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Single and joint effects of petroleum hydrocarbons and cadmium on the polychaete *Perinereis aibuhitensis* Grube

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Abstract

Using the concentration gradient and combined pollutant exposure method, the single and joint effects of petroleum hydrocarbons (PHCs) and cadmium (Cd) on polychaete *Perinereis aibuhitensis* Grube, an ecologically keystone species in estuarine and coastal environment, have been investigated. The results indicate that the toxicity of PHCs to *P. aibuhitensis* is stronger than that of Cd to the organism. There are positive correlations between the mortality of worms and the exposed concentration of single Cd or PHCs in solution. Similarly, the accumulation of Cd or PHCs in worms increased with increasing Cd- or PHC-exposed concentrations. All the correlation relationships can be described using unitary quadratic equations (Y or $Z = aX^2 + bX + c$). It is calculated, on the basis of these expressions, that the median lethal dose (LC_{50}) of *P. aibuhitensis* exposed to a single Cd or PHCs is 793.4–13567.3 and 28.0–119.9 μg/L, respectively. The exposed time has some stimulative effect on the two pollutants and on the mortality of the worms. Thus, even a low concentration of a single Cd or PHCs may have strong toxic effects on the worms when the exposed time becomes longer. The accumulation of Cd or PHCs in worms differs with an increase in exposure time at the given exposed concentration of a single Cd or PHCs. Noticeably, the accumulation of PHCs in worms decreases with an increase in exposure time at the given high concentration of PHCs in solution. The joint effect of PHCs and Cd on *P. aibuhitensis* is very complicated and changes with the exposed concentrations of the two pollutants. At the given concentration of PHCs, the joint toxicity of the two pollutants on the worms changes from synergism to antagonism with an increase in Cd concentration. The accumulation of Cd in the worms significantly decreases with the addition of PHCs to exposure solution.

Key words: petroleum hydrocarbon; cadmium; combined pollution; *Perinereis aibuhitensis* Grube; ecotoxicology

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Introduction

Estuaries represent habitats at risk, receiving anthropogenic effluents from various rivers and waters, translocated from remote and nearby domestic, agricultural, and industrial sources of pollution (Zhou *et al*., 2003). Thus, estuarine and coastal organisms have to adapt to highly changing natural conditions and also have to cope with pollutant inputs (Ruiz *et al*., 1993; Zhou *et al*., 2004; Sun *et al*., 2006). Because of the high productivity and crucial role of estuaries in the life history of many invertebrates, fish, and birds (Evans *et al*., 1979; Summers, 1980; Baird *et al*., 1985; Mc Lusky, 1989), the sustainability of estuarine biodiversity is vital to the ecological health of coastal regions.

Among the polychaetes inhabiting estuarine regions, *Perinereis aibuhitensis* Grube is a common species, but it plays an important role in this community as it can regulate other aquatic and terrestrial populations (Commito and Shrader, 1985; Ambroses, 1986), by providing food for many shore birds and oceanic fishes (Michaud and Ferron, 1990). As an ecologically keystone species, *P. aibuhitensis* is well adapted to stressful environmental conditions, with a broad distribution and factors which make them good potential sentinels. Moreover, *P. aibuhitensis* is also regarded as a good bioindicator of metal and organic contamination in estuaries (Gillet, 1987). Generally speaking, these organisms are very sensitive to the presence of metals or organic pollutants in the environment, and the concentrations of metals or organic pollutants accumulated in these organisms are proportional to metal or organic pollutant levels in the environment (Bryan and Hummerstone, 1971, 1973; Nejmeddine *et al*., 1998; Song *et al*., 2005). On the basis of laboratory and field studies, the bioindicator function of polychaetes has been proposed to assess pollution caused by polycyclic aromatic hydrocarbons (PHAs), polychlorinated biphenyls (PCBs), and other organochlorinated compounds (Rossi and Anderson, 1978; Farrington *et al*., 1986; Essink, 1989; Goerke and Weber,

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1990; Rubinstein *et al*., 1990; Reish and LeMay, 1991).

