals that the contents are related closely to the host's health[9,10]. In the present study, the effect of exposure to raw sewage on plasma cortisol and element levels of goldfish were investigated.

Materials and Methods

Goldfish, *Carassius auratus*, weighing about 8g were used in the experiment. Fish were adapted to 20°C for 2 weeks in an indoor tank with 60ℓ of dechlorinated water. During the rearing period, they were fed with a commercial diet. They were examined after being fasted for 24 hours. The experiment was performed at a constant temperature of 20°C. Throughout the experimental period, fish were fasted.

Raw sewage collected at the influx of a sewage plant in Miyagi Prefecture. For the test group, this sewage was diluted to 10% and 50% with temperature adjusted dechlorinated water. Experimental fish were exposed to undiluted and diluted raw sewages for 3 days. Throughout the experimental period, control fish were kept in dechlorinated water. Each tank had twenty fish. Half of the rearing water was exchanged softly every day. The dissolved oxygen levels (DO) were not less than 6mg/l during the experiment. Water quality of experimental tank was measured by the methods[11] described previously.

Blood was collected from the heart of alive fish in the experimental groups using a heparin-treated syringe. After measurement of haemoglobin (Hb) concentration, the levels of eight elements in plasma were measured by PIXI[11]. Plasma cortisol was also measured by the method using Gammacoat [^{125}I] cortisol[12].

Results

Water quality in tanks at the beginning of this experiment is shown in Table 1. Parameters of raw sewage in the initial period were several hundreds times for BOD and ammonia-N, hundred times for total-N, several ten times for total-P, K and Mg, ten times for Na, Ca, Cl and Fe and two to three times for Cu and Zn in comparison with those of the control.

Goldfish exposed to undiluted raw sewage exhibited occasionally laboured respiratory movement in subsequent several hours. No fish exposed to 10% and 50% raw sewages exhibited significant changes in behavior. No fish died during this experiment. In goldfish exposed to undiluted raw sewage, a slight hemolysis was observed.

Changes in Hb, plasma cortisol and elements of goldfish exposed to sewages are shown in Table 2. When fish were exposed to undiluted raw sewage, decreases in Hb and plasma Zn and increases in plasma P, Br and cortisol were found after 3 days. In goldfish exposed to 50% raw sewage for 3 days, significantly higher values for plasma P, Br and cortisol and lower values for Fe and Zn than those of the control were found. Elevated plasma cortisol was closely related to increase in the concentration of sewage. No statistically significant changes in these blood parameters were found in fish exposed to 10% sewage for 3 days, however, a slight increase in plasma cortisol was observed.

Discussion

Chemicals such as ammonia and copper exert genaral stress effects, that is, increase cotrisol and catecholamines in plasma. Raw sewage contained much higher level of ammonia-N compared to that of the control. Significant increase in plasma cortisol in goldfish exposed to 50% and undiluted sewages was found. In fish exposed to 10% sewage, a slight increase in cortisol was also observed. It is thus considered that goldfish exposed to sewages incurred considerable stress and might have physiological abnormalities even in 10% sewage even with sufficient DO in water.

In this study, plasma Zn decreased 3 days after the exposure to 50% and undiluted sewages. Catecholamines stimulate the urine excretion of K, Mg and Zn[13,14]. Adrenocorticosteroid decreases Zn in plasma[15]. These could account for the decreased Zn. It has been suggested that elevated cortisol[16] and lowered Zn[9,10] levels in plasma reduces powers of biodefence. It may thus be possible that goldfish exposed to sewages are subjected to suppression of the immune response. Elevated glucocorticoids in plasma also facilitate P release from the bones[17,18]. The reason why plasma Br increased and Fe decreased is not clear. Changes in plasma P, Br and Fe in the test group may have resulted from transfer to these elements between plasma and various tissues and/or are inversion of these elements from environmental water during the exposure.

It follows from the present data that goldfish exposed to sewages incur physiological abnormalities, even to 10% sewage for 3 days. Immediate construction and improvement projects of municipal and regional sewage treatment plants with high treatment capacity are thus required.

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Table 1. Water quality in tank at the beginning of this experiment.

		Control	Raw sewage
pH		6.9	7.1
BOD	(mg/l)	0.2	140
Total-N	(mg/l)	0.2	23.2
Ammonia-N	(mg/l)	0.04	16.1
Total-P	(mg/l)	0.1	7.2
Na	(mg/l)	18	127
K	(mg/l)	1.4	36
Ca	(mg/l)	3.5	37
Mg	(mg/l)	0.9	28
Cl	(mg/l)	10	90
Fe	(mg/l)	0.02	0.24
Cu	(mg/l)	0.01	0.02
Zn	(mg/l)	0.01	0.03

Table 2. Changes in haemoglobin (Hb), plasma cortisol and elements of goldfish exposed to sewages for 3 days.

		Control	Raw sewage		
			10%	50%	100%(undiluted)
Hb	(g/100ml)	7.3±0.4	6.9±0.8	6.4±0.9	6.2±0.7*
P	(mg/l)	174±34	195±46	242±38*	229±34*
Cl	(mg/l)	3530±345	3625±420	3360±320	3375±315
Ca	(mg/l)	123±27	141±41	140±36	127±28
K	(mg/l)	118±29	132±36	107±39	126±28
Fe	(mg/l)	0.3±0.1	0.4±0.2	0.2±0.1*	0.3±0.1
Cu	(mg/l)	0.1±0.1	0.1±0.1	0.2±0.2	0.1±0.1
Zn	(mg/l)	2.4±0.5	1.9±0.8	1.5±0.6*	1.6±0.5*
Br	(mg/l)	1.2±0.4	1.5±0.7	2.1±0.6*	1.8±0.5*
Cortisol	(ng/ml)	69±23	82±36	126±43*	162±65*

Data are given as mean ±S. D., N=8.
* Significant difference from those of the control ($p<0.05$).