

Influence of Cadmium on Starch Accumulation in Zn/Cd Hyperaccumulator *Thlaspi caerulescens*

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Background and aims

Cadmium is non-essential metal, and toxic for plants already at very low concentration. It has negative effect on plant growth and development (Adriano 2001), causing imbalance of protein and carbohydrate ratio (Costa and Spitz 1997), and disrupt carbohydrate metabolism since in plant cell cadmium can replace essential metal ions as zinc, and cause damage to chloroplast membranes (Seregin and Ivanov, 2000).

Some plant species evolved ability to hyperaccumulate Cd in their shoots and significant amount is stored in photosynthetically active mesophyll cells (Reeves and Baker 2000; Vogel-Mikuš et al. 2008; Küpper et al. 2004). Increased tissue cadmium concentrations can reduce plant biomass and cause starch accumulation in non-tolerant plants (Rai et al. 2005), however there is no information available on carbohydrate metabolism in Cd hyperaccumulating plants.

The aim of this study was therefore to investigate the influence of different Cd concentrations on total starch accumulation in Zn/Cd hyperaccumulator *Thlaspi caerulescens*.

Iodine staining

More intensive iodine coloration was observed on leaf cross sections treated with 300 μ M Cd when compared to the control treatment. Black precipitates are starch granules in chloroplasts.