With rapid industrial, agricultural, and urban development, the polychaetes including *P. aibuhitensis* are being increasingly threatened by excessive anthropogenic contamination, involving heavy metals and petroleum hydrocarbons (PHCs), including straight and branched chain saturated alkanes from methane (CH_4), ethane (C_2H_6), and propane (C_3H_8) to $C_{76}H_{154}$. According to documented literature (Newton, 1990; Zhou, 1995; Sun *et al*., 2001; Guo and Zhou, 2003; Zhang *et al*., 2006), PHCs are the most harmful to the growth and development of oceanic plants and animals, and human health. Because of its high biological toxicity, large-area pollution, and long persistence in the environment, PHCs are becoming one of the most intractable oceanic organic pollutants. Similarly, cadmium (Cd) is not only a nonessential element with high toxicity, but also an important pollutant in the Chinese coastal areas (Guo *et al*., 2006). It has been indicated in the authors' previous investigation (Sun *et al*., 2006; Zhou, 2006) that the simultaneous presence of PHCs and Cd in estuarine environment is becoming frequent.

Until now, pollution-monitoring studies using *P. aibuhitensis* have been scarce and the relationship between the mortality of *P. aibuhitensis* and the environmental levels of pollutants, such as, PHCs and Cd, is still vague. The aim of this study was to examine single and joint toxic effects of PHCs and Cd on *P. aibuhitensis*.

1 Materials and methods

1.1 Tested materials

After having collected polychaete *P. aibuhitensis* samples from a seaboard mudflat in Ganyu County, Jiangsu Province, China, the tested samples were transported to the laboratory, using cool boxes with sediment from the sampling site. Prior to the pollutant-exposed experiments, the worms were rinsed with seawater from the sampling site and maintained in the artificial seawater at a salinity of 16‰ for a few days. During acclimation to laboratory conditions, the worms were not fed, to assess only one route of contamination absorption into the living organisms.

The tested PHCs were obtained from a gas station in Shenyang City, Liaoning Province, China. The tested form of Cd was $CdCl_2 \cdot 2.5H_2O$, which was bought from a chemical corporation in the same city. All reagents used in the study were of analytical grade.

All beakers used in the experiments were acid-washed and soaked in the appropriate exposure medium (Mouneyrac *et al*., 2003), to avoid any adsorption of metals onto beakers.

1.2 Pollutant-exposed experiments

Table 1 Treated groups of petroleum hydrocarbons (PHCs) and Cd and their combinations

Pollutant	Dose tested in μg/L
PHCs	0, 5, 10, 50, 100, 500
Cd	0, 200, 500, 1000, 5000, 20000
Cd + PHCs	200+50, 500+50, 1000+50, 5000+50, 20000+50, 200+100, 500+100, 1000+100, 5000+100, 20000+100

The exposed levels (Table 1) of the tested pollutants were set according to the current situation of pollution in Chinese coastal areas. The worms, each weighing 0.5–1.0 g, were put into 250 ml glass beakers with 100 ml of artificial seawater containing exposed levels of pollutants at a salinity of 16‰ as mentioned earlier. There were three worms in each glass beaker, and nine worms in each treatment group. The pollutant-exposed experiments were carried out for two weeks at 15°C according to the temperature of the sampling site. During the experiments, the worms were not fed. Furthermore, the exposed solutions containing PHCs and/or Cd were changed every two days. The worms were checked daily, deaths were recorded, and the mortality was calculated.

1.3 Determination of pollutants

All surviving worms were frozen at –20°C, and then dried to constant weight at 60°C. After having dried and weighed them, half of the worms surviving in the pollutant-exposed experiments were digested in concentrated HNO_3 and $HClO_4$ at 100°C. The digested solution was made up to the known volume with double distilled water and analyzed for Cd by flame atomic absorption spectrophotometry (FAAS, WFX-120A, Ruilin, Beijing).

Another half of the worms were rinsed with distilled water and sipped up with filter paper. After saponification and extraction using petroleum ether, the concentration of PHCs in the extracted solution was determined using the spectrophotometer method at 256 nm.

