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THE EFFECT OF SIMULTANEOUS EXPOSURE OF COPPER AND LEAD TO HYBRID TILAPIA (*Oreochromis* sp.)

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Abstract: Generally, in the environment, aquatic organisms are continuously being exposed to more than one heavy metal. The main objectives of the study are to observe the simultaneous effects of copper (Cu) and lead (Pb) and the relationship between these metals in a series of concentrations. Metal accumulations in the fish gills (exposed to Cu alone, exposed to Pb alone, exposed to synthetic solution of both Cu and Pb) were measured for 21 days of exposure period. Results of the exposure experiment indicated that Pb accumulated more than Cu in single-metal exposure ($p < 0.05$) throughout the exposure period, whereas Cu seems to facilitate the uptake of Pb and vice versa throughout the exposure period. For the first 14 days of exposure, the Cu concentration was higher in the fish gills than Pb. However, the accumulation of Pb in the fish gills increased drastically during the last 7 days of exposure period in all concentrations. This clearly shows a possible alteration of the physiological absorption process.

KEYWORDS: Copper, Lead, conference, water

Introduction

The quality of human life is directly or indirectly dependent on the health, vitality and vigour of the environment around us. The aquatic environment, which covers two-thirds of the planet, is inhabited by the majority of extant species in their ecological niches. Moreover, many of them are important sources of human food while at the same time playing vital roles for ecosystem functioning, human health and civilization. As a consequence, disposal of anthropogenic chemicals and wastes continue to increase. The aquatic environment is often the ultimate recipient of this increasing range of anthropogenic contaminants, a large proportion of which are potentially carcinogenic and genotoxic substances (Jha, 2004). Metal ions are essential as trace elements, but at higher concentrations, they become toxic. Heavy metals in ionic forms are difficult to remove from the environment and ultimately indestructible, unlike many other pollutants that can be chemically or biologically degraded. Today, heavy metals constitute a global environmental hazard (Yoshida *et al.,* 2005).

Fishes are often at the top of the aquatic food chain and may concentrate large amounts of some metals from the water. Furthermore, fish are one of the most indicative factors in aquatic systems, for the estimation of trace metals pollution and risk factors in freshwater systems, for the estimation of trace metals pollution and risk potential of human consumption (Yilmaz, 2005). Generally, in the environment, fish are continuously exposed to more than one heavy metal. Many studies have demonstrated the sensitivity of fishes to single metals at concentrations in the 0.01–0.1 mg/l range for Hg and Cu, and 0.1–10 mg/l for Cd and Pb (Gulden and Hasso, 2005). In contrast, few studies on fishes have addressed the interactions of metals, despite the fact that effluents running into the aquatic environment normally contain more than one trace metal in significant amounts. The toxicity of a chemical can be enhanced (positive interaction or synergism), reduced (negative interaction or antagonism), or unaffected (no interaction) by the presence of another toxicant.

In this study, we examine how the concurrent presence of lead with copper could affect lead accumulation and vice versa in hybrid tilapia. The usual experimental approach to study interactions consists of testing the toxicity of the chemicals in combination and comparing the results with those from tests with single chemicals. Additive effects are considered to exist when no significant deviations from the expected combined effect are found.

Materials and Methods

Fish and experimental design

Hybrid tilapia (*Oreochromis niloticus* x O. *aureus*) at the age of 2 to 3 months were obtained from a local farmer in Kuala Terengganu. Upon arrival to the institute, the fish were housed in 250 litre aquaria in a designated flow-through system, 30 days prior exposure for acclimatisation to laboratory conditions. Fish were subjected to a photoperiod of 12 h light/12 h dark and were fed daily. Every effort was made to provide optimal conditions for the fish and no fish mortality occurred during the acclimatisation period.

