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Joint toxic action of binary metal mixtures of copper, manganese and nickel to *Paronychiurus kimi* (Collembola)

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ABSTRACT

The joint toxic effects of binary metal mixtures of copper (Cu), manganese (Mn) and nickel (Ni) on reproduction of *Paronhchiurus kimi* (Lee) was evaluated using a toxic unit (TU) approach by judging additivity across a range of effect levels (10–90%). For all metal mixtures, the joint toxic effects of metal mixtures on reproduction of *P. kimi* decreased in a TU-dependent manner. The joint toxic effects of metal mixtures also changed from less than additive to more than additive at an effect level lower than or equal to 50%, while a more than additive toxic effects were apparent at higher effect levels. These results indicate that the joint toxicity of metal mixtures is substantially different from that of individual metals based on additivity. Moreover, the close relationship of toxicity to effect level suggests that it is necessary to encompass a whole range of effect levels rather than a specific effect level when judging mixture toxicity. In conclusion, the less than additive toxicity at low effect levels suggests that the additivity assumption is sufficiently conservative to warrant predicting joint toxicity of metal mixtures, which may give an additional margin of safety when setting soil quality standards for ecological risk assessment.

1. Introduction

Metal contamination of soil has become a widespread environmental problem throughout the world and most metals found in contaminated soil occur as a mixture of multiple metals originating from industrial, mining or agricultural activities. Thus, organisms in soil are likely to be exposed to mixtures of metals rather than a single metal. However, the current regulatory standards for metals have solely been based on single-metal toxicity data (Backhaus and Faust, 2012) and our understanding of how different combinations of metals exert joint toxic effects is still limited. Moreover, in view of currently available data on the toxicity of metal mixtures, multiple metals can interact with each other in different ways, which lead to synergism, antagonism, or potentiation effects on soil invertebrates, such as springtails (Baas et al., 2007; Van Gestel and Hensbergen, 1997), potworms (He et al., 2015; Lock and Janssen, 2002; Posthuma et al., 1997), isopods (Odendaal and Reinecke, 2004; Zidar et al., 2009), and earthworms (Khalil et al., 1996; Weltje, 1998). There is other strong evidence that the toxicity of individual metals in a mixture may be different from those of individual metals alone (MacFarlane and Burchett, 2002; Zhou et al., 2006), and the toxicity of metal mixtures cannot be adequately predicted from the effects of individual toxicants (Kraak et al., 1994). From a regulatory perspective, it would, therefore, be desirable to have a better understanding of mixture toxicity when assessing risks associated with meals in contaminated soil.

Two different concepts, concentration addition and independent action, are commonly used to evaluate the toxicity of mixtures with similar or dissimilar modes of action, respectively (Barata et al., 2006, 2007). In reality, however, most metal-contaminated soils are often contaminated with a variety of metals with different modes of action. Moreover, detailed knowledge of the modes of action of individual metals in a given mixture is sometimes not accurate and the concepts of similar or dissimilar modes of action may be dependent on the types of organisms present (Lynch et al., 2016). Despite these limitations, it has been suggested that concentration addition should be a more suited default model in risk assessment of chemical mixtures because of its conservative prediction of mixture toxicity in most cases (Cedergreen et al., 2008) and an extensive report on mixture toxicity for the European Union proposed to use the concept of concentration addition when assessing the risk of mixtures (Verbruggen and Van den Brink, 2010). Several researchers also suggested that concentration addition should be used to predict the toxicity of mixtures irrespective of the modes of action of individual chemicals in the mixture, since concentration addition provides more conservative predictions, and consequently

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prevents an underestimation of the toxic effects of multiple mixtures, giving a realistic worst case scenario (Backhaus et al., 2000, 2004; Faust et al., 2001; Syberg et al., 2008; Versieren et al., in press).

The most commonly used concentration addition is the toxic unit (TU) approach (Sprague, 1970), which is based on the premise that the toxicity of mixtures can be predicted directly from the concentrations of the individual chemicals comprising them. In the TU approach, the concentrations of individual chemicals in a mixture are expressed as fractions of their EC50 values. The toxicity of mixtures is then judged by comparing the EC50 of the mixture ($EC50_{mix}$) with the corresponding sum of TU. When a 50% effect of the mixture ($EC50_{mix}$) is predicted to occur at a TU value of 1, the toxicity of the mixture is defined as being concentration additive or as being either antagonistic or synergistic ($EC50_{mix} > 1$ TU or < 1 TU, respectively) (Borgert et al., 2004). Although the TU approach has been considered a suitable tool for predicting the toxicity of mixtures and assessing the interactive effects of individual chemicals in the mixtures, differences in the shape and slope of concentration–response curves of individual metals in the mixtures can affect the assumption of additivity through violation of the TU concept (Van Gestel and Hensbergen, 1997). The type of interaction may also vary with effect level on which the mixture is being judged (Banks et al., 2003; Van der Geest et al., 2000) because the ecotoxicological mode of action may change as various toxicological effects become operative (Barata and Baird, 2000; Barata et al., 2006, 2007).