1.4 Data processing and statistical analyses

The statistical analyses of data obtained from this experiment were carried out using the SPSS11.0, including the calculation of average values, standard deviation (SD), regression, variance analysis, and multiple comparison. ANOVA was used to compare the concentration of PHCs and Cd in the worms and the statistical significance was set at $p < 0.05$.

2 Results and discussion

2.1 Toxic effects of Cd on *P. aibuhitensis*

The results from the pollutant-exposed experiments indicated that Cd had strong toxic effects on polychaete *P. aibuhitensis*. As shown in Fig.1a, there was a positive correlation between the mortality of worms and the concentration of Cd in the solution. In Fig.1b, after a 2-d exposure, the mortality of worms was 0–55.6%, changing with the exposed concentration of Cd; after a 6-d exposure, the mortality of worms was 0–77.8%, changing with the exposed concentration of Cd; and after a 10-d exposure, the mortality of worms reached 33.3%–100.0% when the concentration of Cd in solution was 200–20000 μg/L.

The correlation relationships between the mortality of worms and the concentration of Cd in solution can be

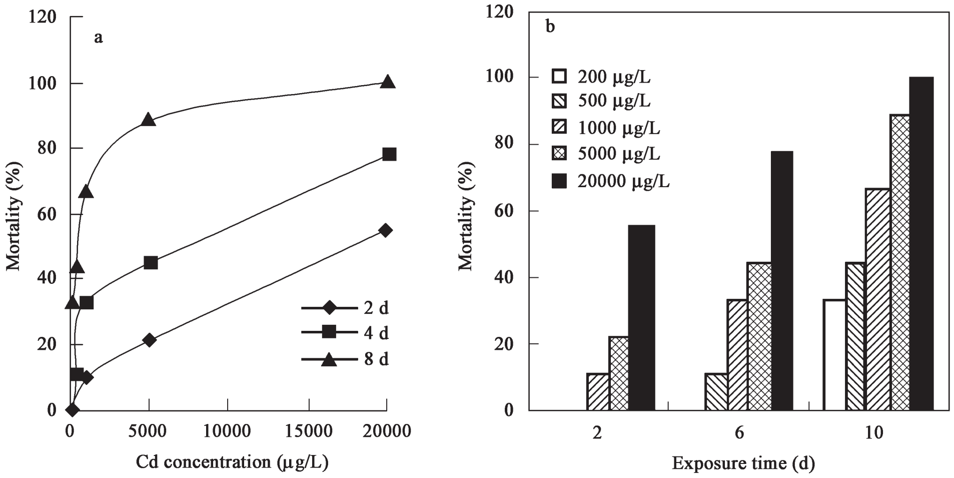

Fig. 1 Relationship between the mortality of *P. aibuhitensis* and the exposed concentration of Cd (a) or the exposed time (b).

expressed using the following regression equation:

$$Y_n = a_1X_1^2 + b_1X_1 + c_1 \tag{1}$$

where, X_1 is the concentration of Cd in the solution (μg/L); Y_n (Y_1, Y_2, and Y_3 in Table 2) is the mortality of worms exposed to Cd for 2, 4, and 8 d, respectively, because of toxic effects of Cd; a_1, b_1, and c_1 are the constants, respectively.

On the basis of the regression equations in Table 2, the LC_{50} values of worms exposed to Cd were calculated. The calculation showed that the LC_{50} values of *P. aibuhitensis* exposed to single Cd were 793.4–13567.3 μg/L. Moreover, the LC_{50} values decreased with increasing exposure time. In other words, even low concentrations of Cd could have strong toxic effects on the worms when the exposure time became longer. According to the LC_{50} values of the worms exposed to single Cd, it could be deduced that *P. aibuhitensis* was not sensitive to the toxicity of Cd pollution. In other words, *P. aibuhitensis* was not a good bioindicator for monitoring Cd pollution in estuarine and coastal environment on the basis of its mortality.