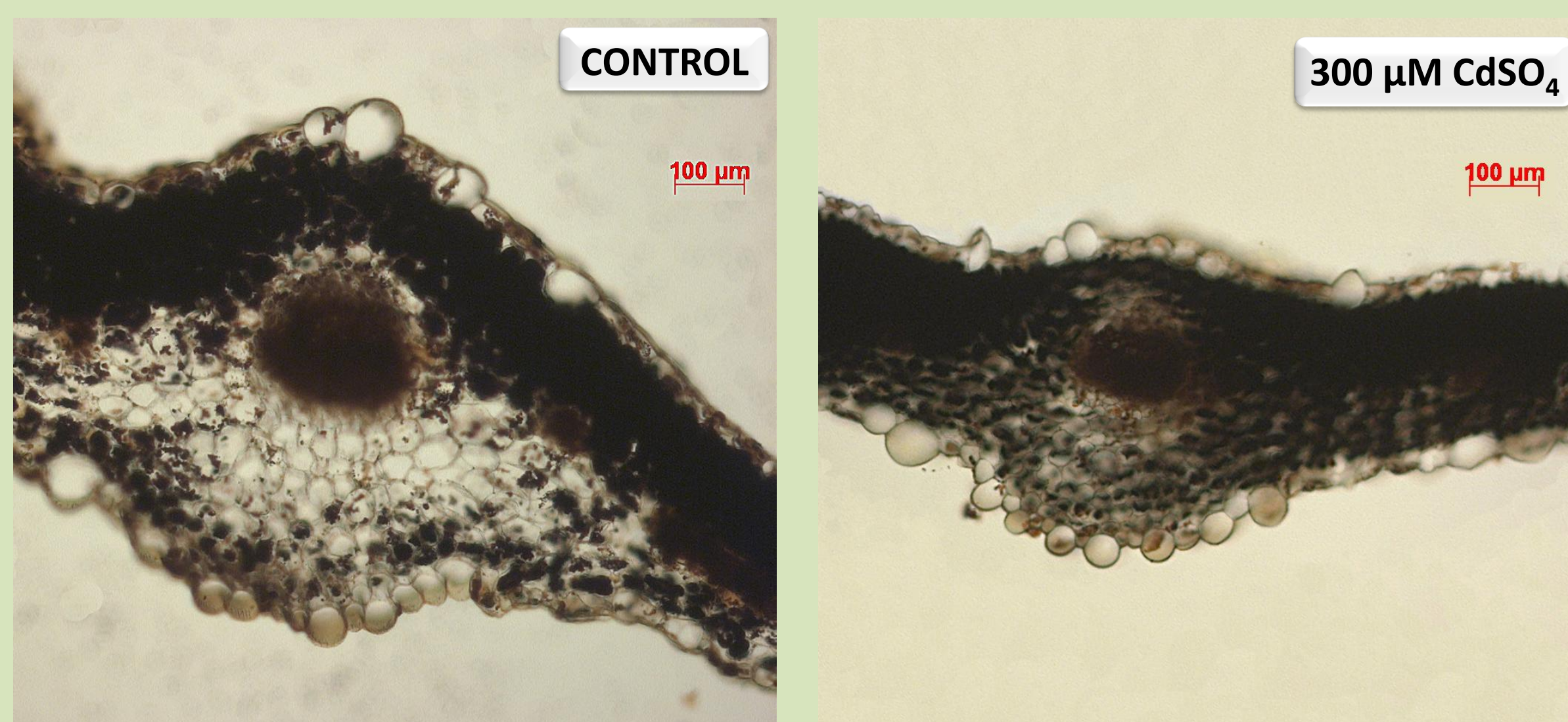


Figure 1. Plant material harvested after four weeks of treatment: Control, 150 and 300 μ M of CdSO_4 . Biomass of plants decreased significantly with increase of Cd in comparison with control.

Materials and Methods

Six weeks after germination of *T. caerulescens* seeds (accession Ganges) the seedlings were transferred to hydroponic system (Tolra et al., 1996). After two weeks the treatments were applied: control, 150 and 300 μ M of CdSO_4 . The nutrient solution was exchanged once per week. Plants were grown in a growing chamber (19 °C, 16-h day length, 50% humidity).

Plants were harvested after four weeks (Fig. 1), weighed and then freeze-dried. Milled plant material was wet-digested (Vogel-Mikuš et al., 2005) and measured for Cd with AAS (PerkinElmer Analyst 100). Total starch was determined spectrophotometrically at 510 nm with commercial kit K-TSTA (Megazyme, Ireland).

The hand cuttings of fresh leaves were stained with Lugol's iodine and examined under light microscope. The data were statistically analysed with one-way ANOVA and Duncan's post hoc test at $p < 0.05$.

Cadmium, Starch, Water Content and Plant Biomass

Dry *T. caerulescens* shoot and roots biomass decreased significantly with increasing Cd concentrations in nutrient solution (Fig. 2). A negative correlation between Cd uptake and water content in shoots was observed, while on the contrary there was a positive correlation between shoot Cd and starch concentration (Fig. 3).

