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# Trace Metals Co-Toxicity in Hard Body Structures of *Liza klunzingeri* (Mugilidae: Perciformes) Mullet Fish

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**Abstract:** The possibility of inorganic pollutants causing stress to the marine ecosystem instigated us to conduct eco-toxicological tests on the predominant trace metals (Cu, Zn, Pb and V) in commercial important fish. Among the four metals, V had the lowest observed effect concentration (LOEC: $LC_{15}$) at 6.3 µg $L^{-1}$. Toxicity tests (96 h) using Multi factor Probit analysis revealed V (6.3 µg $L^{-1}$) had the greatest effect at median lethal concentration ($LC_{50}$) followed by Pb (6.7 µg $L^{-1}$), Cu (7.8 µg $L^{-1}$) and Zn (15.7 µg $L^{-1}$) in *Liza klunzingeri* reared in filtered seawater in the laboratory. Fish exposed to trace metals mixture (1:1 ratio) showed synergistic co-toxicity coefficient (CTC) factor among Cu, Pb and Zn and antagonistic CTC with V mixtures. There was significant trace metals bioaccumulation in fish body structures (otolith, dorsal fins and scales) exposed for 180 day toxicity tests at LOEC. These results suggest there is value in ecologists to characterize *L. klunzingeri* as a bio indicator to metal pollution and enumerate the co-toxicity of trace metals in the marine environment.

**Key words:** Metals toxicity, bioaccumulation, mullet fish

## INTRODUCTION

The factors that cause stress in the Kuwaiti marine environment may be multidimensional in their origin. Sources include industrial and domestic effluent discharges, oil spills, accumulation of organic and inorganic pollutants in Kuwait Bay and in the coastal region due to industrialization and rapid human activities were identified after the Gulf War-I in 1991. Discharges from the power, thermal, desalination and water treatment plants and leakage from oil wells that contained high metal levels were observed in Kuwait marine environment[1-2]. Most metals are essential for the physiological function processes in fish[3]. However, above tolerable limits, these metals were found to be one among the factors that caused 'fish kill' in the year 1999 and 2000 especially to *L. klunzingeri* in Kuwait while, sub-lethal concentrations of metals showed behavioural, biochemical and histological changes in fish[4]. Observation showed the main routes of accumulation of trace metals over time by fish are through the gills, skin, muscles and food[3,5]. Likewise, hard body parts of fish such as otolith, scales and dorsal spines showed significant accumulation of trace metals in the marine environment[6-10]. Studies also observed co-toxic effect of metals in mixture on sensitive fish[11-13]. Besides the direct effect of trace metals, their interaction to seawater variables such as temperature, pH, D.O., salinity, nutrients and biological factors may attribute to enhance the co-toxicity effect on fish[14-15]. Previous publications showed no evidences of co-toxicity of trace metals in *L. klunzingeri* in Kuwait's marine environment. Hence, this study aims to determine the acute toxicity ($LC_{50}$) and bioaccumulation of trace metals, individually and in mixture (co-toxicity) in *L. klunzingeri.*

## MATERIALS AND METHODS

The commercially important fish, mullet, *Liza klunzingeri* were collected from the Kuwait Bay. Ten fish replicates, each weighing 20±3 g and total length 9±3 cm were acclimated for 30 day in the laboratory in 250 L glass tanks containing 0.45 µm filtered and disinfected seawater. The fish were fed (2% body weight) with *Artemia salina* (brine shrimp) nauplii (2 g $day^{-1}$) and formulated fish feed (3 g $day^{-1}$) without trace metals (<0.001 µg $g^{-1}$). Seawater (5%) was exchanged on alternate days and dead fish (if any) removed besides the filters provided inside the tanks. Antibiotics and fungicides were added to waters before toxicity tests to prevent bacterial infection following the guidelines described earlier[16]. In 250 L tanks, seawater parameters were gradually changed over a period of

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15-20day using different regulators that maintained temperature (25°C±2°C), dissolved oxygen (8.1 mg $l^{-1}$), salinity (40‰) and pH (8.2) as against the normal quality of seawater in Kuwait to enable conducive parameters for the fish sustenance in the lab conditions. A backwash polystyrene filter (40 µm) removed the debris from the tank.

