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COCCIDIOSTATS IN POULTRY MANURE ‑ A POTENTIAL IMPACT ON METAL ACCUMULATION IN BENEFICIAL SOIL INVERTEBRATES

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Abstract

Carboxylic ionophore antibiotics are used in veterinary medicine for prevention and treatment of coccidiosis in poultry. The most frequently used coccidiostats in Slovenia and in northern Europe are natural ionophores monensin, salinomycin, and lasalocid. As these substances are only partially metabolised in treated animals, the major part of coccidiostats consumed are excreted in active form. When poultry manure is used as fertiliser, part of the coccidiostats ends up in the environment. Ionophores act as cation transporters across cell membranes, so they can potentially also impact metal transport, especially in metal-burdened environments. We studied the bioaccumulation of copper in earthworms (Eisenia andrei) and isopods (Porcellio scaber) when concurrently exposed to monensin and Cu-contaminated artificial soil. Copper predominantly enters agricultural soils by use of Cu-based herbicides and manure from pig farms where it is used as a growth promoter. In the present study, animals were exposed for 14 or 28 days to environmentally realistic concentrations of Cu (80 and 160 mg/kg dry soil) and monensin (25 mg/kg dry soil). We found that monensin increases the uptake and accumulation of Cu in earthworms, while no impacts on isopods were observed. Thus we may conclude that in Cu-burdened environments like vineyards, Cu intake could decrease the toxicity threshold if monensin-contaminated manure from poultry farms was used frequently.
Keywords: ionophore, monensin, bioaccumulation, earthworms, woodlice

Introduction

Coccidiostats are ionophore antibiotics used to prevent and treat coccidiosis. They are administered mixed with feed in the production of broilers and turkeys. One of the most frequently used natural carboxylic ionophore antibiotics is monensin (Hansen et al., 2009a; Zizek and Zidar, 2013). In the treated animals monensin is only partially metabolised and excreted primarily via faeces, which is usually used as manure on arable land. In this way coccidiostats enter terrestrial ecosystems. When in the environment, coccidiostats undergo both biotic and abiotic degradation (Vertesy et al., 1987; Sassman and Lee, 2007; Hansen et al., 2009b; Hansen et al., 2012). The reported monensin half-lives in soil are between 2 days (Sassman and Lee, 2007) and 24.6 days (Yoshida et al., 2010). The reports on the predicted environmental concentrations of monensin vary greatly and are between 0.05 mg kg-1 soil (Žižek et al., 2011) and 1.12 mg kg-1 soil (EFSA, 2005).
The presence of ionophores in the environment simultaneously with Cu may alter metal assimilation in non-target organisms. Namely, ionophores bind numerous mono- and divalent cations that facilitate the passage of ions through cell membranes (Celis et al., 1974; Elsasser, 1984). Monensin is usually classified as a monovalent ionophore that combines with Na^+ and K^+ ions (Dowling, 1992) but also facilitates the transport of divalent cations like Ca^{2+} (Ambroz et al., 1990). It has also been reported that monensin mediates the transmembrane transport of Pb in rats (Hamidinia et al., 2002; 2006).

Based on the available information on steers (Kirk et al., 1994) and chickens and sheep (Khan et al., 1993), monensin was expected to alter Cu availability to terrestrial invertebrates. Copper predominantly enters agricultural soils by the use of Cu-based herbicides and manure from pig farms where it is used as a growth promoter. Cu concentrations in pig manure can exceed 700 mg kg-1 (Li et al., 2010; Xiong et al., 2010). In vineyards, after frequent and long-lasting use of Cu-based fungicides, Cu concentrations can reach several hundred mg of Cu per kg soil (Komárek et al., 2010).

In the present study, we were interested in the effects of the ionophore monensin on copper accumulation in earthworms and isopods following concurrent administration of the ionophore and metal.