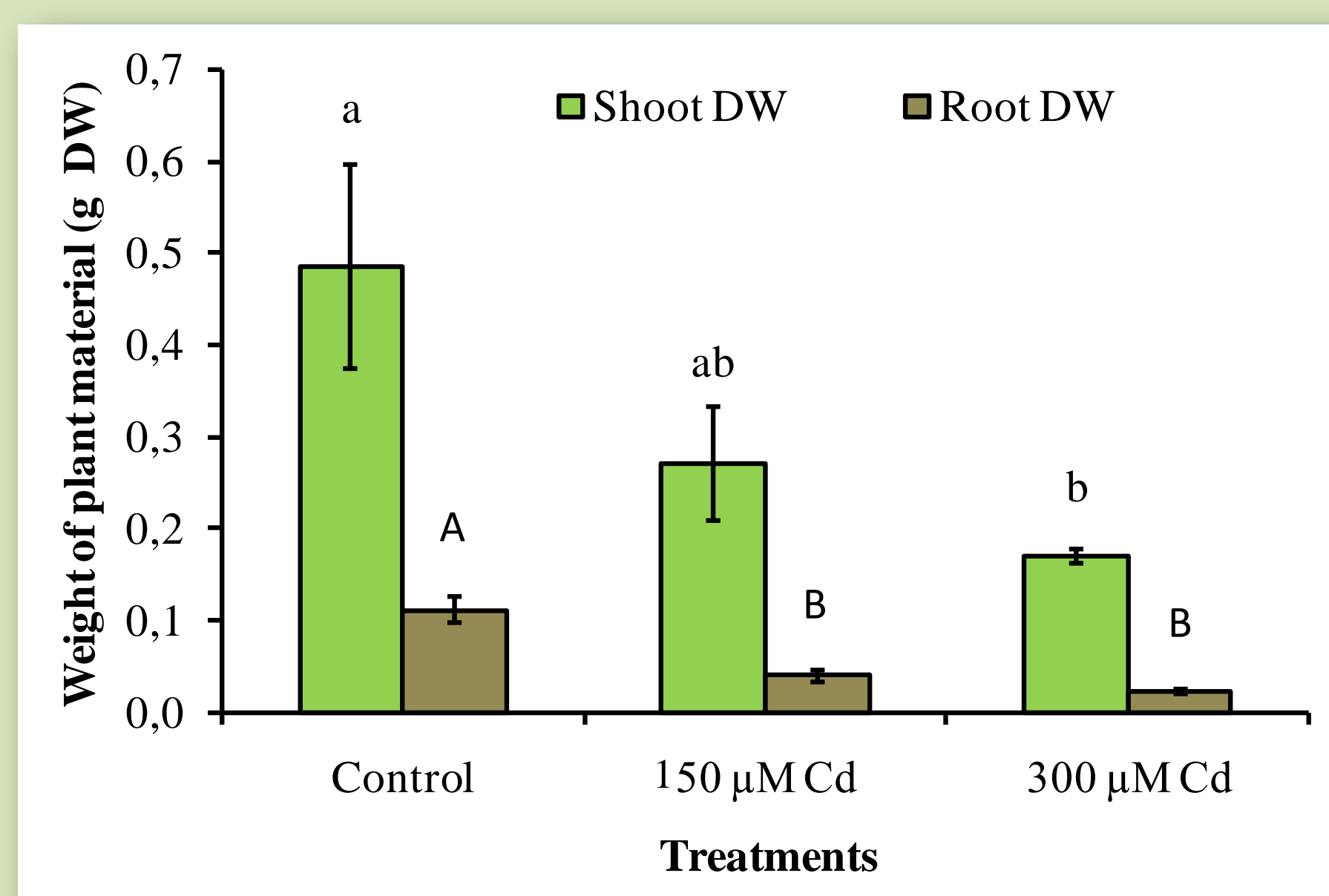


Figure 2. Biomass of shoots and roots (g of dry weight; DW) of *Thlaspi caerulescens*. Mean \pm standard error (n=3). Different letters above bars means significant difference ($p < 0.05$).