A flow-through system (FTS) was constructed to support both acute and sub-lethal toxicity tests with all tests done in triplicate (Figure 1). De-chlorinated tap water was delivered into a 1000 L fibre tank and then pumped up by water pump at the rate of 2 L per hour. The dilution water outlet supplied all five test tanks at a flow rate of 180 ml/hour by adjusting the gravity flow. Toxicant solutions were added into the aquarium by peristaltic pump at a rate of approximately 20 ml / hour. The dilution water and toxicants were mixed in a mixing chamber placed at the top of the first aquarium of each concentration. The test tanks (aquariums) were arranged in series so that the solution flowed into the first replicate test aquarium of the same concentration and this will became the toxicant source for the second tank and later this will be moved to the third aquarium. From the third test aquarium, the solution flowed again into the first test aquarium repeatedly via pipe host from individual water pump, while the source excess flowed out to a treatment tank. The excess flow toxicant or wastewater was treated using charcoal-activated carbon via filter before entering the nearby dumping site. Aeration was provided for each test chamber to supply oxygen. Control fish were held in similar facility without any exposure to Cd. The fish were fed twice a day on fish pellets.

Toxicity Test

Hybrid tilapia were tested against lower concentrations of Cu and Pb based on data obtained from the previous acute-toxicity test (Vijayendran *et al.,* 2006). The test result of the median lethal concentration (LC_{50}) of Cu and Pb at 96 h was 1.832 mgl^{-1} and 33.23 mgl$^{-1,}$ respectively. For the single-exposure test, fishes were exposed to 10%, 20% and 40% of the pre-determined 96 h LC_{50} value. In the simultaneous-exposure case, the fish were exposed to various treatments, one with a constant copper level and varied lead concentrations and the other with a constant lead level and varied copper concentrations (Table 1). In both experiments, 20 healthy fishes of the same size (3.21± 0.05 g) were randomly selected and transferred to the experimental aquaria after acclimatisation phase. Tests were performed in triplicate. Fishes were be starved for 24 hours before experiment but feeding was performed once a day after the first 24 hours. Physical Behaviour of the fishes was observed daily and the fishes were harvested at every 7 days for 21 days.

Analytical procedures

Briefly, fishes from the sub-lethal toxicity test were cleaned with de-ionized distilled water to remove surface-bound metal before being killed. The viscera (kidney and liver), intestines, gills and muscle tissues were dissected using clean equipment. The tissues were then put into an oven to dry at 80°C. The dried, crushed organs and tissues were placed in a Teflon beaker with a mixture of 1 ml of concentrated perchloric acid (Merck, Germany) and 1 ml of nitric acid (65% m/v) (Merck, Germany). The mixture was left to preliminary digestion for 3 hours. Then it was digested at 100°C and placed upon a hot plate until the mixture completely digested. A clean solution was obtained and the mixture was evaporated to nearly dry. Then, the mixture was topped to 50 ml with de-ionized water. Cd concentrations for each sample were measured using ELAN® 6000 Inductively-Coupled Plasma Mass Spectrometer (ICPMS) (PerkinElmer Sciex Instruments, Concord, Ontario, Canada). A six-point calibration curve was produced with a selection of certified primary standard stock solutions (CLARITAS Spex Certiprep). An optimal sample dilution factor of 500 was chosen for tissue samples. To account for instrumental changes during analysis, 100 ml of internal standard containing Indium and Bismuth (1 mgl^{-1}) was added to every sample. With every 5 samples analysed, blanks and standards or calibration checks were included to check for instrument drift and ensure continuity and stability throughout the sample analysis. The metal concentrations in the digestion blanks were typically very low (<1.0 μgl^{-1}) and were subtracted from the sample values. The limits of detection of the ICP-MS were determined (3 standard deviation of the blanks) and were sufficiently low to analyse the sample concentrations. Accuracy of the ICPMS and validity of the process were tested with a reference material, DORM 2 (dogfish muscle; National Research Council Canada).

Statistical analysis

The descriptive statistical analyses were performed using the software SPSS for Windows (Version 11.0). The data of Cd accumulation were tested for homogeneity of variance and normality and were found normally distributed. The data were analysed by use of two-way analysis of variance (ANOVA). Differences between level means per factor were treated using Tukey`s multiple comparison of means.

Results and Discussion

This experiment was designed to measure the accumulation of lead and copper in the gills of hybrid tilapia after exposure to a mixture of the two metals. Aqueous exposure samples were analysed for actual Cu and Pb concentrations throughout the exposure which are reported in table 2. Since most of the analytical confirmations were at least 95% of nominal values, results are reported in terms of nominal concentrations for the rest of the experiment. The uptake rate of copper and lead in fish gills has been plotted against the ambient concentrations of the single and combination metal respectively (Fig, 2 and Fig. 3). A brief examination of Fig. 2 revealed that the accumulation of Cu in the gills increased in a nearly linear manner with the increases in the exposure Cu and a fixed concentration of Pb. The same phenomena was observed in Fig 3 whereby a steady increment of Pb concentration was observed with the increases in the exposure Pb and a fixed concentration of Cu. The values were statistically significant ($p<0.05$) when 2- way Anova was applied. Thus, we suggest that the uptake of one metal by gills was directly proportional to the ambient concentration of the other metal.