**Trace metals analysis in Kuwaiti seawater:** Two litres seawater from Kuwait Bay sites was collected using a VanDorn water sampler during the years 2005-2007 in summer and winter seasons. One-litre seawater added with 25 mL ammonium-pyrrolidine-dithiocarbonate (APDC 2% v $v^{-1}$), 10 mL HCl (0.5 M) and 35 mL methyl-isobutyl-ketone (MIBK 99.5%) was shaken for two minutes in a separator funnel and left undisturbed for 15-20 minutes. Sampled were settled into two separate phases upper and lower solutions (A and B). The upper solution (A) was added with one litre of fresh seawater, APDC, HCl and MIBK and the process repeated. The lower solution (B) was eluted in another separator funnel with the above chemicals. The trace metals concentration collected from both the upper solutions (A and B) in 50 mL volumetric flask was measured with Analytical Jena system -55 and the lower solutions discarded. Quality assurance followed the methodology[17] using blanks, metals standards (ICP grade:1000ppm) and 98% recoveries of samples from trace metals in seawater standard reference material (BCR: seawater CRM-403).

**Acute toxicity test on *L. klunzingeri*:** *L. klunzingeri* (10 numbers) for each toxicity test (96 h $LC_{50}$) were acclimated for 24 h in the lab following the methodology of Finney (1971) and data analyzed using Probit program[18]. Fish were not fed while acclimation and during the acute toxicity test. Different quantities of standard stock solution (1 g $L^{-1}$) of trace metals (Pb, V, Cu and Zn Perkin Elmer) were added to the filtered seawater in the experimental tanks. This produced the $LC_{50}$ test concentration ranging from 6 to 23 µg $L^{-1}$. Similarly, metals solutions were also prepared in mixtures (1:1 ratio) and tested for co-toxicity with replicates of *L. klunzingeri*. The co-toxicity co-efficient (CTC) of trace metals ($_M$) was computed[19].

($CTC_M$) = Actual Toxicity index of metals ($TI_A$) Theoretical Toxicity index of metals (TI)×$100^{-1}$ Wherein, Actual Toxicity ($TI_A$) of metals is given by

($TI_A$) = $LC_{50}$ of A $LC_{50}$ of metals in mixture×$100^{-1}$ and

(TI) = $LC_{50}$ of Std. heavy metal (A) $LC_{50}$ of metals in mixture (A) + $LC_{50}^{-1}$ of Std. heavy metal (B) $LC_{50}$ of metals in mixture (B)×$100^{-1}$

Trace metals solutions were renewed every 24 h to reduce the decrease in toxicant concentrations[9]. The 96 h $LC_{15}$, $LC_{50}$, $LC_{99}$ values were calculated using the Probit Program[18-20,21]. Bioaccumulation test for 180 day with additional dose of trace metals to *L. klunzingeri* exposed from 1 day-180 day that may lead to accumulation of metals while determining the $LC_{50}$ toxicity and co-toxicity tests was prevented by calculating the Cumulative Toxicity Factor (CTF) for the respective exposures (1 day and 180 day). Cumulative Toxicity Factor (CTF) = Acute $LC_{50}$ Cumulative $LC_{50}^{-1}$.

Wherein, cumulative $LC_{50}$ (180-d) = $LC_{50}$ (180 d) $LC_{50}$ (1 d)×$100^{-1}$. The CTF value (<1.5-5, 5-10, 10-20, 20-50, 50-100 and>100) is rated (1-6) based on the nature of toxicant index (non-hazardous, slight, moderate, very hazardous, extreme and highly extreme hazardous) respectively[13].