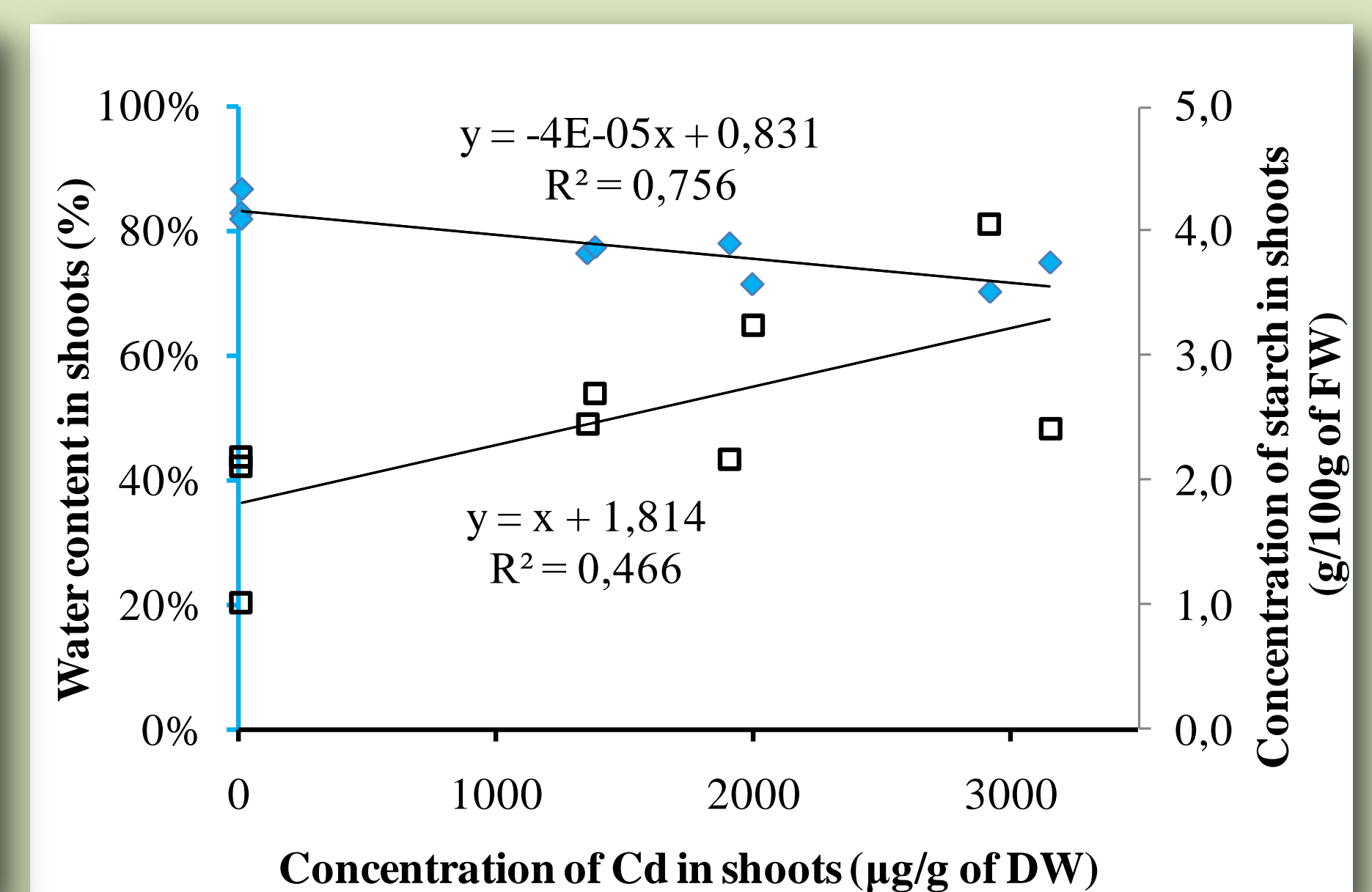
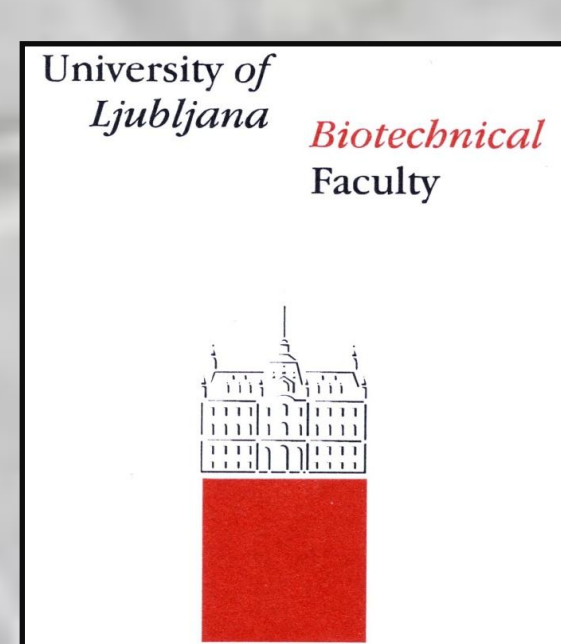
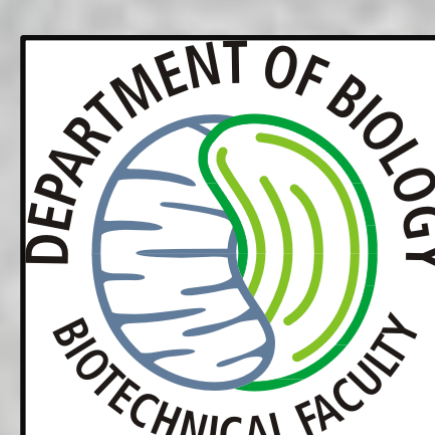


Figure 3. Correlation between shoot Cd concentrations and shoot total starch (\square , on 2^o axis, in mg of starch per 100 mg of fresh weight; FW) and shoot Cd and water content in shoots (\blacklozenge ; on 1^o axis in %) of *Thlaspi caerulescens* in the hydroponic experiment.

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Conclusions

It seems that the mechanisms of Cd toxicity in hyperaccumulating plants are the same as in non-hyperaccumulating plants with exception that they appear at higher tissue Cd concentrations, because metal hyperaccumulating plants had higher ability to store metals in metabolically less active parts of the cells such as vacuoles and cell walls. However further detailed microscopic and biochemical analyses are needed to confirm that.