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Combined Chromium and Phenol Pollution in a Marine Prawn Fishery

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In our world, marine fisheries, including the production of marine prawns, will be an important component part of agricultural production in the 21st century as the marine era (China Environmental Protection Bureau 1995). However, the rising mortality and decreasing yield of marine prawns has taken place in recent years in the China Sea, especially in the Zhoushan Sea, where the biggest fishery in China is housed (Statistical Bureau of Zhejiang Province 1992; 1994). The causes of this mortality and its extension northward remain unclear. It is possible that it is the result of the interaction of multiple pollutants, such as chromium and phenol, in the coastal waters and seawater. This may cause the excessive accumulation of chromium and phenol in marine prawns, decreasing their yield and increasing their death rate. In previous investigations it was found that the concentrations of chromium and phenol in the Zhoushan Sea water were rising. The concentrations of chromium and phenol in polluted prawns were quite high, up to 15.87 and 4.93 mg/kg fresh weight, respectively (Zhou 1994). Chemical pollution of the area has been increasing in recent years, due to the continuous discharge of waste water from rural industries such as printing, manufacture, dyeing, textile, electroplating, meat-processing and tanning enterprises (Zhou 1994; Zhou and Zhu 1997).

We have attempted to find the cause of the decreased yield and increased mortality of marine prawns in the area by setting a 1-year culture experiment. The scientific foundations of the attempt to model the real pollution problem mainly include two aspects: (1) it was indicated that external chromium and phenol are both toxic to marine organisms (Sprague and Drury 1969; Halstead 1972) and marine prawns are sensitive to both chromium and phenol (Zhou and Dai 1995); (2) the interaction of more than one pollutant in an ecosystem may be more harmful to the growth and survival of living organisms than the individual pollutants (Zhou 1995).

MATERIALS AND METHODS

Small marine prawns (*Penaeus japonicus*) and some seawater samples used in the culture experiment were simultaneously collected from surface waters (about 0-10 m depth) of a relatively clean location of the Zhoushan Sea. The background concentrations of chromium and phenol in the collected seawater samples were 0.2

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and 0.006 µg/L, respectively. The original concentrations of chromium and phenol in the small marine prawns were 0.58 and 0.05 mg/kg fresh weight, respectively. When the culture started, various concentrations of $K_2Cr_2O_7$ and/or phenol were respectively added into their corresponding glass vats (12 L each). Each vat contained 5 L of the collected seawater sample and 10 prawns with 9.10 g of the initial total weight.

In the culture experiment, there was one control (no addition of chromium and phenol), 9 single-factor treatments (chromium or phenol addition) and 20 double-factor treatments (addition of chromium and phenol) as listed in Table 1. All the treatments were replicated four times in order to decrease experimental errors to the maximum.

In the process of the culture experiment, the seawater was aerated and saturated with oxygen. Dead prawns were immediately taken away, freeze-dried and weighed. After one year, the living prawns were netted, freeze-dried and weighed. The yield of the prawns included the weight of the dead and living prawns.

Known amounts of all the freeze-dried prawns were separately pounded, then wet-digested with a mixture of concentrated HNO_3 and $HClO_4$ solution and analyzed for chromium and phenol using the diphenyl carbodiazide calorimetric method and the 4-aminoantipyrine calorimetric method, which were developed by Hong (1987) and proved to be reliable analytical methods (Zhou 1994; Zhou and Zhu 1997). Blanks and standard reference materials were included in the digestion procedure and analysed as the samples in order to guarantee the accuracy of the analytical data. As for the standard reference materials from the food monitoring centre of China, one contains 1.00 mg chromium per kg sample (dry wt), another 0.50 mg phenol per kg sample (dry wt). The analytical results showed that the concentrations of chromium and phenol in the blank were close to zero.

Table 1. Concentrations of chromium and phenol in the seawater.