**Bioaccumulation test on *L. klunzingeri*:** Ten *L. klunzingeri* each, were exposed to LOEC (metal concentrations that produced $LC_{15}$) on each trace metals (Pb, V, Cu and Zn) as well as in mixtures (1:1 ratio) to comply with the laboratory standards described on animal sacrifice[16]. Test ran for 180 day in individual tanks. Fish were fed only with *A. salina* nauplii (2 g $day^{-1}$) to ensure either no feed or trace metals (<0.001 µg $g^{-1}$) residues in the tanks. Simultaneously, ten fish were sacrificed (anaesthetized using Quinaldine) and their hard parts (otolith, dorsal spines and scales) removed after 1 day to determine the initial trace metals levels. Bioaccumulation factor ($BAF^1$) of each heavy metal was determined by calculating the actual metal accumulation in fish hard parts from that of seawater[11].

$BAF^1$ = Concentration of metals in fish hard parts (µg $kg^{-1}$) (*B*) Concentration of metals in seawater (µg $L^{-1}$) $(A)^{-1}$.

$BAF^2$ of trace metals mixture in fish exposed for 180 day toxicity test was determined by calculating the metals accumulated after 180 day from that of the metals accumulated in the respective hard parts after one day. Trace metal concentrations in the seawater were also measured during each water exchange. Live fish from the control and tests were killed after 180 day exposure. Paired otolith (0.034 g±0.01), first dorsal fin (0.2 g) and 4-5 scales (0.2 g±0.01) found adjoining the lateral lines from each fish were dried in an oven

(GallanKamp II) at 45°C for 72 h. These parts were pre-digested in Aristar grade 2% $HNO_3$ (v $v^{-1}$) and 1% HCl (v $v^{-1}$) overnight, in polystyrene sterile centrifuge tubes. Samples diluted in de-ionized water (50 mL) and digested in an automatic microwave digester (Spectroprep CEM), was measured in the ICP-MS to determine the metals bioaccumulation. Quality assurance using otolith reference standard (CRM 22) from National Institute of Environmental Studies (NIES) for Pb, Cu and Zn and Ash bone (SRM 4356) for V with recoveries in agreement with certified values ranging 96%-98.78% were obtained to assess the precision of the instrument and qualitative sample results[21].

## RESULTS AND DISCUSSION

**Trace metals analysis in Kuwaiti seawater:** The present study chose essential and non-essential metals (Cu, Zn, Pb and V) because they were:

(1) found to cause marine pollution in Kuwaiti waters[2]
(2) suspected to cause 'fish kill' in Kuwait Bay recently[4]
(3) occurred in untreated industrial, domestic and sewage discharges
(4) occurred in high levels through accidental spills

(4) within the detectable limits of ICP-MS. These trace metals analyzed from the Kuwaiti seawater revealed a mean concentration of Zn (2.9 µg $L^{-1}$±0.05) followed by Cu (2.4 µg $L^{-1}$±0.05), Pb (0.9 µg $L^{-1}$±0.02) and V (0.55 µg $L^{-1}$±0.02). However, these metals were found within the limits of the observations made elsewhere in the globe[12-14,15].

**Acute toxicity to *L. klunzingeri*:** The 96-h $LC_{50}$ tests[18] on *L klunzingeri* showed the concentrations sequence of V (8.5 µg $L^{-1}$)<Pb (9.6 µg $L^{-1}$)<Cu (11.0 µg $L^{-1}$) and Zn (18.6 µg $L^{-1}$). Further, observation revealed *L. klunzingeri* sensitive to V at LOEC (6.3 µg $L^{-1}$) was lower than other metals examined in this study (Table 1). The interaction of V with other organic constituents could attribute to the accumulation of V in the hard parts of the fish when compared to other trace metals. This was statistically tested through Chi-square analysis and supports the earlier findings[8] (Table 1).

