# EFFECTS OF TWO CADMIUM HYPERACCUMULATING PLANTS (*N. CAERULESCENS* AND *A. HALLERI*) IN FEED ON TISSUE BURDEN IN LABORATORY RATS*

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The aim of this work was to determine how two cadmium (Cd) hyperaccumulating plants in feed affect a consumer organism (*Rattus norvegicus* var. *alba*). Using inductively coupled plasma optical emission spectrometry (ICP-OES), Cd concentrations were analyzed in Wistar rat (*Rattus norvegicus* var. *alba*) tissues. Rats were fed the Cd and Zn hyperaccumulating plants *Noccaea caerulescens* or *Arabidopsis halleri.* Rats given *Arabidopsis halleri* took in 4 times as much Cd as did rats fed *Noccaea caerulescens.* However, the muscle, intestinal, kidney, spleen, testicular, bone and liver tissues of rats fed *A.halleri* had 7.3, 5.6, 5.5, 3.5, 3.1, 2.5 and 2.3 times higher Cd concentrations, respectively, than did tissues of rats fed *N. caerulescens*. *A. halleri* burdened the muscle, small intestinal, and kidney tissues with Cd to a greater extent than did *N. caerulescens*. However, the spleen, testes, bone and liver were significantly more burdened with Cd by *N. caerulescens*. In both experimental groups (rats given *N. caerulescens* as well as those given *A. halleri*), the highest Cd concentrations were found (in descending order) in the kidneys > liver > small intestine > spleen > testes > bone > and muscle. This information is vital in situations where, for example, livestock can graze on these plants or when other animals and humans accidentally consume these plants.

*Arabidopsis halleri*, *Noccaea caerulescens,* toxic, cadmium, accumulation

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## INTRODUCTION

Contamination of the environment with heavy metals and other elements has become a problem not only in the Czech Republic (Jankovska et al., 2016; Sloup et al., 2016, 2017; Vymazal, 2017) but also in many other surrounding regions (Malaspina et al., 2014; Chen et al., 2016; Chatterjee et al., 2017). Cadmium (Cd) is an environmental pollutant ranked eighth in the Top 20 Hazardous Substances Priority List (Klaassen et al., 2009), and human activity has markedly increased the distribution of Cd in the global environment. One of the primary means of contamination for humans is through the diet, with most foods potentially containing natural or synthetic chemicals that could represent a toxic hazard to the consumer (Raad et al., 2014).

Plant species that colonize polluted environments are tolerant to pollutats or have developed defence mechanisms. Certain plants are able to hyperaccumulate metal ions that are toxic to other organisms at low doses. This trait could be utilized in the cleanup of metal-contaminated soils. Moreover, the accumulation of heavy metals by plants affects both

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the micronutrient content and toxic metal content of our food (C l e m e n s , 2006). Phytoextraction refers to the uptake of contaminants from soil or water by plant roots and their translocation to any harvestable plant part. Phytoextraction has the potential to remove contaminants and promote long-term cleanup of soil or wastewater.

*Noccaea caerulescens* syn. *Thlaspi caerulescens* (Brassicaceae) is a Cd/Zn hyperaccumulating plant species. It has attracted the interest of plant biologists due to its ability to colonize calamine and serpentine soils, which contain naturally elevated levels of heavy metals such as Zn, Pb, Cd, Ni, Cr and Co (P e n c e et al., 2000).

*Arabidopsis halleri* syn. *Cardaminopsis halleri* (Brassicaceae) serves as a model plant for Zn and Cd hyperaccumulation. It is Zn-tolerant and a Zn-hyperaccumulator (M e y e r et al., 2010). *A. halleri* is also moderately tolerant to Cd (Z h a o et al., 2006). H u g u e t et al. (2012) determined that the mechanisms of Cd storage and detoxification in *A. halleri* differ from those that were previously revealed for Zn. Hyperaccumulators are important tools for the phytoremediation of Cd-contaminated soil (Liu et al. 2011).

The capability to hyperaccumulate heavy metals in *A. halleri* and *N. caerulescens* is achieved by duplications and alterations of the *cis*-regulatory properties of genes coding for heavy metal transporting/excreting proteins (B o t h e , S l o m k a , 2017).

In a previously published study (V a l e k et al., 2015), we monitored the effects of *A. halleri* on rats; in the current study we compared two hyperaccumulating plants and their effects on a consumer organism (rats). The aim of this work was to compare how two hyperaccumulating plants (*Arabidopsis halleri* and *Noccaea caerulescens*) can affect Cd concentrations in the individual tissues of a consumer organism (*Rattus norvegicus* var. *alba*).