Combination		Concentration (µg/L)				
I	Cr	0.2*	50	100	500	1000
	Phenol	0.006*	0.006*	0.006*	0.006*	0.006*
II	Cr	0.2*	50	100	500	1000
	Phenol	5	5	5	5	5
III	Cr	0.2*	50	100	500	1000
	Phenol	10	10	10	10	10
IV	Cr	0.2*	50	100	500	1000
	Phenol	50	50	50	50	50
V	Cr	0.2*	50	100	500	1000
	Phenol	100	100	100	100	100
VI	Cr	0.2*	50	100	500	1000
	Phenol	150	150	150	150	150

* Background concentration, no external addition.

RESULTS AND DISCUSSION

If the single factor treatment of 5 µg/L external phenol addition and no chromium addition was excluded, the yield of prawns in the control (0.2 µg/L chromium and 0.006 µg/L phenol) was highest (Table 2). Obviously, the growth of prawns was inhibited due to the toxic action of chromium and phenol in seawater. Statistical analysis of all the results in Table 2 indicated that the yield of prawns (Y) was negatively correlated with the increase in the concentrations of chromium (X_1) and phenol (X_2) in the seawater, according to the regression equations:

$$Y = -(0.0246X_1 + 0.177X_2) + 70.91 \quad (r = -0.974, n=30) \qquad (1)$$

or

$$Y = -3.30\times10^{-4}X_1X_2 + 59.20 \quad (r = -0.809, n=30) \qquad (2)$$

Equation (1) can be rearranged as follows:

$$Y = -0.0246(X_1 + 7.2X_2) + 70.91 \quad (r = -0.974, n=30) \qquad (3)$$

Or

$$Y = -0.0246(X_1 + X_2) -0.152X_2 + 70.91 \quad (r = -0.974, n=30) \qquad (4)$$

Equation (2) (3) or (4) can tell us that chromium and phenol in seawater had synergically unfavourable effects on the growth of prawns.

Table 2. Mean yield of 10 prawns (g fresh wt) after a 1-year culture.

Concentration of phenol (µg/L)	Concentration of Cr (µg/L) in the seawater									
	0.2		50		100		500		1000	
	$\bar{X}$	CV	$\bar{X}$	CV	$\bar{X}$	CV	$\bar{X}$	CV	$\bar{X}$	CV
0.006	68.67	0.277	68.49	0.453	65.27	1.80	63.81	2.35	49.78	2.59
5	69.11	1.27	67.17	0.309	64.97	0.901	62.33	0.903	46.31	0.525
10	67.26	1.86	65.24	0.796	63.19	1.44	62.20	1.42	41.63	1.09
50	62.77	1.77	62.57	0.643	61.96	2.82	51.71	0.763	29.97	3.34
100	57.32	0.347	54.33	2.71	51.69	1.28	44.78	1.06	27.89	0.412
150	48.01	3.32	41.27	1.53	37.33	1.19	30.19	1.18	18.44	1.27

The single-factor and double-factor data in Table 2 were separately regressed. It was shown that the yield of prawns decreased with the increase in the concentration of phenol in the seawater when chromium was at the background level of no chromium addition. The regression equation is as follows:

$$Y = -0.134X_2 + 69.25 \quad (r = -0.993, n=6) \qquad (5)$$

When phenol in the seawater was at the background level of no phenol addition, the yield of prawns was negatively correlated with the increase in the concentration of chromium in the seawater. The regression equation is as follows:

$$Y = -0.0176X_1 + 69.02 \quad (r =- 0.958, n=6) \qquad (6)$$

When chromium and phenol were simultaneously added into the seawater, the interrelationship between the yield of prawns and the concentrations of chromium and phenol in the seawater could be expressed as

$$Y = -0.0255(X_1 + 7.2X_2) + 71.39 \quad (r = -0.976, n=20) \tag{7}$$

Note: Equation (7) is similar to equation (3).

