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Interactive toxicity of Ni, Zn, Cu, and Cd on *Daphnia magna* at lethal and sub-lethal concentrations

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HIGHLIGHTS

- Feeding behaviour was 27–63 times more sensitive than lethality in Daphnia magna.
- Five out of seven mixtures interacted differently on lethal and sublethal endpoints.
- Metals had the same order of toxicity for both endpoints (Cu > Cd > Ni > Zn).

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ABSTRACT

The toxicity of metal mixtures is currently of particular interest among aquatic toxicologists. To provide insight into whether the interaction of multiple metals is similar at different biological levels, the survival and feeding behavior of *Daphnia magna* were studied following exposure to four metals (Cd, Cu, Ni, Zn) and their binary and quaternary combinations. In terms of survival, Zn-Cu and Cu-Cd mixtures produced more-than-additive mortality, while Ni-Cd mixtures resulted in less-than-additive mortality. Regarding behavior, Zn-Cu and Zn-Cd mixtures produced a more-than-additive reduction in feeding rate. Four (i.e. Zn-Cu, Cu-Cd, Ni-Cd, and Zn-Cd) out of six binary mixtures in the present study interacted differently at the survival and behavioral levels, strengthening the emphasis on carefully selecting the toxicological endpoint when addressing metal mixture toxicity. The results of the present study demonstrated that metals are toxic to feeding behavior of *D. magna* at much lower concentrations (i.e. 27–63 times lower) compared to survival, suggesting that applying sub-lethal endpoints are required for producing protective regulations.

1. Introduction

Aquatic environments are faced with contamination by mixtures of substances, and organisms (e.g., fish and aquatic invertebrates) living in those environments are challenged to simultaneously regulate, detoxify, and metabolize a variety of contaminants that differ in their modes and sites of action. Based on the sensitivity of aquatic biota to single contaminants, considerable effort has been put toward developing regulatory guidelines [1] in order to restrict the presence of contaminants in aquatic systems at concentrations high enough to exert adverse ecotoxicological effects. Moving forward, regulatory authorities must address the sensitivity of aquatic biota to various contaminants when in mixtures, as co-occurring substances may interact to enhance (i.e., potentiate) or attenuate (i.e., antagonize) toxic effects [2]. Unfortunately, there is a current lack of comprehensive knowledge and approaches for testing and predicting mixture toxicity in a regulatory framework, making the development of mixture guidelines a challenging task.

To address this challenge, many researchers have investigated the interactive relationships of similar [3] and dissimilar [4–6] contaminant classes with the goal of identifying the additivity and mechanisms of mixture toxicity. A common recommendation that mixture ecotoxicologists make is to focus on environmentally relevant mixture concentrations to be more representative of sublethal effects that aquatic organisms are most likely to experience in the environment. This is critical because contaminants act differently on different cell types and tissues based on the degree of exposure, and thus, co-occurring contaminants are also likely to display various degrees of additivity at different concentrations, as discussed in Gauthier et al. [2]. Careful consideration must also

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be given to potential differences in mixture additivity at different levels of biological organisation [7].

Trace metals are ubiquitous contaminants in aquatic environments due to their prevalence in mining, industrial, agricultural and urban activities, as well as natural sources (e.g., mineral weathering) [8,9]. Specifically, copper (Cu), cadmium (Cd), nickel (Ni), and zinc (Zn) are considered particularly important metal contaminants [10,11] due to their relatively common occurrence in water bodies at concentrations high enough to cause adverse ecotoxicological effects. As such, the toxicity of Cu, Cd, Ni, and Zn in aquatic organisms has been extensively studied. Nonetheless, toxicological effects on sub-lethal endpoints, such as food consumption, an endpoint that provides information on potential energy status and physiological condition of the individual, remain under-investigated, particularly when multiple contaminants are present.

As feeding rates are indicative of the overall energy status of an animal, food consumption has the potential to be used as a non-invasive and sensitive endpoint in investigating the toxic effects of contaminants on primary consumers, such as the cladoceran, *Daphnia magna* [12]. Daphniids are widely used as model organisms for determining aquatic ecosystem dynamics and health [12]. They are an ideal laboratory organism and have been used extensively in ecotoxicological studies and government standardized test method protocols [13,14]. Several studies show that contaminants reduce the feeding rate of *Daphnia* at well below lethal concentrations [12,15]. Reduction of the feeding rate reduces the total activity, growth, and reproduction of *Daphnia* and consequently their individual fitness and population dynamics could be jeopardized.

The aim of the present study was to first characterize the individual toxicities of Cd, Cu, Ni, and Zn at lethal and sub-lethal concentrations on *D. magna*. Feeding rates were measured as a sub-lethal endpoint. We then investigated the interactive toxic effects of Cd, Cu, Ni, and Zn in binary and quaternary mixtures, and assessed the additivity of the mixtures by comparing the mixture and singular effects using a generalized linear model (i.e., survival) and segmented regression (i.e., food consumption). Finally, we compared the findings of interactions of similar binary mixtures on survival and feeding behavior to investigate whether or not metals interact in a similar fashion at different endpoints.

