Toxicity effects of trace metals on Zebra fish (*Danio rerio*) embryo

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Introduction

Water quality deterioration is a major problem in the most of the countries, due to organic and inorganic contaminants. Among all the toxicant trace metals are threaten to the aquatic ecosystem and organisms. Metals are natural component of aquatic ecosystem. Trace metals such as Copper (Cu), Zinc (Zn) and Chromium (Cr) are important for the metabolic and other biological activities of lives whereas Mercury (Hg), Lead (Pb), Cadmium(Cd) are biologically non-essential metal that can be toxic to biota even at very low concentration. High concentration of some essential trace metals can be toxic when it exceeds the limits(Ebrahimi and Taherianfard, 2011).High accumulation of trace metal in both biotic and abiotic components causes serious health consequences. Thus, assessment of their toxicity has become an important component of water pollution monitoring. Now in most of the toxicity studies Zebra fish (*Danio rerio*) embryo used as an alternative model for the fish acute toxicity to determine the toxicity of pollutants. Therefore this study was focused on determination of the acute toxicity of Cu, Zn, Cd, As, Pb and Hg that produce lethal effect on zebra fish embryos during four day period.

Materials and Methodology

Zebra fish breeding was carried out and eggs were collected using a small pipette. The stock solution of 1000 ppm of selected Cu, Zn, Pb, Cd, As and Hg were prepared a day before the test by dissolving Copper sulfate ($CuSO_4.5H_2O$), Zinc sulfate ($ZnSO_4.7H_2O$), Lead nitrate ($Pb(NO_3)_2$, Arsenic pentoxide (As_2O_5), Cadmium chloride ($CdCl_2.½ H_2O$) and Mercury chloride ($HgCl_2$) in deionized water. The working treatment solution was prepared daily by serial dilution of the stock solution. Initially a range finding test was carried out in the nominal concentrations of (1000, 100, 10, 1 and 0.1 mgL^{-1}) for 96 hours. Ten eggs per concentrations were used. Deionized water was used as a negative control. Each treatment had three replicates. Acute Cu, Zn, Pb, As, Cd and Hg toxicity experiments were performed for a 4-day period using *Danio rerio* embryo. Ten test concentrations of Cu, Cd (0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 10.0 mgL^{-1}), Zn, Pb, As (2.0, 4.0, 8.0, 16.0, 32.0,64.0, 125.0,250.0, 500.0 and 1000.0 mgL^{-1}) and Hg (0.002, 0.004, 0.008, 0.016, 0.032, 0.064, 0.125, 0.250, 0.500 and 1.0 mgL^{-1}) were prepared by diluting the stock solution in deionized water. Deionized water was used as negative control and as internal plate control. As a positive control concentration of 15% ethanol(Hassan *et al*., 2008) was used with each embryo batch for testing. Fertilized zebra fish embryos were immersed in the test solutions before cleavage, by the 16 cell-stage. At least twice the number of eggs needed per treatment group (40 eggs) was randomly selected and transferred into the respective concentrations and controls within the 90 minutes of post fertilization. 24-well plates were filled with 2 mL per well freshly prepared test solutions. In each plate twenty wells were used for test concentrations and four were used as internal plate control. Another plate was used as positive control. The eggs in standard 24- well plate were covered by shelf adhesive foil and maintain at a temperature to 26 ± 1 °C. The mortality of the fish eggs were recorded at 24, 48, 72 and 96 hours of exposure using a stereo microscope with the indicator of lethality; coagulation of fertilized egg, lack of somite formation, lack of detachment of the tail and lack of heart beat as described in OECD/ OCDE 236, 2013; guideline for the test of chemical, Fish Embryo Acute Toxicity (FET) tests. Dead eggs were removed immediately. Three replicates were performed individually. In this study the acute toxic effect of each metal on the *Danio rerio* embryo was

determined by Probit Analysis LC_{50} determination method. Data analysis was performed by using statistical software SPSS15.

Results and Discussion

According to the analysis in the range finding test, minimum concentration that can cause 100 % mortality was recorded. Based on the result obtained, range for As, Pb and Zn is below the 1000.0 mgL^{-1}, for Cd and Cu less than 10.0 mgL^{-1} and for Hg less than 1.0 mgL^{-1}. The relationship between the metal concentration and mortality rate of each trace metal was recorded based on the four apical endpoints. It shows that mortality rate is increasing with the increasing concentration and time of exposure. Acute toxicity of As, Cd, Hg, Pb, Cu and Zn showed that mortality is directly proportional to the concentration of the trace metals. The probit analysis revealed that there is a significance difference between the trace metals and control group ($P< 0.05$).According to the analysis of median lethal concentration (LC_{50}), Hg is highly toxic to the *Danio rerio* embryo and followed by the Cu, Cd, Zn, As and Pb. The toxicity trend of LC_{50} 96 hrs observed was Hg (0.0217 mgL^{-1}) < Cu (0.099 mgL^{-1}) < Cd (0.407 mgL^{-1})< Zn (14.021 mgL^{-1}) < As (34.840 mgL^{-1}) <Pb (41.697 mgL^{-1}). LC_{50} and upper and lower confidence limits revealed a decreasing trend from 24 to 96 hrs(Table 1). Fish embryo stage is highly sensitive to metal pollution. The chorion does not fully protect the embryo against metal penetration. In this study, the most toxic trace metal to *Danio rerio* embryo is Hg. The LC_{50}values of Hg at 24, 48, 72 and 96 hrs wereless than 0.04 mgL^{-1}.It shows that even at lower concentration it can cause high mortality rate on *Danio rerio* embryo.