We believe that the death of some prawns in the culture experiment can be attributed to the chronic toxic effects of low concentrations of chromium and phenol in the seawater. The mortality of the cultured prawns (M) was not only dependent on the concentration of chromium in the seawater, but also the function of the concentration of phenol in the seawater (Table 3):

$$M(\%) = 0.0328(X_1 + 5.3X_2) - 5.50 \quad (r = 0.874, n=30) \tag{8}$$

or

$$M(\%) = 4.72X_1X_2 + 2.82 \quad (r = 0.911, n=30) \tag{9}$$

Equation (8) or (9) can tell us that chromium and phenol in seawater were syngercially responsible for the mortality of prawns.

Table 3. The mortality (%) of prawns after a 1-year culture.

Concentration Of phenol (μg/L)	Concentration of Cr (μg/L) in the seawater									
	0.2		50		100		500		1000	
	$\bar{X}$	CV	$\bar{X}$	CV	$\bar{X}$	CV	$\bar{X}$	CV	$\bar{X}$	CV
0.006	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	27.5	0.96
10	0.00	0.00	0.00	0.00	2.50	0.50	20.0	0.82	30.0	0.82
50	0.00	0.00	0.00	0.00	10.0	0.82	30.0	0.00	47.5	0.50
100	0.00	0.00	10.0	0.00	22.5	0.50	37.5	0.50	50.0	0.82
150	0.00	0.00	17.5	0.50	30.0	0.82	40.0	0.82	57.5	0.50

We found that there was no death of marine prawns under the single-factor experimental conditions. Thus, the double-factor (chromium and phenol) data in Table 3 were regressed alone. The regression equation is as follows:

$$M(\%) = 0.0363(X_1 + 5.3X_2) - 5.26 \quad (r = 0.956, n=20) \tag{10}$$

Compared with equation (8) equation (10) gets closer to the real interrelationship between the death of prawns and interaction effects of chromium and phenol in seawater.

Chromium and phenol in the seawater not only have synergically adverse effects on the growth and survival of prawns, but also promote the accumulation of chromium and phenol in prawns. Regression analysis of all the results in Table 4 showed that the concentration of chromium in prawns (X_1') was positively correlated with an increase in the concentrations of chromium and phenol in seawater, according to the regression equation:

$$X_1' = 5.77\times10^{-3}X_1 + 5.90\times10^{-3}X_2 + 0.580 \quad (r = 0.983,\ n=30) \qquad (11)$$

Equation (11) can be rearranged as follows:

$$X_1' \approx 5{,}77\times10^{-3}(X_1 + X_2) + 0.580 \quad (r=0.983,\ n=30) \qquad (12)$$

Thus, we infer that chromium and phenol in the seawater had concentration addition effects on the accumulation of chromium in prawns.

Table 4. Accumulation of chromium (I) and phenol (II) in prawns after a 1-year exposure to various treatments (mg/kg freeze-dried wt).

Concentration of phenol (μg/L)	Concentration of Cr (μg/L) in the seawater									
	0.2		50		100		500		1000	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
(I)										
0.006	0.63	0.125	1.09	0.096	1.23	0.092	3.07	0.203	4.83	0.070
5	0.51	0.120	1.21	0.119	1.38	0.255	4.06	0.804	6.21	0.983
10	0.67	0.056	1.03	0.206	1.41	0.115	3.98	0.246	6.33	0.460
50	0.79	0.089	1.17	0.148	1.49	0.085	4.17	0.845	6.37	0.475
100	0.83	0.110	1.24	0.127	1.53	0.164	4.38	0.308	7.69	0.465
150	1.04	0.164	1.41	0.141	1.79	0.142	4.55	0.348	7.76	0.479
(II)										
0.006	0.07	0.037	0.05	0.037	0.06	0.016	0.13	0.050	0.32	0.074
5	0.09	0.033	0.11	0.040	0.13	0.059	0.21	0.088	0.37	0.039
10	0.15	0.042	0.21	0.051	0.33	0.074	0.57	0.099	0.65	0.104
50	0.26	0.055	0.35	0.063	0.48	0.070	0.87	0.147	0.98	0.213
100	0.45	0.105	0.53	0.206	1.13	0.113	1.43	0.181	1.51	0.215
150	1.53	0.163	1.66	0.068	1.63	0.133	1.79	0.122	1.82	0.190

