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Copper–zinc coergisms and metal toxicity at predefined ratio concentrations: Predictions based on synergistic ratio model

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ABSTRACT

A significant number of studies have centred on the single actions of heavy metals against test animals in predicting aquatic toxicity. However, practical existence of environmental toxicants is in multiple mixtures and variable undefined ratio combinatorial concentrations. Pollution abatement approaches in setting representative safe boundaries for metal contaminants is crucial with factual data on predictively modelled exposures of organisms to multiple mixtures. In continuance of our approach to toxicity of individual heavy metals, we determined the toxicity of binary mixtures of copper and zinc at predetermined ratios against tilapia species and also evaluated the coergisms based on synergistic ratio model for effective formulations of safe limits. *Orecohromis niloticus* species were exposed to copper and zinc (Cu:Zn) at ratios of 1:1 and 1:2 on $96hLC_{50}$ index and mortality response analysed following the probit-log-dose regression with metal–metal interactions effectively modelled. The $96hLC_{50}$ values for Cu:Zn were calculated to be 68.898 and 51.197 mg/l for ratios 1:1 and 1:2, respectively. The joint action toxicity of the metal mixtures was observed to differ from the metals acting singly against the same animal species. Synergistic coergisms were realized in most of the ratio mixtures except the antagonistic effect displayed by the combination of Cu:Zn in the ratio 1:1 when compared to the single action of copper. Biological toxicity of heavy metals however still appears uncertain, and consideration of multiple mixtures and interactions of toxicants in natural milieu is very crucial in environmental management of the existing and emerging contaminating metals.

1. Introduction

Heavy metal toxicity reports have been largely based on single action of the individual elements. However, metal mixtures, which are practically obtainable in the real environment, could have wide differential toxicity with variable interactive mechanisms wholly dissimilar from the biological actions of the metals acting singly (Manzo et al., 2010; Norwood et al., 2003). Contaminants are introduced into the aquatic ecosystems as complex blends and the development of realistic safe limits for environmental management needs to be considered based on the toxicity assessment of metal mixtures rather than relying solely on single actions against the biodiversity of ecosystems (Otitoloju, 2002). Heavy metal coergisms are very diverse and affect various ecological systems. Reactions between heavy metals and plant materials, and microorganisms have been documented (Parris et al., 2004; Krupa et al., 2002; Saikkonen et al., 1998). Chemical mixtures exist in the environment where their individual elemental concentrations are considered non-toxic (Montvydienė and Marčiulionienė, 2007). Conversely, these substances with low concentration levels present may elicit toxicity due to additivity or synergism of the constituents (Montvydienė and Marčiulionienė, 2004; Rajapakse et al., 2002). The high tendency for concurrent exposure to multiple environmental chemicals demands the development and use of models that provide understanding into the toxicity of substance mixtures (Rider and LeBlanc, 2005). Joint action studies of heavy metals with life animal species evaluate toxicology of contaminant mixtures known to exist in the natural setting and incorporate it regarding possible interactions into public health assessment of locations where people may be exposed to multiple combinations (ATSDR, 2004). Results can then be used to determine criteria to define environmental safe margins.

Single actions of zinc and copper have each received extensive attention. The two metals were identified to be most recurrently occurring binary mixtures in complete exposure pathways at hazardous waste sites (ATSDR, 2004). High copper concentrations in ambient water are very common (Schiff et al., 2007), and possible coergisms and, by extension, additivity in public health concerning sites-exposure of people to the metal combinations has been

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presented (ATSDR, 2004). Relevance of heavy metal interactive effects is of significance in aquatic environment with sundry organisms and ecosystem regulatory capacities. Earlier studies recognized toxicities of single application of zinc and copper to tilapia species and rainbow trout (Ezeonyejiaku and Obiakor, 2011; Ezeonyejiaku et al., 2011; Bagdonas and Vosyliene, 2006). Correspondingly, published studies indicated the synergistic and antagonistic effects of copper and zinc mixtures at predetermined ratios to catfish *Clarias gariepinus*, critically depicting the role of ratio concentrations in biological toxicity (Ezeonyejiaku et al., 2014a, 2014b; Lin et al., 2005).

The synergistic dynamics of copper and zinc have been studied in aquatic ecosystems based on different models such as isobologram, synergistic ratio, concentration addition and response, and interactions described in different range of life species at predefined ratios of concentration interfaces (Dondero et al., 2011; Xu et al., 2011; Otitoloju, 2002). Genotoxicity of binary mixtures of the metals were also shown to be consistent with the synergistic model (Obiakor et al., 2010; Bagdonas and Vosyliene, 2006). Ratio of Zn:Cu at 1:1 (wt/wt) tested against Mangrove periwinkle *Tympanostonus fuscatus* showed the binary interactive mixture to significantly depart from the action of the individual constituent metals when acting singly and zinc was found to consistently reduce the toxic effect of copper (Otitoloju, 2002). Reproductive anomalies on *Ceriodaphnia dubia* and *Daphnia carinata* were demonstrated following the combination of copper and zinc at a given ratio ($1.3+13.0$ μg/l of Zn+Cu) (Cooper et al., 2009).

