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Effects of Cd & Ni toxicity to *Ceratophyllum demersum* under environmentally relevant conditions in soft & hard water including a German lake

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ABSTRACT

Even essential trace elements are phytotoxic over a certain threshold. In this study, we investigated whether heavy metal concentrations were responsible for the nearly complete lack of submerged macrophytes in an oligotrophic lake in Germany. We cultivated the rootless aquatic model plant *Ceratophyllum demersum* under environmentally relevant conditions like sinusoidal light and temperature cycles and a low plant biomass to water volume ratio. Experiments lasted for six weeks and were analysed by detailed measurements of photosynthetic biophysics, pigment content and hydrogen peroxide production. We established that individually non-toxic cadmium (3 nM) and slightly toxic nickel (300 nM) concentrations became highly toxic when applied together in soft water, severely inhibiting photosynthetic light reactions. Toxicity was further enhanced by phosphate limitation (75 nM) in soft water as present in many freshwater habitats. In the investigated lake, however, high water hardness limited the toxicity of these metal concentrations, thus the inhibition of macrophytic growth in the lake must have additional reasons. The results showed that synergistic heavy metal toxicity may change ecosystems in many more cases than estimated so far.

1. Introduction

Many heavy metals like copper (Cu), nickel (Ni), manganese (Mn), molybdenum (Mo) and zinc (Zn) are essential trace elements needed in nutrition of plants, and even cadmium (Cd) has been found to have a metabolic significance in Zn-depleted diatoms (Lane and Morel, 2000). When heavy metals are limited, normal plant growth is often not possible. Over a certain threshold, however, they can lead to growth inhibition and other toxic effects (e.g. reviewed by Chen et al., 2009; Küpper and Kroneck, 2005). This happens either by directly affecting proteins and other cell components, or by interfering with uptake of other essential metals, which leads to their limitation in the plant.

Heavy metals can accumulate in the environment due to industrial activities, pollution and mining. Pesticides and fertilizers containing or contaminated with heavy metals (Frank et al., 1976; Gimeno-Garcia et al., 1996; Mantovi et al., 2003) can lead to heavy metal accumulation in crop plants and threaten human health by entering the food chain (McLaughlin et al., 1999; Senesi et al., 1999). Levels of heavy metals in water and soil can increase rather easily in many areas of the world, reaching μM concentrations that are lethal to many water plants (reviews e.g. by Andresen and Küpper, 2013; Küpper and Kroneck, 2005). The outcome of a large scale meta-study about lakes in Scandinavia and north-western Russia showed that heavy metal pollution is a minor issue on the broad scale, but still can cause ecological problems on the local scale (Skjelkvåle et al., 2001). Nickel concentration in Finnish streams and lakes were in a normal range (low nM), but one peak could be directly traced back to a nickel refinery in north Russia (Skjelkvåle

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et al., 2001; Tarvainen et al., 1997). This shows how industrial point sources can influence the aquatic environments. Many previously contaminated areas still show elevated levels of heavy metals exceeding limits set by environmental authorities (Bachor et al., 2012).

In photosynthetic organisms, heavy metal induced damage is diverse. Among various possible targets, the inhibition of the photosynthetic apparatus is especially deleterious, for example by non-functional replacement of Mg^{2+} by other metal ions in chlorophyll (reviewed e.g. by Küpper and Kroneck, 2005). In many plants Cd exposure led to reduced chlorophyll content (Lagriffoul et al., 1998). Another proposed mechanism of Cd-induced stress is the formation of reactive oxygen species (ROS), although for Cd this can only be indirectly via malfunction of photosynthesis as Cd is biologically redox-inert. ROS can lead to lipid peroxidation (Mediouni et al., 2008), causing membrane damage followed by ion leakage (Kumar and Prasad, 2004). Long-term effects of Cd toxicity, and plant acclimation to it, have been studied in detail in the Cd/Zn hyperaccumulator *Noccaea* (formerly *Thlaspi*) *caerulescens*. However, in some studies, Cd seemed to have a positive effect (Ornes and Sajwan, 1993) when present only in low concentrations.

Ni is an essential element in plants being the central ion in the active centre of urease. Most plants need only minute amounts of Ni and limitation is rarely achieved, most easily occurring in Ni-hyperaccumulators that are adapted to high soil Ni (Küpper et al., 2001). At higher concentrations, Ni can inhibit cell proliferation (Rao and Sresty, 2000) and disrupt photosynthesis (Chen et al., 2009; Küpper and Kroneck, 2005). The symptom of chlorosis results from Fe and/or Mg deficit, which are imported less when concentrations of Ni in the environment are above optimum (Piccini and Malavolta, 1992).