## MATERIAL AND METHODS

### Experimental design

*Arabidopsis halleri* and *Noccaea caerulescens* aboveground biomass was sampled in the flowering stage under natural conditions of an area contaminated with Cd and Zn in the vicinity of Příbram (Czech Republic). These plant samples were later dried at laboratory temperature and homogenized.

### Experimental animals

The present experiment was conducted on 12 male Wistar rats over a six-week period. The rats were randomly divided into two experimental groups (*Arabidopsis halleri* group and *Noccaea caerulescens* group). Rats from the experimental groups (*Arabidopsis halleri* group and *Noccaea caerulescens* group) were fed a mixture of ST-1 (60%) and dried and homogenized plants (40%). Rats given the *A. halleri* plant took in 4 times as much Cd as did rats fed the *N. caerulescens* plant (33.5 mg vs 7.8 mg Cd in 6 weeks).

### Animal welfare

During the experiment, all animals were placed in individual cages. The room housing the cages was air-conditioned. A constant temperature (22–24°C) and humidity level (approximately 70%) were maintained during a constant day/night cycle (8:00–20:00 h). The animals were provided free access to water. All experiments with laboratory animals were conducted in compliance with the current laws of the Czech Republic (Act No. 246/1992 coll. on the Protection of Animals against Cruelty).

### Sampling and analytical procedure

Six weeks into the study, the rats were euthanized and tissues were taken from the following 7 organs with Teflon tools: the liver, small intestine, kidneys, spleen, muscle, testes, and bone tissue (marrow and osseous tissues). All samples were immediately placed in a freezer at –20°C and subsequently freeze-dried. The samples were then pulverized, and 400–500 mg aliquots were decomposed through microwave assisted digestion using a mixture of 65% $HNO_3$ (8.0 ml) and 30% $H_2O_2$ (2.0 ml), purchased from Analytica Ltd. (Prague, Czech Republic) using an Ethos 1 (MLS GmbH, Leutkirch, Germany), at 220°C for 45 min. The digests were poured into 20 ml glass tubes and diluted to 20 ml with distilled water. Certified reference material BCR 185R bovine liver was added to the samples for quality assurance analysis.

Element content in the digests was determined using inductively coupled plasma-atomic emission spectrometry (ICP-OES) (Agilent 720; Agilent Technologies Inc., USA) equipped with a two channel peristaltic pump, a Struman-Masters spray chamber, and a V-groove pneumatic nebulizer made of inert material. To detect low Cd concentrations in the digests, we implemented electrothermal atomic absorption spectrometry (ETAAS) through the use of a VARIAN AA280Z (Varian, Australia) equipped with a GTA120 graphite tube atomizer.

### Statistical analysis

Cd concentrations and their statistical differences were compared within groups using the nonparametric Mann-Whitney U test. The differences were considered significant at $P < 0.05$. All computations were carried out using STATISTICA version 10 program (Statsoft, USA).

## RESULTS

Cd accumulation in rat tissues (bone, small intestine, kidney, liver, spleen, testis, muscle) after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*) is shown in Figs. 1–7. Rats fed *N. caerulescens* as well as those fed *A. halleri* had the highest Cd concentrations in the following tissues in descending order: kidney > liver > intestinal > spleen > testis > bone > muscle tissues (Figs. 1–7). Total Cd intake by rats given *N. caerulescens* over a six-week period was 7.8 mg Cd per kg. The mean Cd concentrations in rat tissues were as follows (in $mg.kg^{-1}$): 0.003 in the muscle, 0.06 in the bone, 0.07 in the testes, 0.15 in the spleen, 0.50 in the small intestine, 2.22 in the liver, and 4.46 in the kidneys (Figs. 1–7). The sum total of Cd levels in the investigated organ tissues was 7.463 mg Cd per kg, which accounts for 96% of the Cd taken up by *N. caerulescens*.

Total Cd intake from *A. halleri* over a six-week period totalled 6.03 mg Cd per rat (180 g), which corresponds to 33.5 mg Cd per kg. Cadmium concentrations in rat tissues were as follows (in $mg.kg^{-1}$): 0.02 in the muscle, 0.15 in the bone, 0.22 in the testes, 0.52 in the spleen, 2.80 in the small intestine, 5.19 in the liver, and 24.58 in the kidneys. The sum total of Cd in the investigated organ tissues was 33.462 mg Cd per kg, which accounts for 99.9% of the Cd taken up by *A. halleri*.

*A. halleri* burdened the muscle, small intestinal, and kidney tissues with Cd to a greater extent than did *N. caerulescens*. However, the spleen, testes, bone and liver were significantly more burdened with Cd by *N. caerulescens*.