The mortality of worms also increased with increased exposure time at the given concentration of Cd in solution (Fig.1b). When the concentration of Cd in solution was lower than 500 μg/L, there were no deaths of the tested worms after a 2-d exposure; however, the deaths of the tested worms took place in all the treatments after a 10-d exposure. Undoubtedly, the ecological hazard of Cd could occur even when the concentration of Cd in estuarine and coastal environments was greatly lower than the acute lethal concentration under experimental conditions, because the exposure time of Cd in estuarine and coastal environment could become long-term.

The accumulation of Cd in the polychaete *P. aibuhitensis* increased with an increase of Cd concentration to which the worms were exposed (Fig.2a). However, it was indicated by variance of analysis that the differences were not obvious, although Cd accumulation in worms exposed to 20000 μg/L was significantly ($p < 0.05$) higher than that in worms exposed to other concentrations. The correlation relationship between the accumulation of Cd in worms and exposed concentration of Cd in solution can be expressed using the following regression equation:

$$Z_1 = 4.53X_1^2 - 20.58X_1 + 22.54 \\ (n = 8,\ R^2 = 0.958,\ p < 0.01, T = 2) \tag{2}$$

where, Z_1 is the concentration of Cd accumulated in worms, μg/g dw; T is the exposure time, d. According to Eq. (2), the significant ($p < 0.01$) nonlinear relationship between the accumulation of Cd in worms and the concentration of Cd in the exposure solution could be observed.

The accumulation of Cd in worms differed with increased exposure time at the given concentration of Cd. When the concentration of Cd was lower than 1000 μg/L, the differences were not significant. However, the accumulation of Cd in worms significantly ($p < 0.05$) increased when the concentration of Cd in the solution reached 5000 μg/L. As shown in Fig.2a, the accumulation of Cd in worms after an 8-d exposure reached 7.4–155.4 μg/g when the concentration of Cd in solution was 200–20000 μg/L. It can be concluded that the polychaete *P. aibuhitensis* may be considered as a good biomonitor of Cd, with body concentration changing over a wide concentration range, in response to changes in Cd concentration in the solution.

Table 2 Relationships between the mortality of *P. aibuhitensis* and the concentration of Cd or PHCs in solution

Pollutant	Exposed time (d)	Regression equation (n=8)	R^2	LC_{50} (μg/L)
Cd	2	$Y_1 = -1.0 \times 10^{-7}X_1{}^2 + 0.005X_1 + 0.571$	0.979	13,567.3
	4	$Y_2 = -3.0\times10\text{-}7X_1{}^2 + 0.009X_1 + 0.718$	0.899	6,916.8
	8	$Y_3 = -5.0\times10\text{-}7X_1{}^2 + 0.013X_1 + 40.23$	0.905	793.4
PHCs	2	$Y'_1 = -0.0013X_2{}^2 + 0.611X_2 - 4.57$	0.999	119.9
	4	$Y'_2 = -0.0031X_2{}^2 + 1.050X_2 + 0.0044$	0.964	57.3
	8	$Y'_3 = -0.0028X_2{}^2 + 0.918X_2 + 26.49$	0.960	28.0

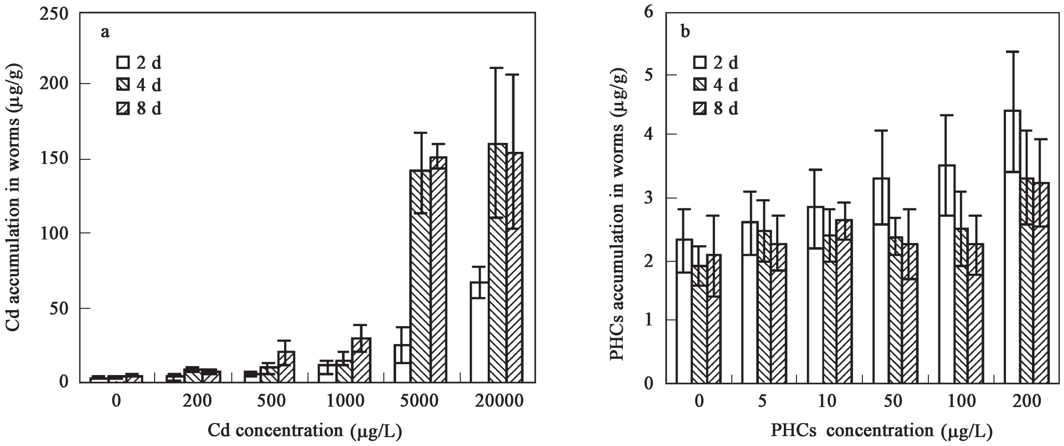

Fig. 2 Accumulation of Cd (a) and PHCs (b) in *P. aibuhitensis* under experimental conditions.

