Effects of Some Heavy Metals on the Growth of Grass Shrimp

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Introduction

Grass shrimps are distributed widely. They are an important source of food. Rearing grass shrimps has increased over the last few years, especially in the coastal provinces of the Cuu Long Delta in Vietnam resulting in significantly improved household incomes. However, contamination of water resources is a common occurrence and large numbers of grass shrimps are killed as a result of pollution. This has happened all over the country since 1993. Studies found the cause of these deaths and solutions were proposed. Grass shrimps however continued to die in large numbers. The research, presented in this paper indicates that the cause of these deaths may be due to heavy metal contamination. Phyto-toxicological studies using shrimps were undertaken to measure the effects of chemical and chemical and environmental variables in estuarine and marine environments on shrimp mortality.

Test materials and variables

Grass shrimps, marine and brackish water species, 22 days old from a shrimp farm in Baclieu Province, Vietnam were used during the experiments. The test shrimps have an average length of 2cm and weight of 0.1 g. The shrimps are grown under experimental conditions for several days before the beginning of tests.

The water supply used during the period of experimentation is near-shore seawater from Baclieu Province with low turbidity, high DO and low BOD. Salinity 19 (± 0.038), pH ranges from 8.0 – 8.5 and temperature is maintained at 28 – 30^{o}C. In addition, the water is filtered and chlorinated. The test shrimps are held in a glass tank of at least 8 litres capacity with flow through air and 2 to 3cm of sand over the bottom and a screen at the surface to prevent loss of test subjects. Ten shrimps are retained in each tank. Synthetic tablet food is provided to the shrimps four times per day.

Heavy metal compounds used throughout the experiments were $NaAsO_2$, $Cd(NO_3)_2$, $Cu(NO_3)_2$; $Cr(NO_3)_3$, $Fe(NO_3)_3$, $Hg_2(NO_3)_2$, $Pb(NO_3)_2$ and $Zn(NO_3)_2$ as manufactured by BUDAFEST (Hungary). All heavy metal compounds are dissolved to pre-selected concentrations using distilled water and further diluted with near-shore seawater from Baclieu Province.

During the range finding toxicity tests over a 48 hours test period grass shrimps are exposed to a heavy metal concentrations ranging from 0.01, 0.10, 1.00, 10.00 and 100.00 mg l^{-1}. For the short term definitive tests, for 96 hours a series of different heavy metal concentrations is applied with different dilution factors. All tests are undertaken in triplicate.

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Evaluation

The symptoms of shrimps after contact with the different heavy metal solutions are observed and noted, as well as the number of dead and/or affected shrimps in each container at 3, 6, 9, 12, 24, 48, 72 and 96 hours after the beginning the test. Dead shrimps are removed immediately. Temperature, pH, DO, BOD, salinity, $N\text{-}NH_3$ and $N\text{-}NO_2^-$ are measured daily and at the beginning and end of each test.

Nonparametric statistical test for variance in concentrations of heavy metals between the average lethal threshold for shrimps and the analytical statement are used to evaluate the impact of treatments on shrimp mortality. A graph showing the toxicity of each heavy metal as expressed by a linear equation $y = ax + b$ is constructed with mortality rates on the y-axis (y=M) and heavy metal concentration on the x-axis (x= lg C).

LC_{50} is determined from the linear equation of toxicity of each heavy metal on shrimps in the aquatic environment at different points in time. LC_{50} is defined as the lethal concentration at which 50% of the shrimp population dies after an exposure time of 96 hours.