Toxicity ($LC_{50}$) at 96 h between the trace metals mixture (1:1 ratio) were V: Pb (8.9 µg $L^{-1}$)<V: Cu (9.1 µg $L^{-1}$)<Pb: Cu (10.3 µg $L^{-1}$), V: Zn (11.8 µg $L^{-1}$)<Pb: Zn (13.2 µg $L^{-1}$)<Cu: Zn (15.3 µg $L^{-1}$) (Table 1). Observation also revealed similar sequence of $LC_{50}$ to fish exposed for 180 day but with an increased concentration in trace metals in single exposures as well as in mixtures (Table 2).

Table 1: Lethal concentrations ($LC_{15}$ and $LC_{50}$ at 96 h exposure) of trace metals to *Liza klunzingeri* (10 fish) using Probit Program[18]

| Metals | conc. (µg $L^{-1}$) [1] | LC point | 95% C.I. limits upper | lower | $\chi 2$ calculated |
|---|---|---|---|---|---|
| V | 6.2 | 15 | 7.4 | 2.8 | 1.90 |
| | 8.4 | 50 | 9.5 | 7.0 | |
| Pb | 6.7 | 15 | 8.0 | 4.3 | 0.30 |
| | 9.6 | 50 | 11.3 | 8.2 | |
| Cu | 7.7 | 15 | 9.3 | 4.6 | 0.66 |
| | 10.9 | 50 | 12.6 | 9.1 | |
| Zn | 15.7 | 15 | 17.0 | 12.9 | 0.16 |
| | 18.6 | 50 | 20.0 | 17.2 | |
| V: Pb | 5.8 | 15 | 7.2 | 3.0 | 0.76 |
| | 8.9 | 50 | 10.5 | 7.1 | |
| V: Cu | 5.7 | 15 | 7.3 | 2.6 | 0.71 |
| | 9.1 | 50 | 10.9 | 7.2 | |
| V: Zn | 8.6 | 15 | 10.1 | 5.3 | 0.07 |
| | 11.7 | 50 | 13.3 | 9.9 | |
| Pb: Cu | 7.4 | 15 | 8.7 | 4.9 | 0.55 |
| | 10.2 | 50 | 11.7 | 8.8 | |
| Pb: Zn | 9.6 | 15 | 11.3 | 5.7 | 0.57 |
| | 13.2 | 50 | 15.0 | 11.2 | |
| Cu: Zn | 12.0 | 15 | 13.6 | 8.6 | 0.72 |
| | 15.2 | 50 | 16.8 | 13.6 | |

[1]conc.: estimated exposure concentration; C.I: Confidence interval; $\chi 2$: Calculated Chi square for heterogeneity[18]

Table 2: Lethal concentrations ($LC_{15}$ and $LC_{50}$ at 180 day exposure) of trace metals to *Liza klunzingeri* (10 fish) using Probit Program[18]

| Metals | conc. (µg $L^{-1}$) [1] | LC point | 95% C.I. limits upper | lower | $\chi 2$ calculated |
|---|---|---|---|---|---|
| V | 7.5 | 15 | 8.2 | 6.0 | 0.81 |
| | 9.1 | 50 | 9.9 | 8.4 | |
| Pb | 7.2 | 15 | 8.5 | 4.7 | 1.25 |
| | 10.3 | 50 | 12.3 | 8.9 | |
| Cu | 9.0 | 15 | 10.3 | 6.6 | 0.47 |
| | 11.9 | 50 | 13.5 | 10.6 | |
| Zn | 16.0 | 15 | 20.6 | 17.7 | 0.30 |
| | 19.0 | 50 | 27.3 | 20.8 | |
| V: Pb | 6.6 | 15 | 7.9 | 4.2 | 0.29 |
| | 9.4 | 50 | 10.9 | 7.9 | |
| V: Cu | 6.7 | 15 | 7.8 | 4.5 | 0.31 |
| | 9.4 | 50 | 10.9 | 8.0 | |
| V: Zn | 9.7 | 15 | 11.0 | 7.0 | 0.21 |
| | 12.7 | 50 | 14.3 | 11.3 | |
| Pb: Cu | 7.0 | 15 | 8.5 | 3.9 | 0.82 |
| | 10.4 | 50 | 12.2 | 8.6 | |
| Pb: Zn | 10.7 | 15 | 12.0 | 8.2 | 0.20 |
| | 13.5 | 50 | 14.9 | 12.1 | |
| Cu: Zn | 13.0 | 15 | 14.4 | 10.4 | 0.35 |
| | 16.0 | 50 | | 17.5 | 14.6 |