Materials and methods

Test animals

The earthworm species used in the experiments was Eisenia andrei (Oligochaeta: Annelida, Lumbricidae) from the laboratory culture at the Veterinary Faculty, University of Ljubljana. Sexually mature animals with a visible clitellum and weighing between 200 and 400 mg were used in the experiments. Porcellio scaber, Latr. (Isopoda, Crustacea), originated from the laboratory culture at the Department of Biology, Biotechnical faculty, University of Ljubljana. All tests were performed with adult animals of both sexes weighing between 30 and 50 mg. Pregnant females and animals in premoult and ecdysis period (Zidar et al., 1998) were excluded.

Soil preparation

The standardised natural soil Lufa 2.2 (Speyer) was used in the study. Monensin in the form of monensin A sodium salt (90% purity, Sigma-Aldrich, Germany) was introduced to the soil dissolved in acetone at a concentration of 25 mg kg-1 dry soil. Soil for the experiment was spiked with the acetone solution (5 mL solution per 100 g soil), thoroughly mixed and left overnight in a fume hood to evaporate the acetone. Copper was added as water solution of Cu(NO3)2.3H2O salt (99.5% purity, Merck, Germany) in concentrations 80 and 160 mg kg-1 dry soil. After application of chemicals the soil moisture content was adjusted to 60% of the water holding capacity. Soil pH was 6.5 – 7.

Experimental design

Rectangular polypropylene vessels (160 × 110 mm) with a volume of 1.5 L were filled with approximately 1 kg of wet soil and 20 animals were added. Animals were exposed to untreated soil, solely monensin (25 mg kg-1), solely Cu (80 or 160 mg kg-1), or to Cu combined with monensin. Altogether 240 earthworms and 240 isopods were divided into 12 exposure groups per animal species. During the exposure earthworms were fed with dry horse manure with around 8 mg Cu kg-1 dry weight. Manure was mixed with soil once per fortnight. Isopods were fed with dry and partly decomposed hazel leaves with around 8 mg Cu kg-1 dry weight. Leaves were renewed every 7 days. Animals were sampled after 14 and after 28 days. Ten animals from each treatment were used for metal analyses while the rest of the surviving animals were used for histological analyses (not part of this paper).

Metal analyses

After exposure, the animals were left on a wet filter paper for 24 hours to purge their gut. Then they were lyophilised, weighed and completely digested in a concentrated nitric acid/perchloric acid mixture (7:1 v/v). After evaporation of the acid, the residue was dissolved in 0.2% HNO3. Cu concentrations in whole animals were determined by flame atomic absorption spectrometry (Perkin Elmer AAnalyst 100).

Data analysis

The Mann-Whitney test was used to compare the impact of coccidiostats on metal assimilation. The decrease or increase in body metal concentrations was assigned to monensin if statistically different ($p<0.05$) from the solely metal exposed animals. All the calculations were performed with the SPSS 17.0 statistic software package (USA).

Results and discussion

Copper concentration in earthworms exposed to solely monensin contaminated soil did not differ from control earthworms (Figs 1 and 2). In animals simultaneously exposed to 25 mg monensin kg-1 soil and 160 mg Cu kg-1 soil for 14 days we recorded a significantly (Man-Whitney) higher Cu accumulation compared to solely Cu-exposed animals (Fig. 1). After 28 days of exposure (Fig. 2) monensin in soil combined with Cu resulted in higher Cu accumulation in earthworms although the difference was significant (Mann Whitney test) only at the lower Cu concentration. These results are in concordance with the results reported by Elsasser (1984). In monensin-treated chickens and sheep liver concentrations of Cu increased compared to control.