Results

Arsenic toxicity and its effect (As^{3+})

Arsenic although widespread in plant and animal tissue, has become synonymous with "poison" in the public mind. In spite of its toxicity, it has been employed for its medical virtues in the form of organic arsenicals, and in partial prevention of selenosis. It appears that the stable, soluble inorganic arsenites and arsenates are readily absorbed by the digestive tract, abdominal cavity and muscle tissue. Arsenate has a low order of toxicity and does not inhibit any enzymatic activities due to its lack of affinity to hydroxo and thiol groups. However, ATP synthesis is inhibited by AsO_4^{3-} through uncoupling oxidative phosphorylation and the replacement of the stable phosphoryl group. In contrast, arsenite inhibits thiol–dependent enzymes, binds to tissue protein as keratin disulfides in skin and nails and is retained in the body for a prolonged period. The experiment shows that the mortality rates depend on the time of immersion and on the concentration of arsenic. At 0.01 mg As l^{-1}, 10% shrimp mortality occurs after 72 hours and 20% after 96 hours. Increasing the solution As concentration results in increased mortality rates. At 1 mg As l^{-1} 10% shrimp mortality occurs after 6 hours and 100% mortality occurs after 72 hours.

Table 1. Experimental data from hypothetical toxicity test of As^{3+} subjected to probit analysis

Solution As concentration (mg l^{-1})	No. of test shrimps	Shrimp mortality rate (%) over time (hrs)							
		3	6	9	12	24	48	72	96
0.0	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.01	10	0.0	0.0	0.0	0.0	0.0	0.0	10.0	20.0
0.03	10	0.0	0.0	0.0	0.0	6.7	10.0	23.3	40.0
0.1	10	0.0	0.0	0.0	0.0	10.0	33.3	40.0	50.0
0.3	10	0.0	0.0	6.7	20.0	43.3	53.3	66.7	70.0
1.0	10	0.0	10.0	30.0	53.3	80.0	86.7	100	100

Cadmium toxicity and its effect (Cd^{2+})

Cadmium (Cd) being the middle member of the periodic sub-group consisting of Zn, Cd, and Hg reveals intermediate properties. All three elements display a profound capacity of combining with SH. The stability of such complexes increases in the order Zn < Cd < Hg. In this experiment, shrimps become disorientated 8hrs after coming into contact with $Cd^{2+}$and die after 48hrs. At a solution concentration of 0.005 mg Cd l^{-1} 10% shrimp mortality occurs after 72hrs and 20% mortality after 96hrs.

Table 2. Experimental data from hypothetical toxicity test of Cd^{2+} subjected to probit analysis

Solution Cd concentration (mg l^{-1})	No. of test shrimps	Shrimp mortality rate (%) over time (hrs)							
		3	6	9	12	24	48	72	96
0.0	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.005	10	0.0	0.0	0.0	0.0	0.0	0.0	10.0	20.0
0.015	10	0.0	0.0	0.0	0.0	0.0	13.3	26.7	30.0
0.05	10	0.0	0.0	0.0	0.0	20.0	36.6	50.0	66.7
0.15	10	0.0	0.0	0.0	16.7	23.3	53.3	66.7	83.3
0.5	10	0.0	0.0	10.0	33.3	80.0	96.7	100	100

Copper toxicity and its effect (Cu^{2+})

Copper is found in enzymes capable of carrying oxygen as hemoglobin. Copper is also essential in a number of enzymes. Excessive intake of copper however results in its accumulation in the liver. Generally, copper toxicity increases when Mo, Zn, and SO_4^{2-} intake is low.

In this experiment, a solution concentration of 0.1 mg Cu l^{-1} which is known to be toxic to shrimps was used. At this concentration 3.3% shrimp mortality was observed after 48 hrs and 36.7% mortality observed after 96 hrs. At a solution concentration of with 10 mg Cu l^{-1} 16.7% shrimp mortality occurred after 6 hrs and 100% mortality occurred after 72 hrs.