This is consistent with the previous studies by Bryan and Hummerstone (1973) and Bryan *et al*. (1985), which stated that polychaetes were a good biomonitor of Cd availability in sediments.

Although they accumulated a large amount of Cd in their bodies, the polychaete *P. aibuhitensis* were not sensitive to the toxicity of Cd. To a certain extent, the control of intracellular Cd toxicity and detoxification of accumulated Cd in the worms could possibly be attributed to the formation of Cd-binding ligands that could sequester Cd toxicity in the worms. These cellular ligands could be found in the particulate fraction of the cells in the form of mineral deposits and granules of lysosomes (George, 1990; Mason and Jekins, 1995), and in the cytosol, in the form of metallothioneins (Engel and Roesijadi, 1987). On the other hand, the worms could possibly excrete mucus in response to metal exposure. According to Mouneyrac *et al*. (2003), the mucus could bind dissolved trace metals and reduce their availability for uptake. Thus, it was possible that the secretion of mucus by *P. aibuhitensis* might be an important physiological mechanism, coping with very high Cd in the solution.

2.2 Toxic effects of PHCs on *P. aibuhitensis*

The results from the pollutant-exposed experiments also indicated that PHCs had strong toxic effects on *P. aibuhitensis*. The mortality of the tested worms increased with increased concentration of PHCs (Fig.3a). After a 2-d exposure, the mortality of worms was 0–66.7%, changing with the exposed concentration of PHCs; after a 4-d exposure, the mortality of worms was 0–88.9%, changing with the exposed concentration of PHCs; and after an 8-d exposure, the mortality of worms reached 22.2%–100.0%, when the concentration of PHCs was 5–200 μg/L.

The correlation between the mortality of *P. aibuhitensis* and the concentration of PHCs in solution can be described as follows:

$$Y_m = a_2X_2^2 + b_2X_2 + c_2 \tag{3}$$

where, X_2 was the concentration of PHCs in solution, μg/L; Y_m (Y'_1, Y'_2, and Y'_3 in Table 2) was the mortality of worms exposed to PHCs for 2, 4 and 8 d, respectively; a2, b2, and c2 were the constants, respectively. On the basis of the regression equations in Table 2, the LC$_{50}$ values of the worms exposed to PHCs were calculated. The calculation showed that the LC$_{50}$ values of *P. aibuhitensis*

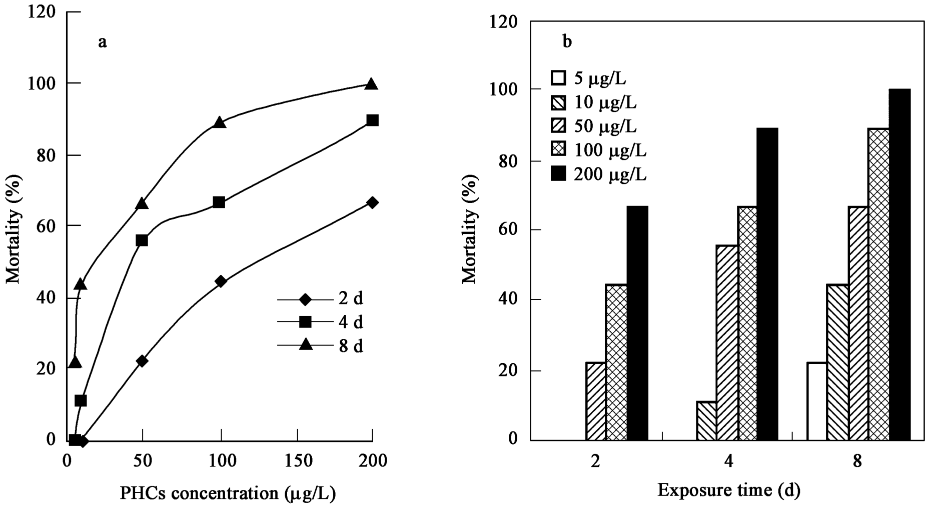

Fig. 3 Relationship between the mortality of *P. aibuhitensis* and the concentration of PHCs (a) or the exposed time (b).