Models of calculating the metal–metal interactions exist with in-built variations in their reliability and application (Otitoloju, 2002). A notable model in wide application as described in substantial reviewed literature is concentration addition and this has been comprehensively used in chemical interactive effects and toxicity (Le et al., 2013; Mebane et al., 2012); however, other models are also used (Bao et al., 2008; Montvydienė and Marčiulionienė, 2007). A synergistic ratio model after Hewlett and Plackett (1959) is described as a better candidate for joint action toxicity evaluations for setting safe limits of pollutants (Otitoloju, 2002) as it is directly computed from toxicity indices to measure the type and strength of interaction where it exists including binary and multiple mixtures. Moreover, it provides a general theoretical basis for the interpretation of quantal response statistics for chemical mixtures (Hewlett and Plackett, 1959). The authors inferred that study of joint drug (heavy metals as used here) action requires a universal biological depiction of the way in which one drug applied singly produces a response in an individual organisms. Our previous studies of copper and zinc single toxicities on tilapia species measured the $96hLC_{50}$ values following the probit-log-dose regression, deriving 58.837 and 72.431 mg/l for copper and zinc, respectively (Ezeonyejiaku and Obiakor, 2011; Ezeonyejiaku et al., 2011). With body of research evidence bordering on different contaminant mixture interaction models and since the current study continues our approach of 2011, the objective was to determine the toxicity of binary mixtures of copper and zinc at predetermined ratios to *Oreochromis niloticus* and evaluate the coergisms of the two metals based on synergistic ratio model to underpin management strategies of aquatic ecosystems and formulation of safe precincts.

2. Materials and methods

2.1. Culture procedures of test systems and acclimatization to microcosm conditions

Juvenile tilapia species, *Oreochromis niloticus*, Linnaeus, 1758 (Chordata, Actinopterygii, Perciformes, and Cilchlidae) of fairly similar live-weight (23.07 ± 1.46 g wet weight) and length (13.06 ± 1.45 cm) were collected irrespective of gender from an uncontaminated hatchery of known stock history and transferred to the laboratory in oxygenated plastic containers. They were kept in holding tanks of 40-litre capacity and filled to a depth of 12 cm with aerated dechlorinated tap water for fourteen days to allow them acclimatize to laboratory conditions. The priority of juvenile fish species was considered on their test sensitivity, which can differ from one life stage to another and the use of juveniles not only increases sensitivity, but also allows standardisation (Verslycke et al., 2003). Holding tanks were aerated continuously and water quality monitored for deviations using appropriate digital read-out metres. Renewal of water was done on daily basis during the acclimatisation and stopped following commencement of the 96 h static experiment. Laboratory culture was maintained under a 12-h light: 12-h dark photoperiod. Light quality and intensity were ambient laboratory illumination and level, respectively.

Adequate procedures of culture management were followed to avoid poor water quality in the culture system. Ammonia and ammonium concentrations got checked throughout the microcosm study period. Accumulated materials from the bottom of tanks were siphoned daily with glass siphon tube attached to the tank leading to the floor. Water of the same quality with the culture was added as needed to compensate for evaporation. Cultures were fed ad libitum twice daily with commercial feed pellets and feeding stopped on starting the assay, throughout the 96 h exposure dose response evaluation of the heavy metal mixtures. Fish were observed daily for abnormal appearance or behaviour and care was taken to keep the mortality rate of fish not more than 5% in the last four days before the experiment commenced. Culture density was kept at twenty fish per tank.

2.2. Test chemicals

Chemically pure salts of zinc sulphate ($ZnSO_4 \cdot 7H_2O$) and copper sulphate ($CuSO_4 \cdot 5H_2O$) of analytical grades were used as toxicants. The heavy metal (toxicant) stock solutions were prepared in deionised water at desired concentrations. The confirmatory test concentrations of metals were determined by atomic absorption spectrophotometer and required serial dilutions made from the stock solution with dechlorinated tap water.

Based on the single $96hLC_{50}$ values of copper and zinc (Ezeonyejiaku and Obiakor, 2011; Ezeonyejiaku et al., 2011), mixtures of these metals were then prepared in equitoxic concentrations (50, 60, 70, 90, 100 mg/l and untreated control) at ratios of 1:1 and 1:2. Predetermined concentrations of zinc and copper mixture to be tested at predefined ratios were calculated, measured out and dissolved in a conical flask before making it up to required volume. The test solution was thoroughly mixed by stirring with glass rod for 2–3 min before transferring the mixture to appropriate bioassay tank (Otitoloju, 2003). We selected copper and zinc as choice metals based on documented reports of their ubiquity in Nigerian aquatic environment and toxicity to organisms (Obiakor et al., 2015; Ezeonyejiaku et al., 2014a, 2014b; Obiakor et al., 2014; Ezeonyejiaku and Obiakor, 2013; Obiakor et al., 2013; Ezeonyejiaku et al., 2011; Otitoloju, 2003; Khunyakari et al., 2001; Horne and Dunson, 1995).

2.3. Single action toxicity of copper and zinc

The single action toxicity of zinc and copper had earlier been demonstrated and documented (Ezeonyejiaku and Obiakor, 2011; Ezeonyejiaku et al., 2011) and the $96hLC_{50}$ observed as follows: zinc – 72.431 mg/l and copper 58.837 mg/l.