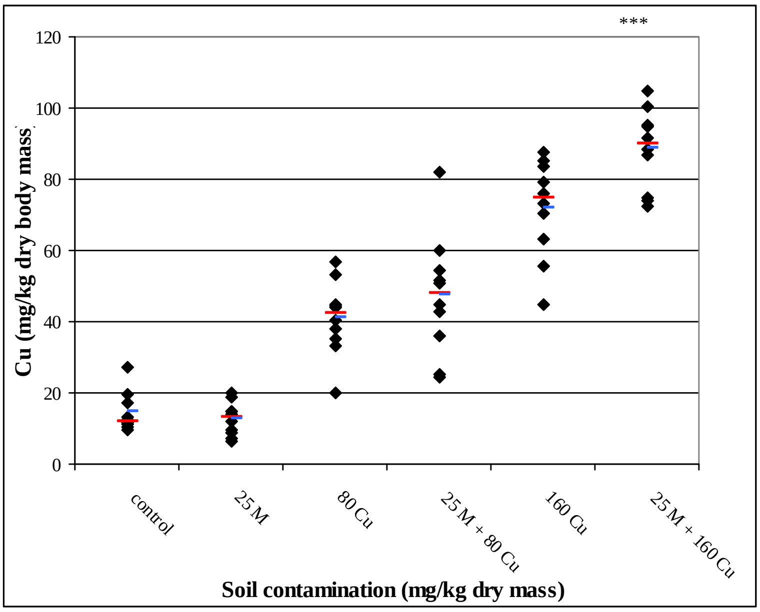

Figure 1: Whole body Cu concentration in earthworms (Eisenia andrei) after 14 days exposure to solely Cu or monensin contaminated Lufa 2.2 soil or concurrently exposed to monensin and Cu contaminated soil (nominal values). (diamonds – individual values; longer red line – median value; shorter blue line – average; *** - $p<0.001$)

Copper accumulation in animals concurrently exposed to monensin and Cu for 28 days was in general lower compared to that after 14 days. As monensin half-life in soil at similar conditions is 23.5 days (Žižek et al., 2011), more than half of monensin probably degraded in 28 days and thus its influence on Cu accumulation was lower but still prominent. Earthworms exposed to only Cu for 28 days did not differ from earthworms exposed for 14 days which indicates that Cu body concentrations had probably reached equilibrium between Cu assimilation and excretion.

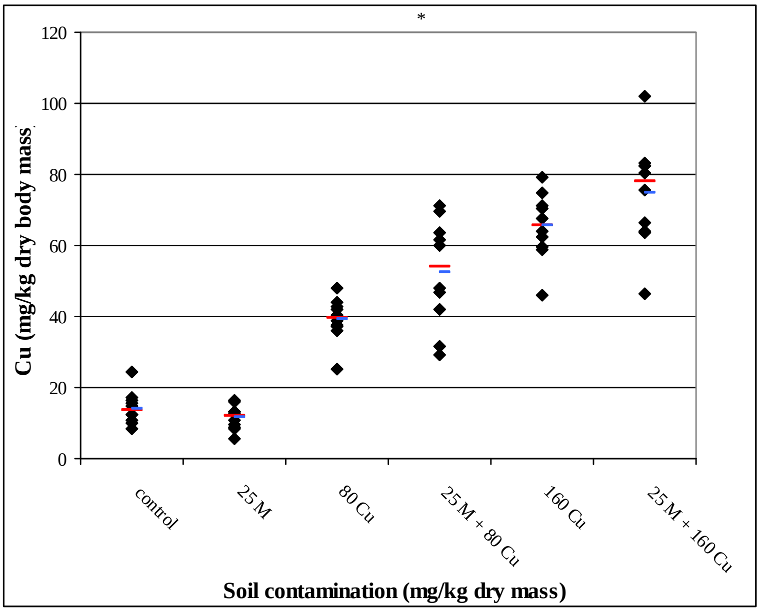

Figure 2: Whole body Cu concentration in earthworms (Eisenia andrei) after 28 days exposure to solely Cu or monensin contaminated Lufa 2.2 soil or concurrently exposed to monensin and Cu contaminated soil (nominal values). (diamonds – individual values; longer red line – median value; shorter blue line – average)

In isopods no influence of monensin on copper accumulation was detected. Measured Cu body concentrations did not differ between groups in spite of different copper contamination of soil. Copper concentrations vary greatly among control animals and within other groups. Whole body concentrations were also in general higher than in the study of Zidar et al. (2003), where animals were individually exposed via food. This discrepancy might be related to cannibalism as mortality in isopods was high (up to 50 %). Dead animals were immediately eaten by other animals in the vessel that thus ingested substantial amounts of Cu.