exposed to single PHCs were 28.0–119.9 μg/L. Moreover, the LC_{50} values decreased with increasing exposed time. In other words, even low concentration of PHCs could have strong toxic effects on the worms when the exposed time became longer. According to the LC_{50} values in Table 2, it could be deduced that the toxicity of PHCs to *P. aibuhitensis* was stronger than that of Cd to the organism. Thus, *P. aibuhitensis* could be considered as a bioindicator for monitoring pollution of PHCs in estuarine and coastal environments, in view of its morality.

The mortality of worms also increased with increased exposure time at the given concentration of PHCs (Fig.3b). After a 2-d exposure, there were no deaths of the tested worms when the exposed concentration of PHCs was lower than 10 μg/L. After an 8-d exposure, the death of the tested worms occurred in all the treatments. Thus, it was not difficult to understand that the ecological hazard of PHCs could take place even when the concentration of PHCs in estuarine and coastal environment was lower than 10 μg/L, because the exposure time of PHCs in the estuarine and coastal environment was long-term and not shorter than 8 d.

There was a difference in the accumulation of PHCs in *P. aibuhitensis* exposed to increasing PHCs concentrations (Fig.2b). However, the difference was not significant by variance analyses. For example, the change in PHCs accumulated in the worms after an 8-d exposure was only 2.07–3.24 μg/L when the concentration of PHCs in solution was 5–200 μg/L. In other words, the accumulation of PHCs in *P. aibuhitensis* did not differ greatly in response to a series of increasing exposed concentrations of PHCs. In this sense, *P. aibuhitensis* did not seem to be not a good potential biomonitor of PHCs pollution in estuarine and coastal environment.

The correlation between the accumulation of PHCs in worms and the concentration of PHCs in solution can be expressed using the following regression equation:

$$Z_2 = 0.0482X_2^2 + 0.0531X_2 + 2.25 \quad (n = 8, R^2 = 0.980, p< 0.01, T = 4) \tag{4}$$

where, Z_2 is the accumulation of PHCs in worms, μg/g; T is the exposure time. From Eq.(4), the significant ($p <$ 0.05) nonlinear relationship between the accumulation of PHCs in worms and the concentration of PHCs in exposed solution could be observed.

The accumulation of PHCs in worms also differed with increased exposure time at the given concentration of PHCs (Fig.2b). It was indicated by the variance of analysis that the accumulation of PHCs in worms was not significantly different when the concentration of PHCs in solution was 5 and 10 μg/L, respectively. However, when the exposed concentration of PHCs was equal to and higher than 50 μg/L, the accumulation of PHCs in worms decreased significantly ($p < 0.05$) with exposure time at the given concentration of PHCs. In other words, the accumulation of PHCs in worms decreased with increased exposure time at the given high concentration of PHCs in the environment. It could be elementarily presumed that the elimination of PHCs in *P. aibuhitensis* was probably responsible for the induction of metabolism and excretion under the stress of high PHCs, although the relevant physiological mechanism of this phenomenon is to be explored in the future.

2.3 Joint effects of PHCs and Cd on *P. aibuhitensis*

The joint action of PHCs and Cd on *P. aibuhitensis* was very complicated and changed with the exposed concentrations of the two pollutants (Table 3). At the given concentration of PHCs, the joint effects of the two pollutants on *P. aibuhitensis* changed from synergism to antagonism with increasing the Cd concentration. When the concentration of Cd was equal to or lower than 500 μg/L, the joint effects of the two pollutants were synergistic. At a low exposed concentration of Cd, the joint toxicity of PHCs and Cd on *P. aibuhitensis* was higher than the toxicity of Cd alone. However, the joint toxicity of PHCs and Cd was lower than the toxicity of Cd alone when the concentration of Cd was equal to or higher than 1000 μg/L. In other words, the joint effects of the two pollutants on the worms were antagonistic at the high exposed concentration of Cd.