Table 3. Experimental data from hypothetical toxicity test of Cu^{2+} subjected to probit analysis

Solution Cu concentration (mg l^{-1})	No. of test shrimps	Shrimp mortality rate (%) over time (hrs)							
		3	6	9	12	24	48	72	96
0.0	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1	10	0.0	0.0	0.0	0.0	0.0	3.3	10.0	36.7
0.3	10	0.0	0.0	0.0	0.0	26.7	30.0	46.7	56.7
1.0	10	0.0	0.0	0.0	20.0	46.7	60.0	70.0	83.3
3.0	10	0.0	10.0	20.0	26.7	40.0	90.0	90.0	93.3
10.0	10	0.0	16.7	30.0	46.7	90.0	96.7	100	100

Chromium toxicity and its effects (Cr^{3+})

Chromium (Cr) is one of the least toxic of the trace elements. Generally the mammalian body can tolerate 100-200 times its total body content of Cr^{3+} without harmful effects. However, chromium (VI) compounds are approximately 100 times more toxic than Cr (III). The stomach acidity leads to reduction of Cr (VI) to Cr (III) of which gastrointestinal absorption is less than 1%. In this experiment, at a Cr solution concentration of 0.1 mg Cr l^{-1} 3.3% of the test shrimp population died after 48 hrs with 30% mortality at 96 hrs. At 10 mg Cr l^{-1}, 20% mortality was observed after 3 hrs with 100% mortality at 72 hrs.

Table 4. Experimental data from hypothetical toxicity test of Cr^{3+} subjected to probit analysis

Solution Cr concentration ($\mu g\ l^{-1}$)	No. of test shrimps	Shrimp mortality rate (%) over time (hrs)							
		3	6	9	12	24	48	72	96
0.0	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1	10	0.0	0.0	0.0	0.0	0.0	3.3	13.3	30.0
0.3	10	0.0	0.0	0.0	10.0	10.0	30.0	40.0	50.0
1.0	10	0.0	0.0	10.0	10.0	20.0	40.0	70.0	80.0
3.0	10	0.0	10.0	10.0	20.0	20.0	56.7	80.0	93.7
10.0	10	20.0	20.0	30.0	60.0	73.3	96.7	100	100

Iron toxicity and its effects (Fe^{3+})

Iron (Fe), the most abundant transition element and is essential in biologic systems (haemoglobin in blood, the oxygen-carrying protein molecule regarded as the most important iron (II) complex). In this experiment, high solution Fe concentrations of 3.0 mg Fe l^{-1} resulted in 93.3% shrimp mortality after 96 hrs. However, low solution concentrations of 0.1 mg Fe l^{-1} resulted in 16.7% mortality at 72 hrs and 23.3% mortality after 96 hrs.

Mercury toxicity and its effect (Hg^{+})

Mercury (Hg) is considered a nonessential but highly toxic element for living organisms. Even at low concentrations, Hg and its compounds present potential hazards due to accumulation in the food chain. Poisoning by methyl mercury compounds presents a bizarre neurological picture as observed in large-scale outbreaks in Japan and Iraq. The higher toxicity of Hg as compared to Cd cannot be attributed to the smaller ionic radius and greater penetration of Hg^{2+} ion. The profound capacity of the soft acid (acceptor) CH_3Hg^{+} to bind soft ligands such as –SH groups of proteins is a more plausible explanation for the high toxicity of methyl mercury compounds. Mercury in the test solution rapidly affected the test shrimps. Immediate reactions to mercury in solution were observed. At 0.5 mg Hg l^{-1}, 30% mortality rate was observed after 3 hrs and 100% mortality after 72 hrs. At solution Hg concentrations of 0.005 mg Hg l^{-1} 10% mortality was observed at 72 hrs and 16.7% at 96 hrs.

Figure 1. Fe^{3+} median lethal concentration (LC_{50}) determinations at three representative times by probit analysis and line of best fit.