Correspondingly, the concentration of phenol in prawns (X_2') was positively correlated with an increase in the concentration of phenol and chromium in seawater:

$$X_2' = 9.65\times10^{-3}X_2 + 4.91\times10^{-4}X_1 - 6.38\times10^{-3} \quad (r = 0.956,\ n=30) \qquad (13)$$

Equation (13) can be rearranged as follows:

$$X_2' = 9.65\times10^{-3}(X_2 + 0.051X_1) - 6.38\times10^{-3} \quad (r = 0.956,\ n=30) \qquad (14)$$

It was thus concluded that chromium and phenol in the seawater had partial addition effects on the accumulation of phenol in prawns.

In particular, when the concentration of chromium in the seawater was at the background level, the accumulation of chromium in prawns increased with the increase in the concentration of phenol in the seawater:

$$X_1' = 2.86\times10^{-3}X_2 + 0.595 \quad (r = 0.943,\ n=6) \qquad (15)$$

Similarly, when the concentration of phenol in the seawater was at the background level, the accumulation of phenol in prawns also increased with the increase in the concentration of chromium in the seawater:

$$X_2' = 2.59\times10^{-4}X_1 + 0.0407 \quad (r = 0.971,\ n=6) \qquad (16)$$

In essence, the decrease in the growth of prawns and the death of prawns should be more directly attributed to the accumulation of chromium and phenol in prawns. Statistical analysis based on all the results in Table 2 and 4 indicated that the yield of prawns was simultaneously related to the concentrations of chromium and phenol accumulated in the tested prawns, according to the regression equation:

$$Y = -(2.83X_1' + 15.48X_2') + 71.70 \quad (r = -0.961, n=30) \tag{17}$$

Similarly, statistical analysis based on all the results in Table 3 and 4 indicated that the mortality of the cultured prawns was positively correlated with the accumulation of chromium and phenol in prawns:

$$M(\%) = 4.61X_1' + 15.37X_2' - 8.65 \quad (r = 0.925, n=30) \tag{18}$$

Either under the single-factor or the double-factor conditions, the decreased yield and the increased mortality of prawns were significantly related to the accumulation of chromium and phenol in prawns (Table 5).

Table 5. Relationship between the yield or mortality of prawns and accumulation of chromium and phenol
conditions.

Situation	Chromium in seawater	Phenol in seawater	Regression equation and correlation coefficient
Single Factor (1)	0.2 μg/L (unchangeable & background)	0.006, 5, 10, 50, 100, 150 μg/L	19) $Y = -42.99X_1' + 94.21$ $(r = -0.961, n=6)$ 20) $Y = -14.01X_2' + 68.15$ $(r = -0.949, n=6)$
Single Factor (2)	0.2, 50, 100, 500, 1000 μg/L	0.006 μg/L (unchangeable & background)	21) $Y = -4.16X_1' + 72.24$ $(r = -0.937, n=5)$ 22) $Y = -67.68X_2' + 71.73$ $(r = -0.980, n=5)$
Double Factor	50, 100, 500, 1000 μg/L	5, 10, 50, 100, 150 μg/L	23) $Y = -(2.84X_1'+16.39X_2') + 72.82$ $(r = -0.955, n=20)$ 24) $M(\%) = 4.62X_1'+17.06X_2' - 8.64$ $(r = 0.963, n=20)$

To summarize, we can infer from the above analyses that the accumulation of chromium and phenol in prawns results from the interaction of chromium and phenol in seawater, and the main reason of the decreased yield and the increased mortality of prawns lies in the excessive accumulation of chromium and phenol in prawns. Moreover, the excessive accumulation of chromium and phenol in prawns means that the prawn products produced for human consumption are of poor quality and are possibly toxic. Thus, the key objective of food safety is to stop the excessive accumulation of chromium and phenol in prawns to the maximum. In order to attain this basic goal, the concentrations of chromium and phenol in seawater must be controlled on the basis of combined pollution data, not single-factor results.

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