Sensitivity of *Caenorhabditis elegans* to mixtures of zinc, copper and cadmium

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1. Introduction

Industrial and natural sources of heavy metals cause an increase in metal accumulation, which can lead to serious health hazards for diverse animals including humans, resulting in a persistent (eco)toxicological concern. In contrast to the toxic effects of individual metals, very little is known about their interactions and putative additive effects in the environment. Especially soils, sediments and surface waters can be contaminated with metal mixtures. One of the major challenges in ecotoxicology is to obtain insights in mixture toxicology to set realistic environmental quality criteria. Therefore, the aim of this study was to gain insights into the sensitivity to the selected metals (Cu,Cd,Zn), and to investigate whether and how these sensitivities are affected in mixture exposure scenarios. To assess different endpoints, we fully exploited the benefits of the free-living soil nematode *Caenorhabditis elegans* as a unique model to investigate the effects of metal exposure on survival rate and behavioural responses.

2. Materials and methods

C. elegans (wild type N2 strain) nematodes were cultured at 20°C on nematode growth medium (NGM) agar plates, seeded with *Escherichia coli* (OP50 strain) as food source. Ten adult nematodes were exposed to different concentrations of cadmium (0, 2.5, 5, 10, 100, 400, 800 and 1200 mg/L), copper (0, 0.05, 0.5, 2.5, 25, 50, 100, 200, 400 mg/L) and zinc (0, 5, 10, 25, 50, 100, 200, 400 mg/L), in triplicate. After 0h, 2h, 8h, 24h and 48h of exposure the number of living worms was counted using a stereomicroscope.

For the lethality test of the CuCd mixture all combinations of the concentrations, used for the single metal lethality, were tested except for the 2 highest concentrations of both metals. We determined how survival, at a given concentration of the first metal, changed by adding the second metal and examined if there was an interaction between the two metals. It was also tested which interactive effects of metals occurred in the mixtures ZnCd, ZnCu and ZnCuCd.

For the locomotory analyses, worms were exposed during 24h and 48h to the LC20 concentrations of 24h ($LC20_{24h}$) and during 48h to the LC20 concentrations of 48h ($LC20_{48h}$) of the three metals. Mixtures CuCd, ZnCu, ZnCd and ZnCuCd, made by combinations of the LC20 concentrations of the single metals, were tested. Average crawling speed on agar plates, automatically quantified by using video tracking technology, and trashing behaviour in liquid medium were determined.

3. Results and discussion

3.1. Lethality

Time-resolved dose-response curves were obtained for all single metals and LC10, LC20 and LC50 values were determined. Based on toxicant mass units, cadmium was the least toxic and copper the most toxic to *C. elegans*, which is consistent with data from prior studies [e.g. 1,2]. At each time point, the regression slope of Zn was the most negative, meaning that by addition of Zn mortality increased faster than when Cu or Cd were added. In comparison with $LC50_{24h}$, the $LC50_{48h}$s were strongly decreased for all metals, indicating increased metal toxicity after a prolonged exposure. This decrease was also seen in earlier studies [1,3]. However, the differences in declining rate of LC50, indicate that uptake and elimination rates differ between metals [3].

When Cd concentrations were added to Cu, and vice versa, significant increases in toxicity were observed. In contrast, zinc had a neutral effect on cadmium since no obvious increase in toxicity was noticed. The mortality in CuCd and ZnCu mixtures did not differ significantly from the expected values: these metals appeared to have an additive effect in combination.

3.2. Locomotion

Worms exposed to the $LC20_{24h}$ of Cu and Cd had a significant lower speed and trashing behaviour compared to control (Fig.1). Behaviour seemed to be a less sensitive indicator for Zn toxicity compared to the other metals [2].

In line with the lethality tests, locomotory responses declined even more in binary and tertiary mixtures. Locomotion in the ZnCu and CuCd mixture was lower than in the single metals, while the average speed of the worms exposed to the ZnCd mixture was non significantly different from the speed of worms, exposed to Cd only. This again suggests that zinc has a neutral effect on cadmium and that Cu has additive effects with Zn and Cd.

The exposure to $LC20_{48h}$ did not affect the movement of worms exposed to single metals. When the nematodes were exposed to the CuCd mixture, their average speed declined (Fig.1). Furthermore, the swimming behaviour decreased after an exposure to CuCd, ZnCu and tertiary mixture.

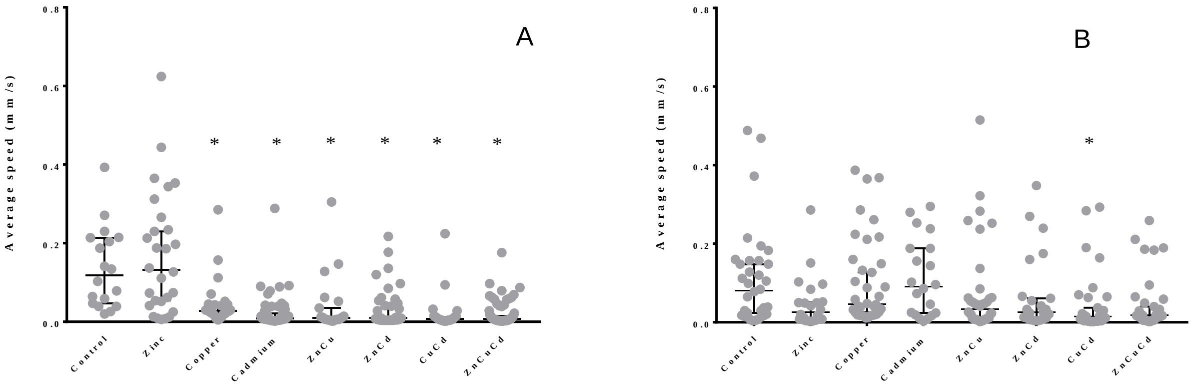

Figure 1: Average speed (mm/s) of worms exposed to $LC20_{24h}$ (A) and to $LC20_{48h}$ (B) of metals and their mixtures. Individual data are shown as well as the median and interquartile range. Asterisks (*) denote significant differences (P< 0.05) compared to control.

4. Conclusions

We analysed the effects of single metals and their mixtures on the lethality and locomotion of *C. elegans*. Mortality rate was the highest for Cu and lethality increased over time for all metals. Different interaction effects were noticed for the mixtures ZnCd, ZnCu, CuCd and ZnCuCd. Our study shows that even at low concentrations the locomotion, both on agar plates and in liquid medium, was disturbed. After $LC20_{24h}$ exposure, a reduced crawling speed, except for Zn, and a reduced trashing behaviour, except for Zn and ZnCd mixture was observed. However, for both locomotion parameters single metal exposure of $LC20_{48h}$ did not have an effect. Since the same trend of mixture effects was noted in locomotion and in lethality tests (at low concentrations), locomotion could probably be considered as a sensitive endpoint in metal toxicities.

5. References

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