

# An 112-Days Experiment on Dietary Cadmium Retention in Hepatopancreas in Adult *Cantareus aspersus* Snails

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*Cadmium is very hazardous pollutant with massive impact on aspects of everyday life. Little knowledge exists on kinetics of dietary cadmium retention in Cantareus aspersus for durations above three months although this species of land snails serves as excellent ecotoxicological model for studying cadmium hazard. Here was used a continuous 112-day exposure study design, in which test snails were exposed to a wide range of dietary cadmium levels, including human- and environmentally-relevant levels. Using Flame Atomic Absorption Spectrometry, cadmium levels in the hepatopancreas were found to increase significantly, and in dose-dependent manner starting from a dietary cadmium dose of 0.2 mg/kg dry weight. The results of the present study render the hepatopancreas of mature snails, C. aspersus, as excellent endpoints for assessing Cd toxicity over a broad range of concentrations. Hence, this species of land snails can be reliably used for both active and passive biomonitoring of environmental cadmium pollution.*

**Keywords:** Cadmium, retention, *Cantareus aspersus*, dietary asperses

Cadmium (Cd) contamination in terrestrial environments is a major global issue; it increases year after year with a massive impact on everyday life ranging from ecological sustainability and food security to human health. This trace metal (TM) occurs naturally in the Earth' crust with an abundance of 0.1 - 0.5 mg/kg dry weight (mg/kg d. wt) [1].

Cadmium is a very hazardous pollutant due to several key reasons, primarily the long biological half-life (10-30 years), high accumulation potential along the terrestrial food webs and high toxicity at levels one-tenth that of lead (Pb), mercury (Hg) or aluminum (Al) [2,3].

There is limited knowledge about the kinetics of cadmium retention in gastropod models exposed for long-term (> 3 months) to elevated levels of cadmium [4]. The hepatopancreas, which is the molluskan homologous organ for both the mammalian liver and pancreas, represents the primary endpoint of Cd retention. In this context, the present experiment aimed to determine the level of dietary Cd which led to an increase in Cd retention in the snail hepatopancreas. Was used a continuous 112-day exposure, multiple dose study design using newly-matured *Cantareus aspersus* snails as invertebrate model. Hepatopancreas cadmium level was assessed using Flame Atomic Absorption Spectrometry.

## Experimental part

### Materials and Methods

The snails (*Cantareus aspersus*) were obtained in September 2018 from the Mokry Dwor farm (Krzymow, Wielkopolska, Poland) and transferred to a climate-controlled room (18-20°C, 12 h/l: 12 h D) in aerated polypropylene 30-liter terrariums (70.5 x 39.5 x 18.5 cm). The used nominal Cd concentrations were 0, 0.02, 0.05, 0.2, 1, 10 and 100 mg/kg d. wt. with three replicates used per each dose diet. These treatments were abbreviated as 0Cd, 0.02Cd, 0.05Cd, 0.2Cd, 1Cd, 10Cd and 100Cd. The rearing method and the corresponding procedures were similar to those used in our previous experiments [1].

After being oven dried at 105°C, for 24 h, the hepatopancreas samples (between 1.2-1.5 g / each

sample) were weighed (to the nearest 0.01 mg) with an analytical balance (TP-214, Denver Instrument GmbH). Following their calcination in a muffle furnace (Nabertherm B150, Lilienthal) at 550°C, for 6 h, the ash obtained was dissolved with nitric acid using the wet digestion approach -as previously described for standard processing of animal samples [5].

Finally, the filtrates were brought to 25 mL using 10 mL 0.5N HNO<sub>3</sub> and cadmium concentrations were determined using an atomic absorption spectrometer (Varian AA240FS) fitted with a Cd-specific hollow-cathode lamp as the source of radiation. The results were expressed as mg/kg d.wt.

### Statistical method

Differences in hepatopancreas cadmium among were analyzed using a Kruskal Wallis test, with posthoc Dunn's tests being applied in case of significant differences.

## Results and discussions

Land snails (*Pulmonata*) have several ecological and biological attributes rendering them suitable study system for Cd hazard. These are: (i) ecologically associated with humans (*i.e.*, synanthropic species), and also the most species-rich group of terrestrial mollusks; (ii) concentrate higher amounts of Cd than most terrestrial higher bilaterians (including commonly used toxicological models such as rats, mice, earthworms, or arthropods); (iii) sensitive to low level Cd exposure of contamination [6-8].

*Cantareus aspersus* (syn. *Helix aspersa*) was used as study system in this present work because it is the most often used terrestrial gastropod in environmental toxicology; it has a well-known biology [9] and is easily reared under laboratory conditions [10].

Commonly used animal models in toxicological studies (*e.g.* rats, mice, fish) have separate sexes, both of which have to be tested for providing relevant results on cadmium retention dynamics. Land snails, by contrast, are hermaphrodites; therefore, their use is a more cost-effective alternative to vertebrate study systems in ecotoxicological studies on cadmium [11].