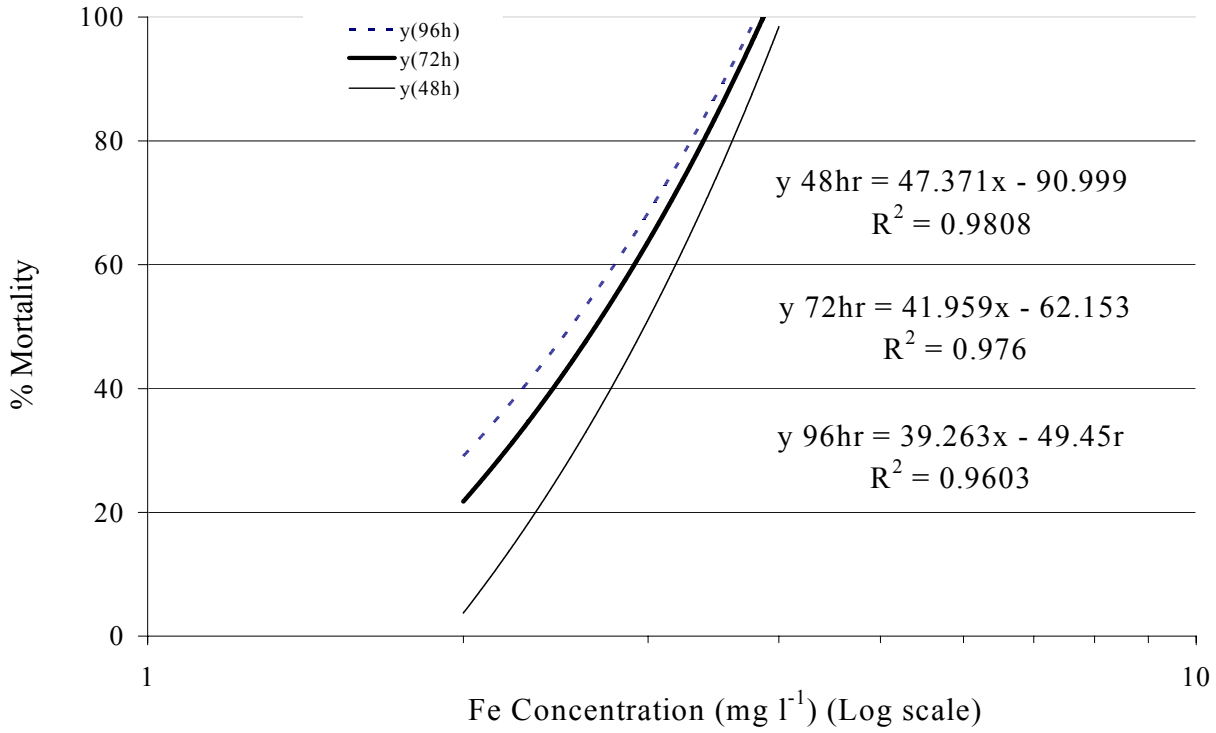
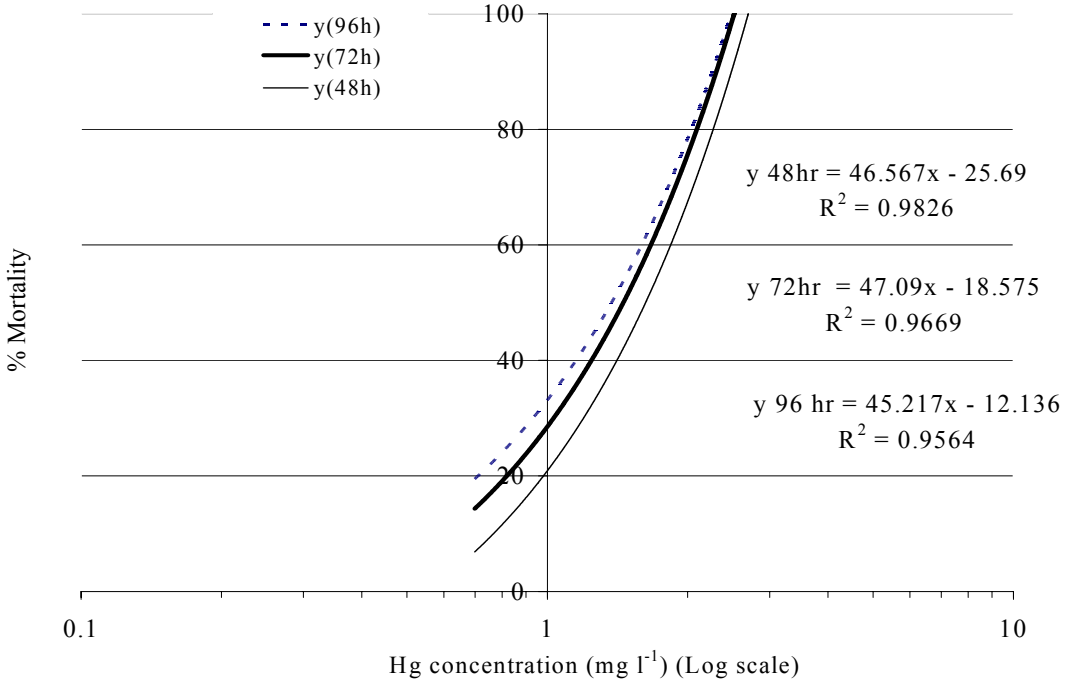