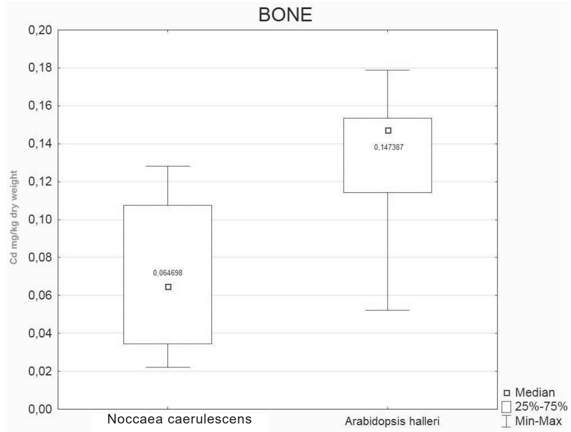


Fig. 1. Cd accumulation in the rat bone after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.02 ($P < 0.05$)

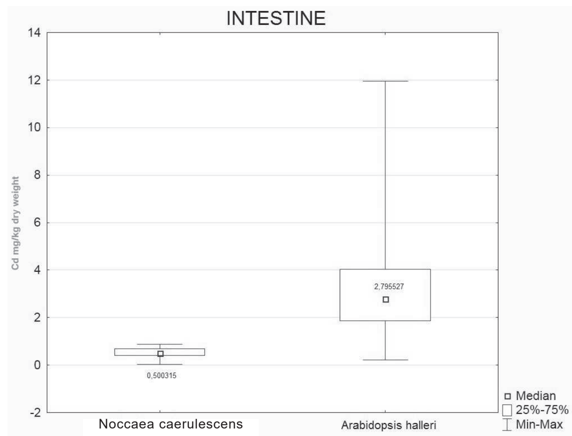


Fig. 2. Cd accumulation in the rat small intestines after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.002 ($P < 0.01$)

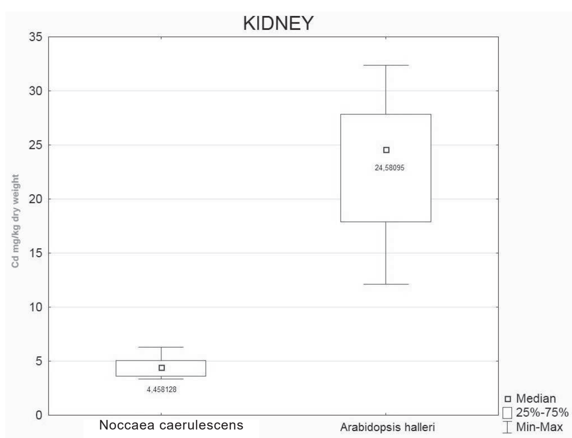


Fig. 3. Cd accumulation in the rat kidney after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.000055 ($P < 0.01$

## DISCUSSION

Toxic metal ions that enter plant roots pose a potential threat to human health (McLaughlin et al., 1999). Cadmium is of particular concern because it is among metals whose ions are most readily taken up by plant roots (Wagner, 1993). *A. halleri* is widely distributed throughout Europe, and it is present in contaminated and non-contaminated areas (Bert et al., 2002).

The ability of *A. halleri* to accumulate and tolerate Cd is comparable to that of the well-known Cd hyperaccumulator *N. caerulescens*. Kupper et al. (2000) reported that *A.halleri* is able to accumulate up to 6000 $mg.kg^{-1}$ of Cd on a dry-weight (DW) basis in the shoots; however, phytotoxicity was observed at this level. Although *A. halleri* can accumulate up to 6000 $mg.kg^{-1}$ DW of Cd when grown in hydroponic solution, plants in their natural European habitats do not normally accumulate more than 100 $mg.kg^{-1}$ DW (Huguet et al., 2012).

In our study, *A. halleri* and *N. caerulescens* grew in the natural conditions of an area contaminated with

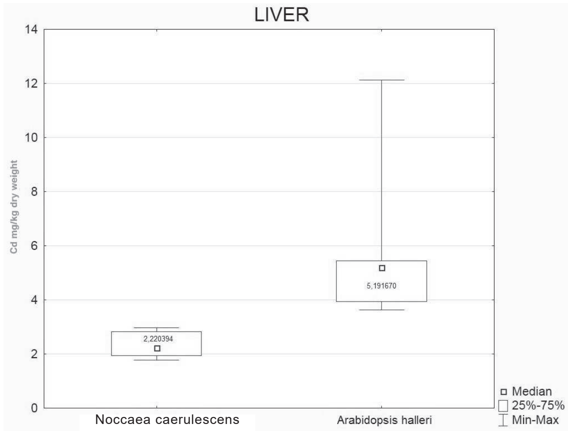


Fig. 4. Cd accumulation in the rat liver after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.000037 ($P < 0.01$)