Among the various trace metals, copper (Cu), manganese (Mn) and nickel (Ni) are naturally-occurring in the environment, and they are often found together in the vicinity of mining areas and smelters in high enough concentrations to pose a serious threat to humans and the environment (Adriano, 2001). These metals are essential to plant and animal species when present in trace concentrations, but they can exert toxic effects on plant and animal life when their concentrations in soil exceed certain thresholds (Adriano, 2001). Though their importance in the environment has been recognized, little information is available on their interactive toxic effects to soil organisms compared to other metals. Moreover, currently available data on the toxicity of mixtures to soil organisms is limited to studies done on a few standardized test species (e.g., *Folsomia candida* and *Enchytraeus albidus*) (Lock and Janssen, 2002; Van Gestel and Hensbergen, 1997).

In the present study, *Paronychiurus kimi* (Lee), a collembolan species native to Korean soils, was used as test species; it is listed as an alternative to *F. candida* for toxicity testing (OECD, 2009). The objectives of the present study were to evaluate the toxicity of binary metal mixtures of Cu, Mn, and Ni to the reproduction of *P. kimi* using a TU approach, and to determine whether the toxicity of binary metal mixtures depends on the effect level at which the mixture is being judged.

2. Material and methods

2.1. Test organisms

P. kimi cultures were started from a population extracted from paddy fields in Korea (Choi et al., 2002). *P. kimi* were cultured on a moist substrate that was comprised of a 4:1:4 ratio (by volume) of plaster of Paris, activated charcoal, and deionized water in plastic Petri dishes (90 × 15 mm) that were filled with approximately 0.5 cm of the media and incubated at 20 ± 1 °C under continuous darkness. Cultures were fed with diluted brewer's yeast weekly. To obtain a large number of synchronized adult *P. kimi* (42–44 days old), hundreds of adults were separately introduced into several breeding substrates and allowed to lay eggs. The adults were then removed after three days. As soon as the eggs hatched (after ≈ 14 days), the juveniles were cultured under the same conditions as described above. Adult cohorts obtained by this method were used throughout this study.

2.2. Artificial soil contamination

Artificial soil used in the tests was composed of the ingredients recommended by the OECD (2009), which was composed of 70% quartz sand, 20% kaolin clay, and 10% finely ground *Sphagnum* peat on a dry weight basis. The soil pH was adjusted to 6.0 ± 0.5 by adding $CaCO_3$ (purity ≥ 99.0%, Sigma-Aldrich, St. Louis, USA). All metals used in the toxicity tests were in the form of chloride salts ($CuCl_2 \cdot 2H_2O$, Sigma-Aldrich, purity > 99%; $MnCl_2 \cdot 2H_2O$, Merck, purity > 98%; $NiCl_2 \cdot 6H_2O$, Merck, purity > 98%). All metal stock solutions dissolved in deionized water were prepared immediately prior to the tests and the concentrations of each metal in the stock solutions were quantified using an inductively coupled plasma-optic emission spectrometer (ICP-OES) (Vista-PRO, Varian, Australia). Since the measured metal concentrations of the stock solutions were in agreement with nominal concentrations (< 5% deviation), the nominal metal concentrations in the soil are used throughout this study.

The binary metal mixtures of Cu, Mn, and Ni were prepared by applying the TU approach using previously reported 28-day EC50 value for the reproduction of *P. kimi* by Son (2009). The concentrations of each metal in the binary mixture were designed to be equivalent toxicity by adding equal fractions of their EC50 values for each metal (i.e. TU). To prepare test solutions for each binary metal mixtures, the correct amounts of each metal stock solution and corresponding amount of deionized water was combined together to reach 50% of the maximum water-holding capacity (WHC_{max}) of the soil. Each of test solution was added to the artificial soil separately and mixed thoroughly. Seven different concentrations for each metal mixture were prepared at concentrations of ∑0TU, ∑0.5TU, ∑0.75TU, ∑1.0TU, ∑1.5TU, ∑2.0TU, and ∑2.5TU (Table 1) with five replicates per each concentration.

2.3. Toxicity tests

Toxicity tests with *P. kimi* were conducted in accordance with the OECD 232 guideline (OECD, 2009). Ten synchronized (42–44 d old) *P. kimi* were introduced into polystyrene containers containing 30 g wet weight of contaminated soil. The test containers were kept in continuous darkness at 20 ± 1 °C for 28 days. The soil moisture content was adjusted weekly by replenishing lost weight with the appropriate amount of deionized water. The containers were also aerated weekly. Granulated dried brewer's yeast was

Table 1
Equivalent toxicity of metal concentrations of Cu, Mn, and Ni (mg/kg dry wt) and their corresponding sums of toxic units (∑TU) obtained from binary mixture toxicity studies in artificial soil.

Toxic unit	Mixture combination			
TUx - TUy	Cu - Mn	Cu - Ni	Mn - Ni	∑TU[a]
0–0	0–0	0–0	0–0	0
0.25–0.25	69.4–81.4	69.4–13.2	81.4–13.2	0.5
0.375–0.375	104.0–122.1	104.0–19.7	122.1–19.7	0.75
0.5–0.5	138.7–162.8	138.7–26.3	162.8–26.3	1.0
0.75–0.75	208.1–244.3	208.1–39.5	244.3–39.5	1.5
1.0–1.0	277.5–325.7	277.5–52.6	325.7–52.6	2.0
1.25–1.25	346.8–407.1	346.8–65.8	407.1–65.8	2.5

[a] All metals were added at the same fraction of their EC50 values (toxic unit, TU), determined in single-metal toxicity tests, expressed as ∑TU of the individual fractions in the binary mixtures.