This seems to indicate there is a significant synergistic effect between copper and lead in fish uptake of either metal. Erema and Stephen, 2006 reported that the accumulation of Zn by *L.*

saxatilis showed that interaction between Zn and Cu in combined solutions indicated concentration-dependent effects, switching from apparent synergism at low concentrations of Cu to antagonism at high concentrations of Cu. Apart from this, Buhl and Hamilton (1997) reported that the toxicity of two mixtures of nine inorganic on Ptychocheilus lucius and Xyrauchen texanus in a reconstituted water solution and found that both mixtures exhibited either additive or greater-than-additive toxicity in these fishes.

It has been noted that gill surfaces make up more than half of the total external area of a fish and it is obvious to observe an elevated level of metal contents after exposure to the metal (Tao *et al.,* 1999). However, it has been suggested that gills are a temporary target organ to accumulate metal and this metal is transferred to digestive organs (such as intestine, kidney and liver) via circulatory system or the enterohepatic circulation (Chang *et al.*, 2007; Wu *et al.,* 2007). Apart from this, fish release ammonia and carbon dioxide at their gills, which respectively consume H ions to form ammonium and dissociate to bicarbonate, releasing H ions. If water next to the gills is made more alkaline (through NH_3 release) or more acidic (through CO_2 release) there could be effects on metal speciation and solubility, which could influence how metals interact at fish gills.

When a toxic substance is introduced into the aquatic environment three main steps can be identified before a response is produced from an aquatic organism. These involve, respectively: (1) Chemical and physico-chemical processes, in which the substance interacts with other constituents of the water and become available to the organism (Buhl and Hamilton, 1996); (2) Physiological processes, including absorption, transport, distribution, metabolic transformation, accumulation, and excretion (Calamari and Alabaster, 1980) and (3) Intoxication processes, including combination with receptors. Theoretically, the two simplest explanations for the physiological synergistic effects of lead and copper on fish uptake would seem to be as follows: (1) the absorption of lead was facilitated directly by the coexistence of copper and vise versa and (2) certain kinds of physiological alternation on target organism or organs caused by the chronic toxic effect of copper enhanced the uptake rate of lead (Vranken *et al,* 1988). The possible physiological alteration that occurred is the ability of Pb to substitute the Ca ion in the gills as it is believed that Pb has similar characteristics with Ca. Besides that, the interaction between one metal with the complexing ligands, such as dissolved organic matter (DOM) enhance the toxicity of the other metal.

Conclusion

Accumulation of copper and lead in hybrid fish gills exposed to the two metals demonstrated synergistic effects. Copper of various exposure concentrations facilitated fish uptake of lead throughout the experiment and vice versa. Generally, to predict the impact of metal-polluted effluents in the aquatic environment, it would be necessary to test not only binary mixtures, but also combinations of three or more metals, or even combinations of metals with organic pollutants. This approach becomes increasingly difficult because of the exponential multiplication of the number of test groups with the increasing numbers of chemicals in a mixture (Cassee *et al.,* 1999). Furthermore, metals not only interact among themselves, but their combined toxicity also depends on environmental factors which affect metal speciation (such as pH, salinity, and especially the levels and types of dissolved organic matter). Estuarine waters are often rich in organic matter such as humic acids, with the ability to form complex free metal ions and reduce their bioavailability. Estuaries are also exposed to periods of reduced pH and salinity, and these conditions are known to commonly increase metal toxicity compared to oceanic seawater. All these factors should be taken into account in order to extrapolate results from laboratory toxicity tests to the aquatic environment.