[1]conc.: estimated exposure concentration; C.I: Confidence interval; $\chi 2$: Calculated Chi square for heterogeneity[18]

Table 3: Toxicity and bioaccumulation on single trace metals in *L. klunzingeri* hard parts recovered from seawater and in related studies

| Metals Expt. $^{-1}$ | Conc. ($\mu g\ L^{-1}$) | Exposure (d) | Metal levels in hard parts ($\mu g\ g^{-1}$) Otolith | D. Fins | Scales |
|---|---|---|---|---|---|
| **Seawater (*A*)** | | | | | |
| Pb | 0.9±0.02 | | | | |
| V | 0.5±0.02 | | | | |
| Cu | 2.4±0.05 | | | | |
| Zn | 2.9±0.05 | | | | |
| **Toxicity test** | | | | | |
| Pb-LOEC (B) | 6.7 | 1 | 0.32±0.13 | 0.62±0.16 | 0.44±0.22 |
| $BAF^1$ | | | 0.35 | 0.68 | 0.48 |
| LOEC (C) | 7.2 | 180 | 0.35±0.01 | 0.79±0.06 | 0.49±0.05 |
| $BAF^2$ | | | 1.09 | 1.27 | 1.11 |
| V-LOEC (B) | 6.2 | 1 | 0.25±0.07 | 0.46±0.11 | 0.34±0.08 |
| $BAF^1$ | | | 0.45 | 0.83 | 0.61 |
| LOEC (C) | 7.5 | 180 | 0.27±0.01 | 0.53±0.09 | 0.39±0.02 |
| $BAF^2$ | | | 1.08 | 1.15 | 1.14 |
| Cu-LOEC (B) | 7.7 | 1 | 0.62±0.19 | 0.71±0.03 | 0.67±0.09 |
| $BAF^1$ | | | 0.25 | 0.29 | 0.27 |
| LOEC (C) | 9.0 | 180 | 0.63±0.08 | 0.73±0.04 | 0.68±0.11 |
| $BAF^2$ | | | 1.01 | 1.02 | 1.01 |
| Zn-LOEC (B) | 15.7 | 1 | 0.71±0.09 | 0.77±0.10 | 0.75±0.10 |
| $BAF^1$ | | | 0.24 | 0.26 | 0.25 |
| LOEC (C) | 16.0 | 180 | 0.74±0.10 | 0.81±0.13 | 0.79±0.12 |
| $BAF^2$ | | | 1.04 | 1.05 | 1.05 |
| **Other Studies** | | | | | |
| Pb | 8.0 | 30 | 0.4-1.2±0.1[14] | 7.0-60.0±0.02[b,15] | 60.0±2.1[12] |
| V | 10.0 | 30 | -- | 0.01-0.76±0.01[15] | -- |
| Cu | 12.0 | 30 | 0.8-3.2±0.3[14] | 0.85-4.0±0.01[12] | 7.0±2.1[12] |
| Zn | 14.0 | 30 | 2.7-10.8±1.3[14] | 0.5-78.0±0.01[12] | 360±1.30[12] |

LOEC: Lowest observed effective concentration, $BAF^1$: Bioaccumulation factor for individual metals = [concentration in hard parts (B) concentration in seawater (A) $^{-1}$], $BAF^2$: Bioaccumulation factor for metal mixture = [concentration in hard parts after 180 day exposure (C) concentration at LOEC after 1d (B) $^{-1}$][12,14,15]