Table 2: Summarized LC_{50} value of trace metals on *Danio rerio* for a period of 24 – 96 hrs

Trace metals	LC_{50} (95% confident limits – lower, upper)			
	24hrs	48hrs	72hrs	96hrs
Hg	0.0397 (0.0324,0.0488)	0.0229 (0.0245,0.0367)	0.0218 (0.0182,0.0263)	0.0217 (0.0180,0.0260)
Cu	0.1735 (0.1498,0.2011)	0.1306 (0.1128,0.1512)	0.0991 (0.0844,0.1157)	0.0991 (0.0844,0.1157)
Cd	0.5660 (0.4339,0.741)	0.464 (0.3505,0.613)	0.418 (0.3152,0.550)	0.407 (0.3072,0.536)
Zn	23.083 (18.871,28.13)	17.080 (13.936,20.801)	14.021 (11.347,17.147)	14.021 (11.347,17.147)
As	42.915 (36.223,50.97)	38.313 (32.007,45.98)	34.840 (28.903,42.08)	34.840 (28.903,42.08)
Pb	113.8 (88.5,149.6)	63.90 (50.81,81.0)	43.75 (35.75,53.71)	41.70 (33.97,51.34)

In *Danio rerio* embryo toxicity test several sub- lethal and teratogenic endpoints were observed. In control no deformities were observed. Growth retardation, Shrinkage of chorion, Scoliosis, Pericardial edema, Yolk sac edema, Lack of pigmentation, Tail deformities, Heamorrhages, missing formation of lens and Lack of otoliths are the major deformities observed during the study. Growth retardation is mainly caused by growth inhibitorsofCd and Cu (Sikorska and Wolnicki, 2006). Skeletal deformities in fish are good bio indicators of pollution (Bengtsson, 1979; Lemly, 1997; cited in Curtis, 2004). Damage of the vertebral column expressed as curvature of the larval body axis is

caused by all heavy metals toxification (Jezierska *et al.*, 2000; Nguyen and Janssen, 2002; Hallare *et al.*, 2005; cited in Osman, 2007). The tail deformities genetically resulting from the inability of treated embryos to expresses the *evenskippid* gene (Osman, 2007).Pigmentation is controlled by Melanocyte Stimulating Hormone (αMSH) and Melanin-Concentrating Hormone (MCH). Pigmentation changes in fish are often due to stress induced (Nguyen and Janssen, 2002; cited in Osman, 2007).

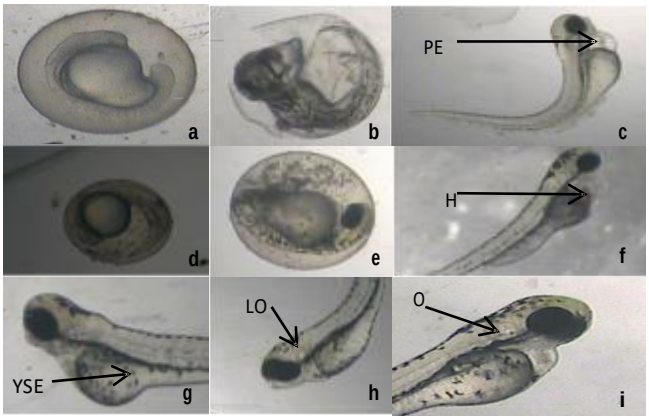

Figure 2: Sub-lethal and terratogenic endpoints ; Growth retardation (a),Shrinkage of chorion (b), Scoliosis and Pericardial edema (c), Lack of pigmentation (d), tail deformities (e), Heamorrhages (f), Yolk – sac edema (g), Lack of otolinths (h), Normally developed *Danio rerio* embryo after 96 hpf

Conclusion

In the present study, a comparison of LC_{50} values, sub-lethal and teratogenic effects indicated that Hg is highly toxic to *Danio rerio* embryo followed by Cu, Cd, Zn, As and Pb. The LC_{50} value of each trace metal is increasing with increasing concentration and time of exposure. This study indicates that *Danio rerio* embryo is a potential indicator for trace metal pollution.

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