These experimental results also indicated that the accumulation of Cd in *P. aibuhitensis* decreased with the addition of PHCs. It was indicated by the variance of analyses that the accumulation of Cd in worms exposed to the combined pollution of Cd and 50 and 200 μg/L PHCs differed insignificantly when the concentration of Cd in solution was lower than 500 μg/L. However, there was a significant ($p < 0.01$) decrease in the accumulation of Cd in worms when the Cd in the solution was higher than 1000 μg/L and the concentration of PHCs in solution was 50 and 100 μg/L, respectively, when compared with the

Table 3 Mortality of *P. aibuhitensis* after a 2-d exposure of Cd or/and PHCs with various doses

Cd		PHCs		Cd+PHCs		Joint effect
Exposed dose (μg/L)	Mortality(%)	Exposed dose (μg/L)	Mortality (%)	Exposed dose (μg/L)	Mortality (%)	
200	0	50	22.2	200+50	44.4	Synergism
200	0	200	66.7	200+200	88.9	Synergism
500	0	50	22.2	500+50	55.6	Synergism
500	0	200	66.7	500+200	88.9	Synergism
1000	11.1	50	22.2	1000+50	0	Antagonism
1000	11.1	200	66.7	1000+200	11.1	Antagonism
5000	22.2	50	22.2	5000+50	0	Antagonism
5000	22.2	200	66.7	5000+200	33.3	Antagonism
20000	55.6	50	22.2	20000+50	11.1	Antagonism
20000	55.6	200	66.7	2000+200	44.4	Antagonism

exposure solution having the same concentration of only Cd. Therefore, it could be concluded that the joint effects of PHCs and Cd on the accumulation of Cd in worms were also complicated.

3 Conclusions

There were positive correlations between the mortality of worms and the exposed concentration of single Cd or PHCs in solution. Moreover, the mortality of worms increased with increased exposure time at the given concentration of single Cd or PHCs in solution. Thus, even a low concentration of single Cd or PHC might have strong toxic effects on the worms when the exposed time became longer.

It was calculated on the basis of the regression equations that the LC_{50} values of *P. aibuhitensis* exposed to single Cd or PHCs were 793.4–13567.3 and 28.0–119.9 μg/L, respectively. Moreover, the LC_{50} values decreased with increasing exposure time. According to the LC_{50} values, it could be deduced that the toxicity of PHCs to *P. aibuhitensis* was stronger than that of Cd to the organism.

The accumulation of Cd or PHCs in the polychaete *P. aibuhitensis* increased with increasing Cd or PHCs concentrations to which the worms were exposed, and differed with increased exposure time at the given exposed concentration of single Cd or PHCs. In particular, the accumulation of PHCs in worms decreased with increased exposure time at the given high concentration of PHCs in the solution. There was a wide range in the concentration of Cd accumulated in worms in response to changes in the Cd concentration of the solution, but the accumulation of PHCs in worms did not differ greatly in response to a series of increasing exposed concentrations of PHCs.

Joint effects of PHCs and Cd were complicated. When the concentration of Cd in solution was equal to or lower than 500 μg/L, there was synergic toxicity between the two pollutants on the mortality of *P. aibuhitensis*. When the concentration of Cd in solution was equal to or higher than 1000 μg/L, joint toxicity of PHCs and Cd on the mortality *P. aibuhitensis* was antagonistic. To a certain extent, the accumulation of Cd in *P. aibuhitensis* was affected by the combined pollution of PHCs and Cd. In particular, there was a significant ($p < 0.01$) decrease in the accumulation of Cd in worms when Cd in solution was higher than 1000 μg/L and the concentration of PHCs in solution was 50 and 100 μg/L, respectively.

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