Table 4: Bioaccumulation of trace metals mixtures in *L. klunzingeri* hard parts at LOEC ($LC_{15}$)

| Metals Expt. $^{-1}$ | Conc. ($\mu g\ L^{-1}$) | Exposure (d) | Metal levels in hard parts ($\mu g\ g^{-1}$) Otolith | D. Fins | Scales |
|---|---|---|---|---|---|
| V: Pb | 5.8 | 1 | 0.31±0.01 | 0.51±0.07 | 0.37±0.12 |
| LOEC | 6.6 | 180 | 0.32±0.01 | 0.54±0.11 | 0.39±0.05 |
| $BAF^2$ | | | 1.03 | 1.05 | 1.05 |
| V: Cu | 5.7 | 1 | 0.45±0.02 | 0.56±0.11 | 0.52±0.04 |
| LOEC | 6.7 | 180 | 0.47±0.03 | 0.60±0.13 | 0.55±0.05 |
| $BAF^2$ | | | 1.04 | 1.07 | 1.05 |
| V: Zn | 8.6 | 1 | 0.30±0.01 | 0.49±0.22 | 0.40±0.03 |
| LOEC | 9.7 | 180 | 0.31±0.11 | 0.52±0.29 | 0.42±0.06 |
| $BAF^2$ | | | 1.03 | 1.06 | 1.05 |
| Pb: Cu | 7.4 | 1 | 0.57±0.24 | 1.72±0.29 | 1.69±0.02 |
| LOEC | 7.0 | 180 | 0.59±0.36 | 1.79±0.35 | 1.71±0.22 |
| $BAF^2$ | | | 1.03 | 1.04 | 1.01 |
| Pb: Zn | 9.6 | 1 | 1.72±0.24 | 1.76±0.52 | 1.74±0.25 |
| LOEC | 10.7 | 180 | 1.75±0.36 | 1.81±0.55 | 1.78±0.48 |
| $BAF^2$ | | | 1.01 | 1.02 | 1.02 |
| Cu: Zn | 12.0 | 1 | 1.84±0.53 | 1.92±0.63 | 1.86±0.55 |
| LOEC | 13.0 | 180 | 1.87±0.56 | 1.98±0.71 | 1.90±0.61 |
| $BAF^2$ | | | 1.01 | 1.03 | 1.02 |

LOEC: Lowest observed effective concentration, $BAF^2$: Bioaccumulation factor for metal mixture = concentration in mixture at LOEC after 180 day exposure concentration at LOEC after 1day$^{-1}$

**Bioaccumulation in *L. klunzingeri*:** Bioaccumulation tests on *L. klunzingeri* for 180 day at LOEC ($LC_{15}$) ensured low mortality. Hard parts showed highest heavy metal concentrations in dorsal fins followed by scales and otoliths (Table 3). Metal concentrations in dorsal fins, scales and otoliths attributed to their assimilation of trace metals along with $Ca^{2+}$ for its composition and formation[6]. BAF in *L. klunzingeri* recovered from seawater as well as in fish exposed for 180 day was found to be lower for Cu and Zn than for Pb and V. This may be attributed to the regulation of Cu and Zn by the fish[10]. However, toxicity and bioaccumulation levels in the present study were found within the limits when compared to the earlier studies conducted elsewhere (Table 3)[12-1415]. BAF was in the sequence of dorsal fin>scales>otolith in trace metals mixture and with single exposed metals (Table 4).