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Literature data have shown that these mollusks are easy to grow under controlled laboratory conditions using agar-based feeds [4, 8, 12]. Indeed, the feed used here was well accepted by the test snails. The cadmium levels in feed were: (1) for the 0Cd treatment, below the detection Threshold (0.01 mg/kg d. wt); (2) for the 0.02Cd treatment,  $0.02 \pm 0.01$  mg/kg d. wt; (3) for the 0.05Cd treatment,  $0.05 \pm 0.03$  mg/kg d. wt; (4) for the 0.2Cd treatment,  $0.18 \pm 0.06$  mg/kg d. wt; (5) for the 1Cd treatment,  $0.98 \pm 0.24$  mg/kg d. wt; (6) for the 10Cd treatment,  $9.82 \pm 1.03$  mg/kg d. wt; (7) for the 100Cd treatment,  $99.52 \pm 3.44$  mg/kg d. wt. These doses are environmentally-relevant since they overlap with the maximum Cd concentrations allowed in vegetal foods, such as leafy vegetables or fruits [13, 14].

The median concentrations of Cd measured in the hepatopancreas were: (1) for the 0Cd treatment, 2.57 mg/kg d. wt; (2) for the 0.02Cd treatment, 3.80 mg/kg d. wt; (3) for the 0.05Cd treatment, 4.85 mg/kg d. wt; (4) for the 0.2 Cd treatment, 4.98 mg/kg d. wt; (5) for the 1Cd treatment, 44.34 mg/kg d. wt; (6) for the 10Cd treatment, 212.14 mg/kg d. wt; (7) for the 100Cd treatment, 406.36 mg/kg d. wt. (fig. 1). The measured values in controls were within the same range with those assessed in one of our previous experiments [1], as well as with those determined in specimens sampled from unpolluted areas [15, 16]. The maximum Cd levels observed here are also of interest from an ecotoxicological point of view since they are among the highest values ever reported in *C. aspersus*. Therefore, these values may be used as a benchmark for future studies investigating Cd retention in this gastropod species.

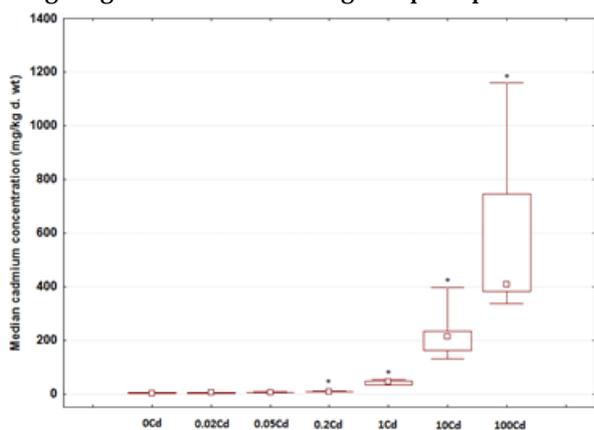


Figure 1. Median Cd concentrations in the hepatopancreas of *Cantareus aspersus* snails. Data are shown as medians (point) with lower and upper quartiles (box) and minimum and maximum values (bars). Marked boxes (\*) indicate significant differences as compared to the reference group (Dunn test,  $*-p < 0.05$ ).

Were identified significant differences in hepatopancreas Cd levels (Kruskal-Wallis,  $p < 0.001$ ).

The measured values increased significantly compared to controls starting from the third Cd treatment (i.e. 0.2Cd treatment) onward, as shown by post hoc analyses using Dunn tests (fig. 1). In a previous study, were investigated the retention of Cd in adult *C. aspersus* snails at similar exposure doses, but for shorter durations, namely 14, 28 and 56 days. Interestingly, the dose from which significant

increases in hepatopancreas Cd were detected was similar [1]. These results suggest the potential existence of threshold level below which these land snails are able to maintain relatively stable cadmium concentrations in the hepatopancreas, which is up to 5 mg/kg d. wt.

Starting from the third dose onward, the test snails concentrated Cd not only in a dose dependent manner, but also at levels far above the measured values in the food. This renders the hepatopancreas of mature snails, *C. aspersus*, as excellent endpoints for assessing Cd toxicity over a broad range of concentrations. Serving as a barometer for dietary Cd exposure – the main path of Cd uptake not only in terrestrial gastropods [6], the hepatopancreas of this species of land snails can hence be reliably used for both active and passive biomonitoring of environmental cadmium pollution.

## Conclusions

The results of the present study support the use of hepatopancreas of mature snails, *C. aspersus*, as endpoints for assessing Cd accumulation and toxicity over a broad range of concentrations. Therefore, this gastropod species can be reliably used for both active and passive biomonitoring of environmental cadmium pollution.

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Manuscript received: 10.01.2019