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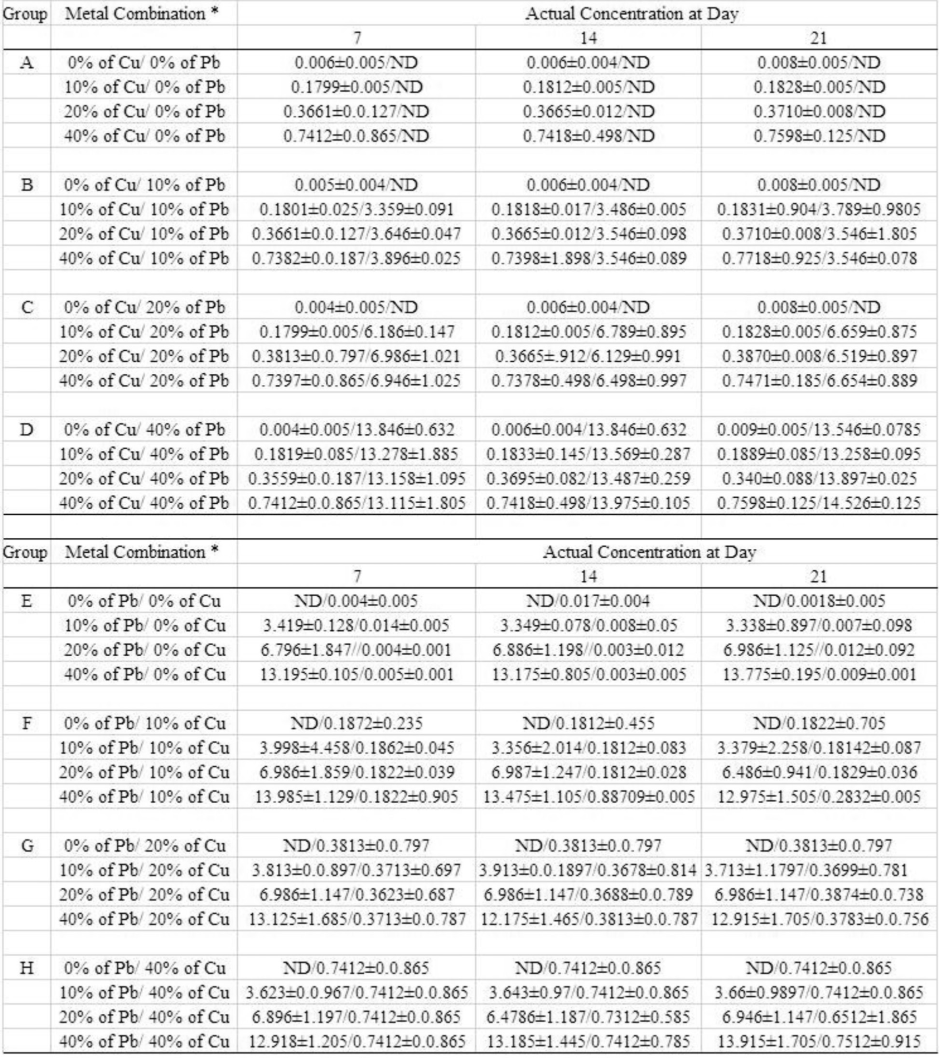
Table 1: Concentrations of Copper and Lead for the simultaneous exposure experiment, Values based on 96 h LC$_{50}$ of each metal.

Group	Copper (mg/l)	Lead (mg/l)
A	10%, 20%, 40%	10%
B	10%, 20%, 40%	20%
C	10%, 20%, 40%	40%
D	10%	10%, 20%, 40%
E	20%	10%, 20%, 40%
F	40%	10%, 20%, 40%

Table 2: Actual Concentration of Copper and Lead for the simultaneous exposure experiment at day 7, 14 and 21