2. Experimental

2.1. Test animals

All experiments were carried out with *D. magna* cultured under laboratory conditions as described by Lari et al. [12]. The culture was maintained at room temperature (20 ± 1 °C), in moderately-hard (90–100 mg/L as $CaCO_3$) reconstituted water, under full-spectrum fluorescent light (750 lx; 16:8 h light:dark). A reference toxicity test with sodium chloride (NaCl) was performed every month to test the sensitivity of the culture. There was no important difference in the sensitivity of daphniids throughout the course of experiments, based on confidence interval overlap (Supplementary Table A). Less than 24-h-old neonates from three to five-week-old *D. magna* were used for all bioassays. The water that was used for culturing *D. magna* was also used for preparing test metal solutions and control water in all experiments. Water quality parameters of the test solutions were measured before starting the tests, and were found to be within the following ranges: pH 8.3–8.7, hardness 90–95 mg/L as $CaCO_3$, conductivity 467–486 μS, alkalinity 159–168 mg/L as $CaCO_3$, dissolved organic carbon (DOC) <1 mg/L and temperature 20.0–20.1 °C.

Table 1
Measured metal concentrations in exposure waters used for single metal lethality tests (n = 3).[a]

Metal	Nominal Concentration (μg/L)	Mean measured Concentration (μg/L)	Standard deviation of the mean (μg/L)
Ni	200	200	0
Ni	400	363	0.5
Ni	800	723	2.1
Ni	1600	1500	1
Ni	3200	2960	3
Zn	100	99	0
Zn	200	193	1
Zn	400	383.7	7.8
Zn	800	728.3	97.3
Zn	1600	1552.3	12.4
Cu	10	10	0
Cu	20	19.3	0.6
Cu	40	37.7	1.5
Cu	80	82	1
Cu	160	161.7	1.5
Cd	20	20.3	0.6
Cd	40	40.3	0.6
Cd	80	83.3	0.6
Cd	160	166.3	12.5
Cd	320	338	2

[a] The quality assurance of this analysis is presented in Supplementary Table E.

2.2. Test chemicals and analysis

To reduce variation in metal concentrations of test solutions, stock solutions of $NiSO_4{\cdot}6H_2O$ (Alfa Aesar, USA), $ZnSO_4{\cdot}7H_2O$ (Fisher Scientific, USA), $CuSO_4{\cdot}5H_2O$ (Sigma-Aldrich, USA), and $CdSO_4{\cdot}8H_2O$ (Alfa Aesar, USA) were made as the source of Ni, Zn, Cu, and Cd, respectively, and were used throughout the study. Water samples from three replicates of each treatment were filtered using a 25 mm syringe filter with 0.45 μm cellulose acetate membrane (VWR, USA). Water samples were acidified by adding 0.2% final concentration, trace metal grade HNO_3 (Fisher Scientific, Canada). Actual concentrations of metals in exposure waters were measured using Spectro Ciros Inductively-Coupled Plasma Optical Emission Spectrometer with an axial view plasma (Spectro Analytical Instruments, Kleve Germany) at the Lethbridge Research and Development Centre, Lethbridge, AB, Canada. DOC was measured pre- and post-exposure in control treatments. A 50 mL sample from three replicates for each treatment was filtered through a 0.45 μm cellulose acetate membrane (VWR, USA) and acidified by adding 0.5% final concentration HCl (Sigma-Aldrich, USA). Samples were analysed for DOC by SGS Canada Inc. (Lakefield, Ontario, Canada).

2.3. Lethality assays

All lethality assays were 48-h static exposures. Five replicates of 6 treatments of 10 daphniids were exposed to 200 mL of different concentrations of Ni, Zn, Cu, and Cd and culture water as a control in 250 mL glass beakers. Based on preliminary range-finding tests, daphniids were exposed to the following decreasing geometrical concentrations series: Ni (3200, 1600, 800, 400, and 200 μg/L), Zn (1600, 800, 400, 200, and 100 μg/L), Cu (160, 80, 40, 20, and 10 μg/L), and Cd (320, 160, 80, 40, and 20 μg/L) (Table 1). Daphniids were not fed during exposures. The number of dead individuals, tallied as immobility with no appendage movement, was counted at the end of the 48-h exposure period and median 50% and 20% lethal concentrations (LC50 and LC20) of each metal were calculated (see sub-section 2.5).

In metal-mixture lethality assays five replicates of 12 treatments of 10 daphniids were exposed to single metals (Ni, Cu, Cd, and Zn), mixtures of metals (Ni-Zn, Ni-Cu, Ni-Cd, Zn-Cu, Zn-Cd, Cu-Cd, and Ni-Zn-Cu-Cd) and culture water as a control as described above