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Statistically significant deviations from additivity: What do they mean in assessing toxicity of mixtures?

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ABSTRACT

There is increasing attention from scientists and policy makers to the joint effects of multiple metals on organisms when present in a mixture. Using root elongation of lettuce (*Lactuca sativa* L.) as a toxicity endpoint, the combined effects of binary mixtures of Cu, Cd, and Ni were studied. The statistical MixTox model was used to search deviations from the reference models i.e. concentration addition (CA) and independent action (IA). The deviations were subsequently interpreted as 'interactions'. A comprehensive experiment was designed to test the reproducibility of the 'interactions'. The results showed that the toxicity of binary metal mixtures was equally well predicted by both reference models. We found statistically significant 'interactions' in four of the five total datasets. However, the patterns of 'interactions' were found to be inconsistent or even contradictory across the different independent experiments. It is recommended that a statistically significant 'interaction', must be treated with care and is not necessarily biologically relevant. Searching a statistically significant interaction can be the starting point for further measurements and modeling to advance the understanding of underlying mechanisms and non-additive interactions occurring inside the organisms.

1. Introduction

Industrial discharges, consumer wastes and the usage of plant protection products or sewage sludge bio-fertilizers may all lead to metal contamination in soil. Metals can be easily adsorbed in soils (Yang et al., 2009) and be accumulated in plants which may result in a threat to the health of the plant itself and consumers in the food chain. In the natural environment, plants are often exposed to multiple metals simultaneously rather than a single metal (Backhaus et al., 2000). Many metals listed individually within the safe range of industrial permits are extremely toxic to certain species and even more so when present in combination (Wong et al., 1987). Thus, to maintain healthy and functioning ecosystems, it is necessary to improve the understanding of combined effects of multiple metals on terrestrial plants.

Since testing is cost- and time-consuming, computational models are developed to help predict toxicological responses and understand the toxicity mechanisms of mixtures. The most frequently used predictive tools for assessing mixture toxicity disregarding interactions are concentration addition (CA) (Loewe and Muischnek, 1926) and independent action (IA) (Bliss, 1939) if the constituents making up the mixture are known. The CA model is used for chemical mixtures for which a similar mode of action is assumed, whereas the IA model is used to predict effects of compounds with a different mode of action (Bliss, 1939). It has been argued that concentration addition should be a more suited default model in risk assessment of chemical mixtures because of its conservatism in most cases (Cedergreen et al., 2008). In addition, it is suggested that dissimilarly acting chemicals rarely exist in complex organisms (Faust et al., 2003). However, the sites or the modes of action are ambiguously defined at the biochemical level and can be dose dependent (Cedergreen et al., 2008). In most cases, the CA and the IA models are used only based on their mathematical connotation as the toxicity mechanisms of metals are still greatly unknown. The conceptually unrelated CA and IA models are single-time point approaches which make them suitable to make predictions for mixture effects based on standardized toxicological tests. Therefore, the CA and the IA models were both used in this research. An elaborate description of these two approaches can be found for example in the papers by Altenburger et al. (2000) and Jonker et al. (2005).

Predicting mixture effects becomes a challenge when a mixture is composed of interacting chemicals that synergize or antagonize the effects of each other. Accurately determining chemical