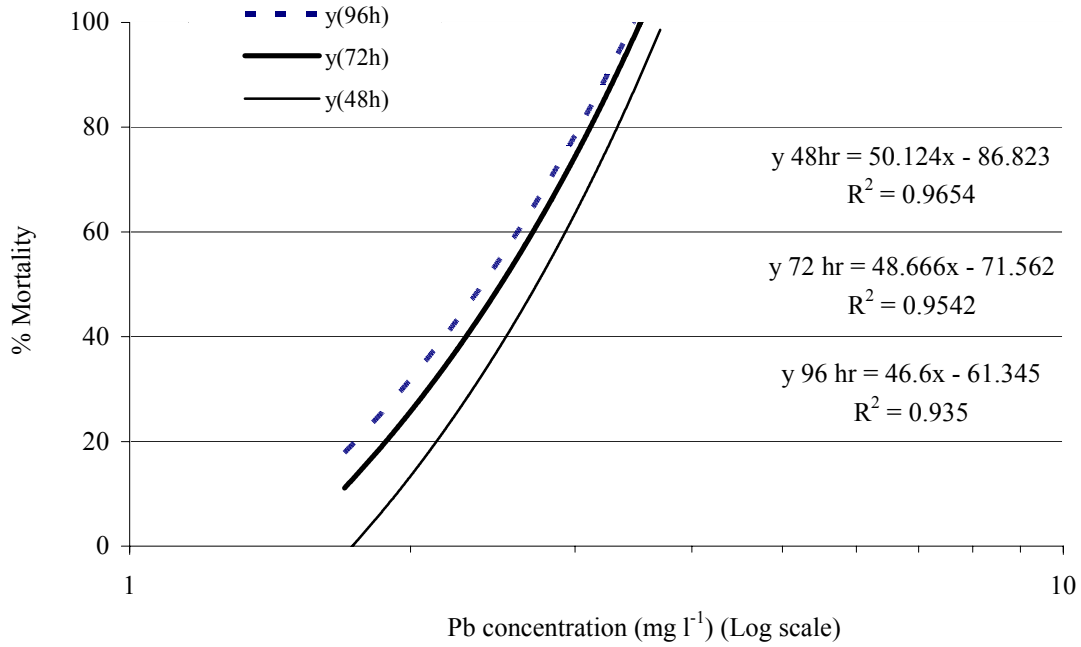
Figure 2. Hg median lethal concentration (LC_{50}) determinations at three representative times by probit analysis and line of best fit.