Group	Metal Combination *	Actual Concentration at Day		
		7	14	21
A	0% of Cu/ 0% of Pb	0.006±0.005/ND	0.006±0.004/ND	0.008±0.005/ND
	10% of Cu/ 0% of Pb	0.1799±0.005/ND	0.1812±0.005/ND	0.1828±0.005/ND
	20% of Cu/ 0% of Pb	0.3661±0.0.127/ND	0.3665±0.012/ND	0.3710±0.008/ND
	40% of Cu/ 0% of Pb	0.7412±0.0.865/ND	0.7418±0.498/ND	0.7598±0.125/ND
B	0% of Cu/ 10% of Pb	0.005±0.004/ND	0.006±0.004/ND	0.008±0.005/ND
	10% of Cu/ 10% of Pb	0.1801±0.025/3.359±0.091	0.1818±0.017/3.486±0.005	0.1831±0.904/3.789±0.9805
	20% of Cu/ 10% of Pb	0.3661±0.0.127/3.646±0.047	0.3665±0.012/3.546±0.098	0.3710±0.008/3.546±1.805
	40% of Cu/ 10% of Pb	0.7382±0.0.187/3.896±0.025	0.7398±1.898/3.546±0.089	0.7718±0.925/3.546±0.078
C	0% of Cu/ 20% of Pb	0.004±0.005/ND	0.006±0.004/ND	0.008±0.005/ND
	10% of Cu/ 20% of Pb	0.1799±0.005/6.186±0.147	0.1812±0.005/6.789±0.895	0.1828±0.005/6.659±0.875
	20% of Cu/ 20% of Pb	0.3813±0.0.797/6.986±1.021	0.3665±.912/6.129±0.991	0.3870±0.008/6.519±0.897
	40% of Cu/ 20% of Pb	0.7397±0.0.865/6.946±1.025	0.7378±0.498/6.498±0.997	0.7471±0.185/6.654±0.889
D	0% of Cu/ 40% of Pb	0.004±0.005/13.846±0.632	0.006±0.004/13.846±0.632	0.009±0.005/13.546±0.0785
	10% of Cu/ 40% of Pb	0.1819±0.085/13.278±1.885	0.1833±0.145/13.569±0.287	0.1889±0.085/13.258±0.095
	20% of Cu/ 40% of Pb	0.3559±0.0.187/13.158±1.095	0.3695±0.082/13.487±0.259	0.340±0.088/13.897±0.025
	40% of Cu/ 40% of Pb	0.7412±0.0.865/13.115±1.805	0.7418±0.498/13.975±0.105	0.7598±0.125/14.526±0.125
Group	Metal Combination *	Actual Concentration at Day		
		7	14	21
E	0% of Pb/ 0% of Cu	ND/0.004±0.005	ND/0.017±0.004	ND/0.0018±0.005
	10% of Pb/ 0% of Cu	3.419±0.128/0.014±0.005	3.349±0.078/0.008±0.05	3.338±0.897/0.007±0.098
	20% of Pb/ 0% of Cu	6.796±1.847//0.004±0.001	6.886±1.198//0.003±0.012	6.986±1.125//0.012±0.092
	40% of Pb/ 0% of Cu	13.195±0.105/0.005±0.001	13.175±0.805/0.003±0.005	13.775±0.195/0.009±0.001
F	0% of Pb/ 10% of Cu	ND/0.1872±0.235	ND/0.1812±0.455	ND/0.1822±0.705
	10% of Pb/ 10% of Cu	3.998±4.458/0.1862±0.045	3.356±2.014/0.1812±0.083	3.379±2.258/0.18142±0.087
	20% of Pb/ 10% of Cu	6.986±1.859/0.1822±0.039	6.987±1.247/0.1812±0.028	6.486±0.941/0.1829±0.036
	40% of Pb/ 10% of Cu	13.985±1.129/0.1822±0.905	13.475±1.105/0.88709±0.005	12.975±1.505/0.2832±0.005
G	0% of Pb/ 20% of Cu	ND/0.3813±0.0.797	ND/0.3813±0.0.797	ND/0.3813±0.0.797
	10% of Pb/ 20% of Cu	3.813±0.0.897/0.3713±0.697	3.913±0.0.1897/0.3678±0.814	3.713±1.1797/0.3699±0.781
	20% of Pb/ 20% of Cu	6.986±1.147/0.3623±0.687	6.986±1.147/0.3688±0.0.789	6.986±1.147/0.3874±0.0.738
	40% of Pb/ 20% of Cu	13.125±1.685/0.3713±0.0.787	12.175±1.465/0.3813±0.0.787	12.915±1.705/0.3783±0.0.756
H	0% of Pb/ 40% of Cu	ND/0.7412±0.0.865	ND/0.7412±0.0.865	ND/0.7412±0.0.865
	10% of Pb/ 40% of Cu	3.623±0.0.967/0.7412±0.0.865	3.643±0.97/0.7412±0.0.865	3.66±0.9897/0.7412±0.0.865
	20% of Pb/ 40% of Cu	6.896±1.197/0.7412±0.0.865	6.4786±1.187/0.7312±0.585	6.946±1.147/0.6512±1.865
	40% of Pb/ 40% of Cu	12.918±1.205/0.7412±0.0.865	13.185±1.445/0.7412±0.785	13.915±1.705/0.7512±0.915