The co-toxicity coefficient value (CTC) was synergistic in trace metals mixture containing Zn, Cu and Pb. The CTC value was observed high in Pb: Cu mixture than for other combinations and supports the earlier studies on the effects of such metals toxicity in fish (Table 5)[3,5,14]. However, CTC revealed antagonistic action of V with Pb, Cu and Zn. During this study, experimental cumulative toxicity was prevented while replenishing trace metals in the test tanks by determining the Cumulative Toxicity Factor (CTF). The cumulative toxicity of these metals to the fish was minimal during the bioaccumulation test conducted for 180 day when compared to that of 1 day exposure. The trace metals determined in the present study at LOEC ($LC_{15}$) revealed CTF index at slight and moderate hazardous to the fish (Table 6). No mortality was observed at LOEC of trace metals addition. The fish were observed to recover soon in seawater in the absence or low trace metals. Thus, this test complied with the laboratory standards described on animal sacrifice[16]. The co-toxicity effect of trace metals mixture to *L. klunzingeri* in Kuwaiti waters may be related to

- the fluctuating seawater salinities (24-40‰)
- temperatures (20°C-40°C)
- fluctuating seasonal changes
- industrial and domestic waste discharges into the Bay apart from the synergistic action between metals

The present investigation showed trace metals mixture in seawater can interact in synergistic and antagonistic ways that indicate *L. klunzingeri* could be a bio-monitoring tool for pollution. Furthermore, mass mortality of *L. klunzingeri* could be validated through trace metals co-toxicity, if large quantities of these trace metals are untreated and accidentally discharged into the Kuwaiti marine environment.

Table 5: Co-toxicity coefficient (CTC) of trace metals in *L. klunzingeri*

| Metals (1:1 ratio)$^{-1}$ | $LC_{50}$ (Present study) | [1]Standard $LC_{50}$ | *CTC Value | Action of CTC (>100 = S, <100 = A) |
|---|---|---|---|---|
| Pb | 10.35 | 1.38 | | |
| V | 9.13 | 30.0 | | |
| Cu | 11.94 | 0.17 | | |
| Zn | 19.05 | 5.0 | | |
| V: Pb | 9.44 | | 32.05 | A |
| V: Cu | 9.45 | | 38.30 | A |
| V: Zn | 12.74 | | 18.71 | A |
| Pb: Cu | 10.40 | | 778.23 | S |
| Pb: Zn | 13.55 | | 194.45 | S |
| Cu: Zn | 16.01 | | 269.48 | S |

[1]Standard $LC_{50}$: [3], A: antagonistic, S: Synergistic, *CTC value[19]

Table 6: Cumulative Toxicity Factor (CTF) of trace metals to *L. klunzingeri*

| Metals (1:1 ratio)$^{-1}$ | $LC_{50}$ (180 d) | $LC_{50}$ (1 d) | Cumulative toxicity | [1]CTF | CTF (%) | Toxicity index ranking |
|---|---|---|---|---|---|---|
| Pb | 10.35 | 9.66 | 107.15 | 0.09 | 9.6 | 2 |
| V | 9.13 | 8.46 | 108.05 | 0.08 | 8.4 | 2 |
| Cu | 11.94 | 10.96 | 108.95 | 0.10 | 10.9 | 3 |
| Zn | 19.05 | 18.61 | 102.33 | 0.18 | 18.6 | 3 |
| V: Pb | 9.44 | 8.89 | 106.18 | 0.08 | 8.8 | 2 |
| V: Cu | 9.45 | 9.10 | 103.78 | 0.09 | 9.1 | 2 |
| V: Zn | 12.74 | 11.77 | 108.21 | 0.11 | 11.7 | 3 |
| Pb: Cu | 10.40 | 10.25 | 101.42 | 0.10 | 10.2 | 3 |
| Pb: Zn | 13.55 | 13.22 | 102.51 | 0.13 | 13.2 | 3 |
| Cu: Zn | 16.01 | 16.01 | 104.74 | 0.15 | 15.2 | 3 |

Cumulative toxicity = $LC_{50}$ (180 d) $LC_{50}$ (1 d) $\times 100^{-1}$; [1]CTF = Acute $LC_{50}$ Cumulative $LC_{50}^{-1}$[13], Toxicity Index : 1: non-hazardous; 2: Slight; 3: Moderate; 4: Very hazardous; 5: extreme

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