Lead toxicity and its effects (Pb^{2+})

Lead (Pb) resembles the divalent alkaline earth group metals in chemical behavior more than its own Group IVA metals. It differs from the Group IIA metals in the poor solubility of Pb salts such as hydroxides, sulfates, halides, and phosphates. Metabolism of Pb and calcium are similar both in their deposition in and mobilization from bone. Since Pb can remain immobilized for years, metabolic disturbances can remain undetected. Under normal conditions more than 90% of the Pb retained in the body is stored in the skeleton. The affinity of Pb^{2+} for thiol and phosphate-containing ligands inhibits the biosynthesis of haem and thereby affects membrane permeability of kidney, liver and brain cells. This results in either reduced functioning or complete breakdown of these tissues, since Pb is a cumulative poison. In this experiment a Pb solution concentration of 0.005 mg Pb l^{-1} resulted in shrimp mortality of 10% after 72 hrs. However, a solution concentration of 5.0 mg Pb l^{-1} resulted in 10% mortality after 3 hrs and 100% mortality after 72 hrs.

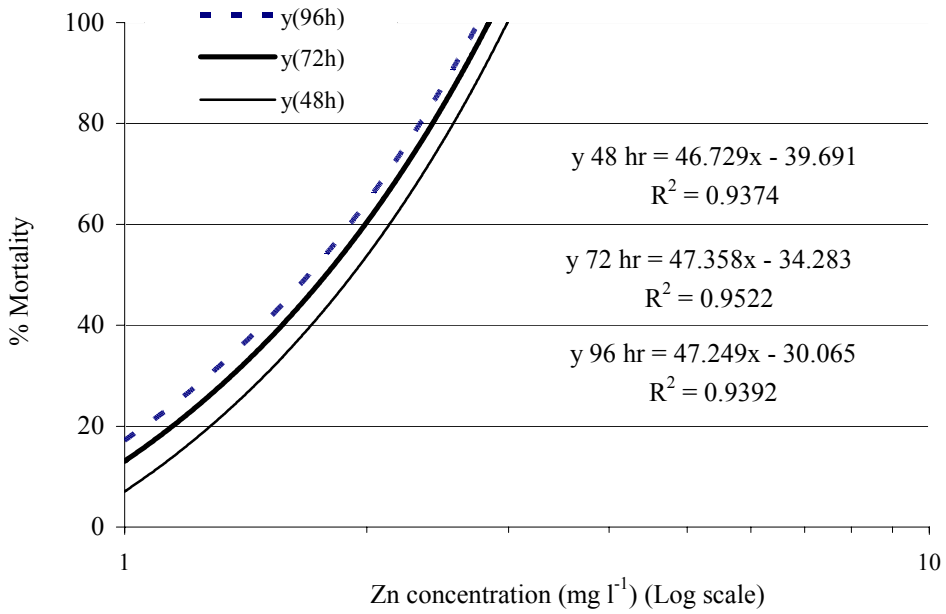
Figure 3. Pb median lethal concentration (LC_{50}) determinations at three representative times by probit analysis and line of best fit.



Zinc toxicity and its effects (Zn^{2+})

Zinc is one of the most abundant of the essential elements required by the human body. Zinc appears to be present in all mammals. As with cobalt (II), Zn (II) has the ability to occupy low symmetry sites in enzymes. In this experiment, a solution Zn concentration of 0.01 mg l^{-1} resulted in 10% mortality at 72 hrs. In contrast, at a solution concentration of 1.0 mg l^{-1} shrimp mortality was 10% at just 3 hrs and 100% at 72 hrs.

Figure 4. Zn median lethal concentration (LC_{50}) determinations at three representative times by probit analysis and line of best fit.



Conclusion

Heavy metals as contaminants in an aquatic environment cause very serious harm to the growth and development of grass shrimps. The results of the laboratory experiments presented in this paper allow the establishment of preliminary lethal concentrations associated with 50% shrimp mortality after 96 hours (LC_{50}-96hr) for different heavy metals as follows:

- As^{3+} = 67.61 µg l^{-1}
- Cd^{2+} = 28.84 µg l^{-1}
- Cu^{2+} = 177.83 µg l^{-1}
- Cr^{3+} = 269.15 µg l^{-1}
- Fe^{3+} = 338.84 µg l^{-1}
- Hg^{+} = 23.44 µg l^{-1}
- Pb^{2+} = 245.47 µg l^{-1}
- Zn^{2+} = 48.89 µg l^{-1}

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