- Value based on 96 h LC$_{50}$ of each metal reported early by Vijayendran et al., 2006. Abbreviation: ND- Non detectable

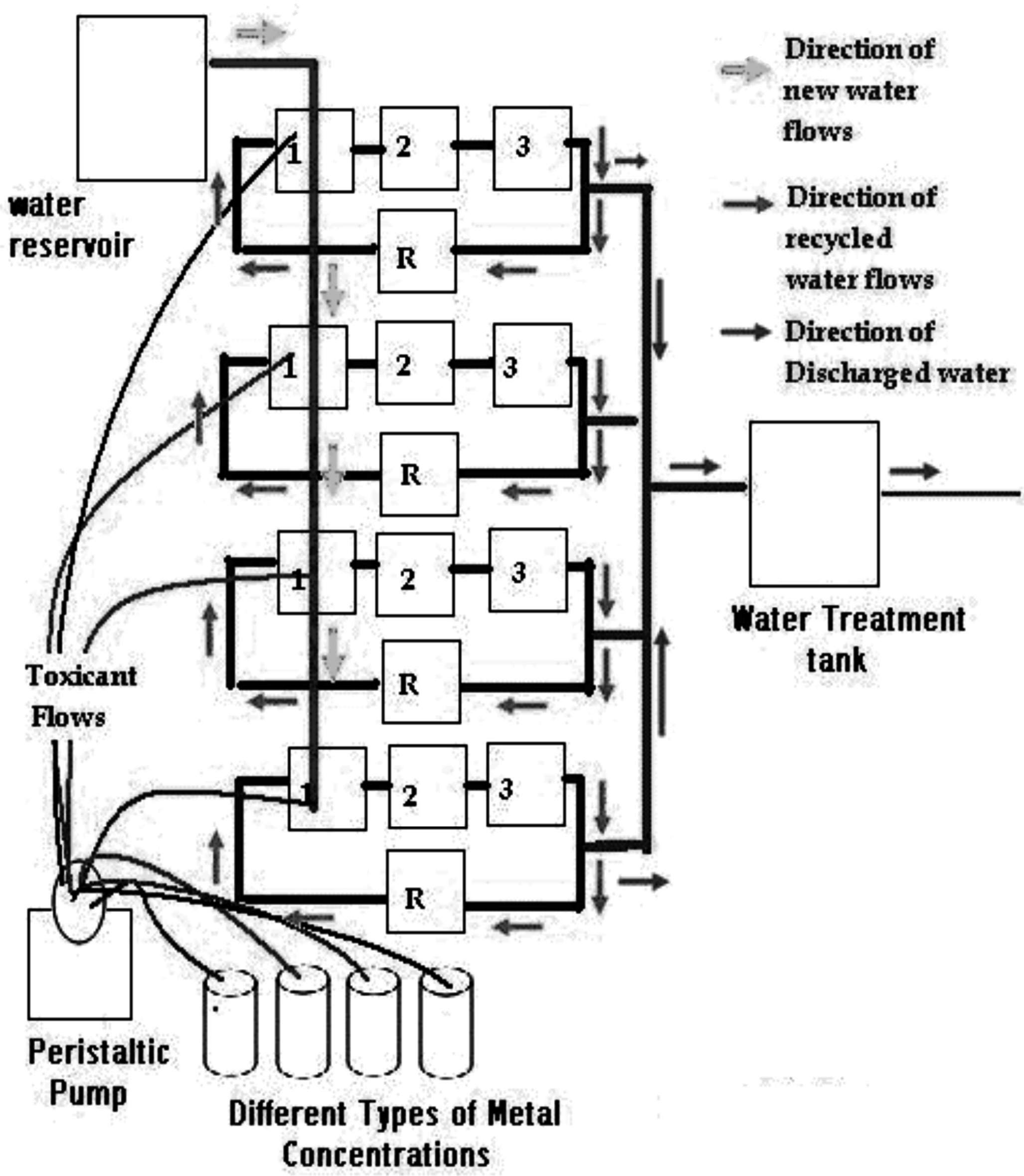


Figure 1. Layout of the Flow-through system

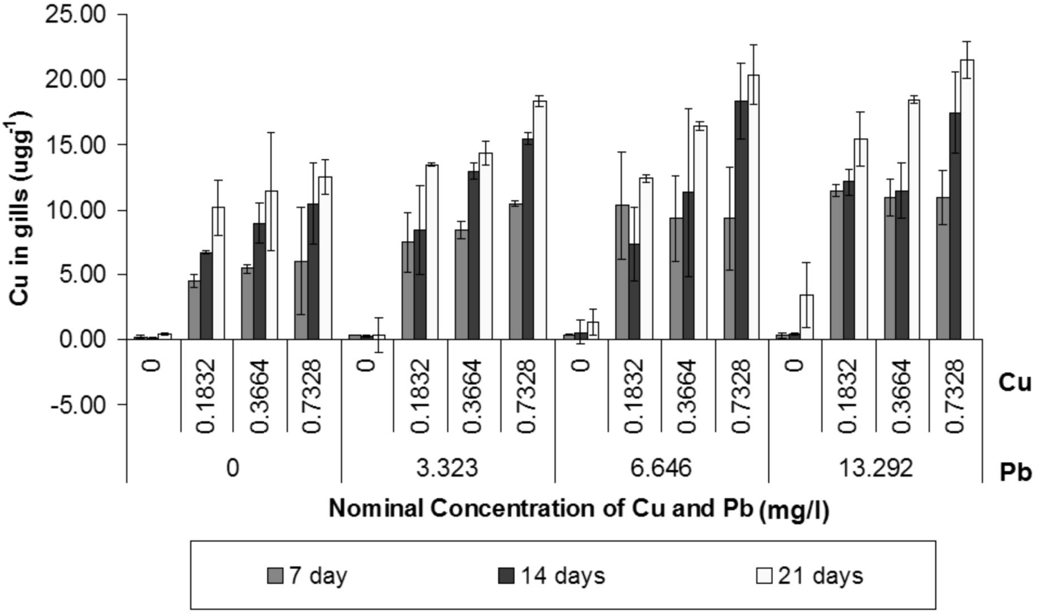