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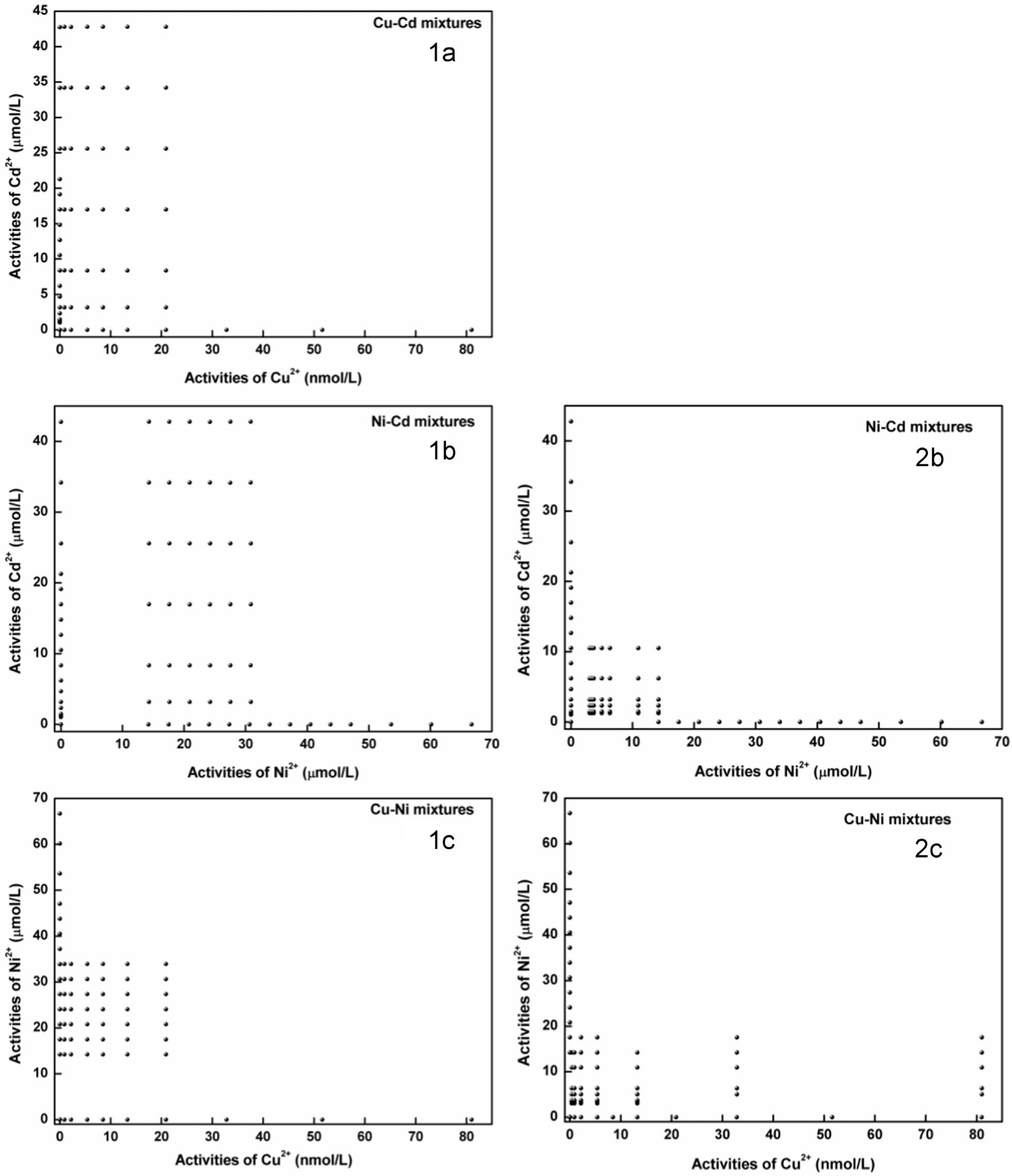

Fig. 1. Set up of experiments for Cu–Cd, Ni–Cd, Cu–Ni mixtures expressed as free ion activities. (1.5-column fitting image).

interactions is not only conducive to adequately describing the relationship between exposure and effect, but also greatly aids risk assessments for chemical mixtures and further studies for underlying mechanisms of chemical toxicity. Synergistic interactions may cause severe effects on organisms (Johnson et al., 2013) which attract the attention of toxicology scientists and policy makers in finding synergism for naturally occurring mixtures. The reference models (i.e. CA and IA) are frequently extended to explore the presence of interaction between mixture components and to explain the variation in assessing mixture toxicity (Jonker et al., 2005; Le, 2012). Deviations from the predictions of reference models are usually interpreted as interactions. The strongest interactions often occur in binary mixtures and the interactive effects may become minor with an increased number of mixture components (Warne and Hawker, 1995; Lydy et al., 2004). Thereupon, experiments in this study were carried out with binary metal mixtures as a foundation for explaining joint effects of complex mixtures. The standardized framework described by Jonker et al. (2005) was applied to analyze the toxicity data of metal mixtures, a detailed description of which is given in our