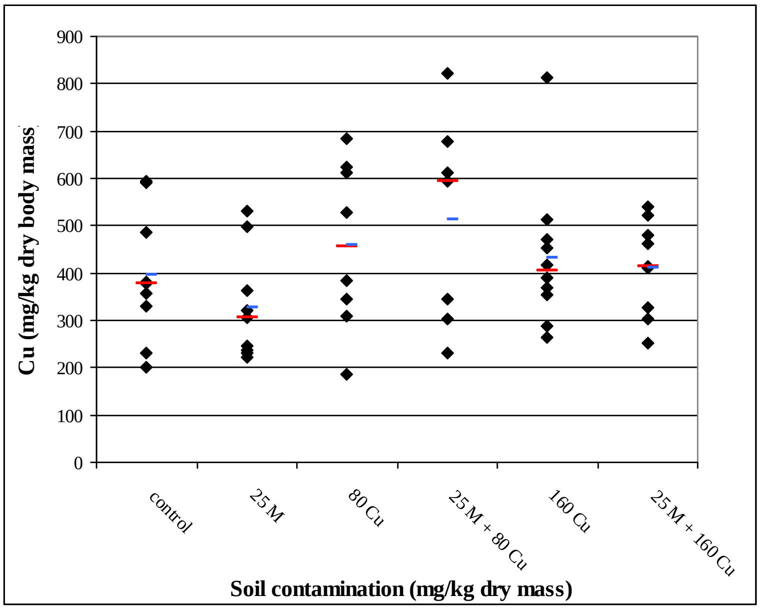

Figure 3: Whole body Cu concentration in isopods (Porcellio scaber) after 14 days exposure to solely Cu or monensin contaminated Lufa 2.2 soil or concurrently exposed to monensin and Cu

contaminated soil (nominal values). (diamonds – individual values; longer red line – median value; shorter blue line – average)

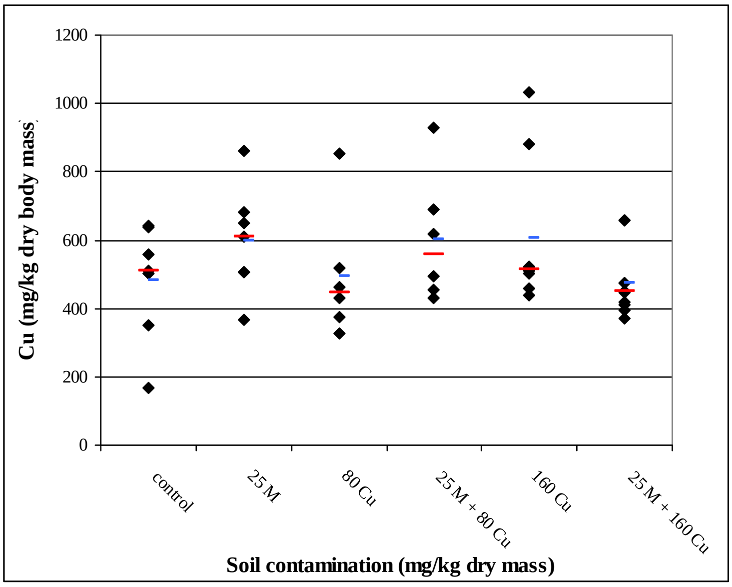

Figure 4: Whole body Cu concentration in isopods (Porcellio scaber) after 28 days exposure to solely Cu or monensin contaminated Lufa 2.2 soil or concurrently exposed to monensin and Cu contaminated soil (nominal values). (diamonds – individual values; longer red line – median value; shorter blue line – average)

Conclusion

We may conclude that monensin increases the uptake and accumulation of Cu in earthworms at environmentally realistic concentrations of Cu and monensin. Therefore, frequent application of manure from poultry farms with no previous composting might increase the uptake and potentially the toxicity of metals in beneficial soil organisms. This could potentially endanger the production potential of arable soil, so care should be taken to use aged or composted manure.

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