Figure 2. Copper uptake by the gills exposed to single and various combinations of Copper and Lead

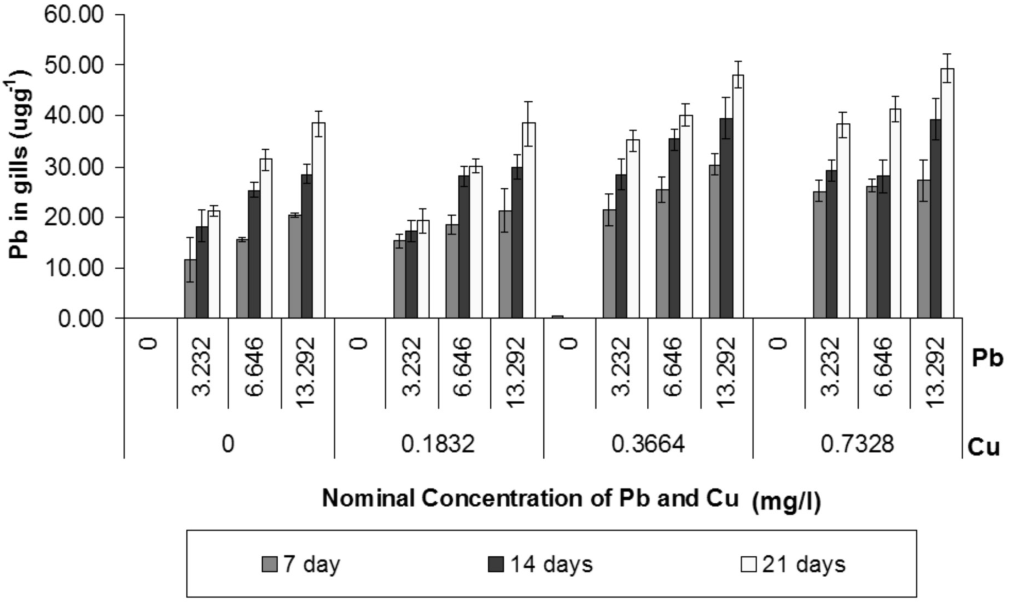


Figure 3. Lead uptake by the gills exposed to single and various combinations of Copper and Lead