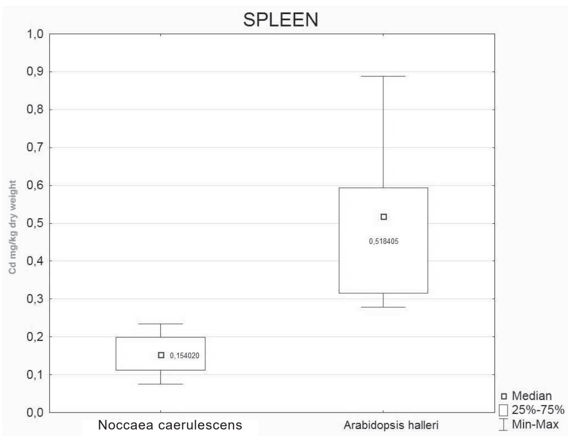


Fig. 5. Cd accumulation in the rat spleen after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.00014 ($P < 0.01$)

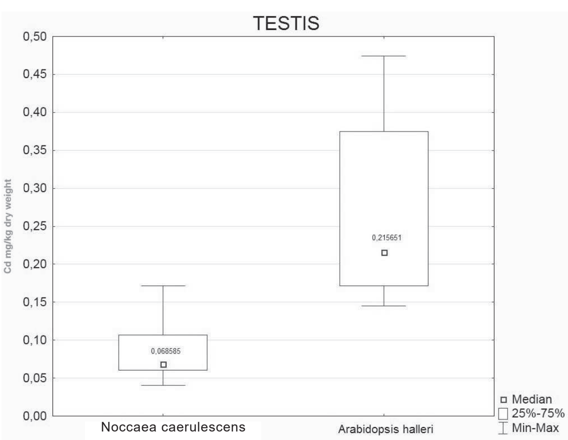


Fig. 6. Cd accumulation in the rat testis after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.00008 ($P < 0.01$)

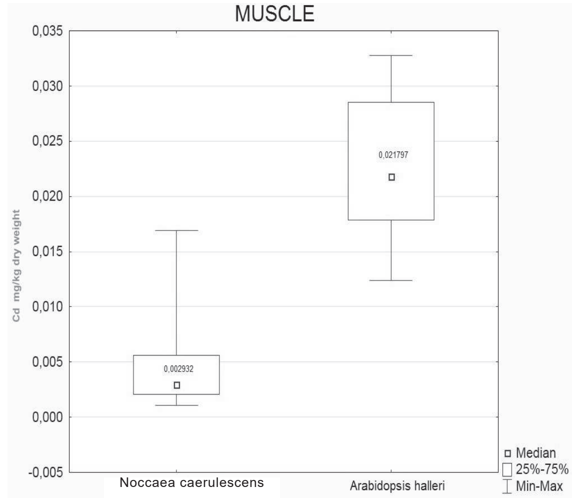


Fig. 7. Cd accumulation in the rat muscle after consumption of hyperaccumulating plants (*Noccaea caerulescens* or *Arabidopsis halleri*). *P*-value: 0.00008 ($P < 0.01$)

Cd and Zn near the city of Příbram, Czech Republic. *A. halleri* grew naturally at this locality, whereas *N. caerulescens* was seeded there.

Although rats given *A.halleri* in feed mixture took in 4 times as much (33.5 mg) Cd over a six-week period as did rats fed *N. caerulescens* (7.8 mg Cd per 6 weeks), kidney, intestinal and muscle tissues of rats given *A.halleri* contained 5.5, 5.6 and 7.3 times higher Cd concentrations, respectively, than those tissues of rats fed with *N. caerulescens*. Contrarily, the liver, bone, testis and spleen tissues of rats fed *A. halleri* contained only 2.3, 2.5, 3.1, and 3.5 times as much Cd, respectively, as did those of rats fed *Noccaea caerulescens* (Figs. 1–7).

These results suggest that *A. halleri* affects the consumer organism through the accumualtion of high levels of Cd, especially in the kidney, intestinal and muscle tissues.

## CONCLUSION

In this study, we revealed that *A. halleri* (Cd/Zn hyperaccumulating plant) affected the consumer organism (*Rattus norwegicus* var. *alba*) with cadmium significantly more than other Cd/Zn hyperaccumulating plant (*N. caerulescens*). There has been little literature (V a l e k et al., 2015) to date that deals with the effects of hyperaccumulating plants on consumer organisms. This study is arguably the first to shed light on these problems. However, further research will be required to fully clarify these problems.

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