However, most studies about Cd and Ni toxicity in plants dealt with acute toxicity, i.e. rather short incubation times of hours to days, and μM or even higher concentrations were used. Further, EDTA, which is often used for iron chelation, also binds to other heavy metals, reducing their bioavailability. For aquatic environments, the maximal allowable concentrations for Cd and its compounds are 4–13 nM (depending on water hardness), for Ni and its (bioavailable) compounds 579 nM (European Commission, 2012). The annual averages are even lower (0.7–2.22 nM Cd; 68 nM Ni). Biotic ligand models (BLMs) can predict concentrations that will have an effect on aquatic organisms (Deleebeeck et al., 2009b; Schlekat et al., 2010).

The present study dealt with an actual environmental problem. Lake Ammelshain (51.296N; 12.615E) is a groundwater lake without any influxes. It is located near Leipzig, Germany. Its size is around 50.6 ha and its maximal depth is 27 m. The lake was formed in the 1930s due to gravel stripping. After the stripping was stopped from 1981 on, the lake turned to an oligotrophic level. Concentrations of chlorophyll and phytoplankton are noticeably low (1 μg L^{-1} Chl; Bernhard, 2010). However, different species of phyto- and zooplankton and even fish can be found in the lake, but besides some *Myriophyllum* plants, no macrophytes (Bernhard, 2010; Nixdorf et al., 2008). To elaborate the reasons for the absence of the macrophyte flora, in particular a possible involvement of Cd and Ni toxicity, relevant parameters of the lake water were measured in August and October 2010, and March and May 2011. Furthermore, lake water samples were taken from the epilimnion and the hypolimnion in summer, autumn 2010 and spring 2011. The model plant *Ceratophyllum demersum* L. was cultivated in these lake water samples and systematic simulations of the lake water for 6 weeks using environmentally relevant conditions of nutrient levels, light and temperature conditions. *C. demersum* is a submerged macrophyte sensitive to heavy metal stress and has no roots, which means that all nutrients are taken up over the whole shoot.

2. Materials and methods

2.1. Determination of physical parameters of the lake

In August and October 2010, as well as March and May 2011, lake parameters were measured. The sampling periods were chosen to be consistent with the year's seasons. In winter the lake was covered with a thick ice layer and sampling was not possible. The winter sample was therefore taken in March, when the ice was gone.

The depth profile was recorded in 50 cm steps with DS5 (Hydrolab, Hach Hydromet, Loveland, CO, USA) and the parameters depth, pH, temperature, electric conductivity, oxygen amounts and saturation (LDO sensor, Hach) and air pressure (ADC Summit Silva, Sollentuna, Sweden) were measured. Light intensity was measured between water surface and 9 m depth in summer, and 27 m depth in winter with a light meter Li-Cor 250 equipped with the spherical underwater sensor Li-193 (Li-Cor Lincoln, NE, USA) and a CTM sensor (Sea&Sun, Trappenkamp, Germany). Water samples for cultivation of plants (30 l in PE barrels) and ICP-MS analyses (50 ml in PFA tubes; AHF, Tübingen, Germany) were taken with a IWS II (Hydro-Bios, Kiel, Germany) in 5 l steps from the epi- and hypolimnion (Wetzel, 2001) when the lake was separated into distinct layers. During the circulation period, epilimnion water was taken as a mixed sample between 0–7 m depth and hypolimnion water as a single sample 1 m above the ground for which the IWS II was controlled by a cable (Sea&Sun, Trappenkamp, Germany). The maximal deviation of the sampling position was less than 0.25 m. The water was very clear, without visible particles and therefore not filtered.

2.2. Plant material and cultivation

We used the submerged, rootless macrophyte *Ceratophyllum demersum* L. for the experiments. The strain was continuously cultivated since 2005 in hydroponic solution with 12 h day/12 h night light conditions provided by two Osram FLUORA® fluorescent and two warm white fluorescent tubes (Osram, München, Germany) and a temperature cycle from 18 °C at 6 a.m, over 20 °C at 9 a.m., to a maximum of 22 °C at 3 p.m., back over 20 °C at 9 p.m. to 18 °C again at 6 a.m.

Plants of stock cultures were cultivated long term in a nutrient solution that was previously optimised (personal communication from HK) for submerged macrophytes and water plants (SMNS) and consists of 0.1 μM B, 100 μM C, 40 μM Ca, 87.5 μM Cl, 0.01 μM Co, 0.02 μM Cr, 0.01 μM Cu, 0.2 μM Fe, 0.2 μM Fe-EDDHA, 500 μM Hepes, 0.05 μM I, 120 μM K, 150 μM Mg, 0.4 μM Mn, 0.01 μM Mo, 30 μM N, 60 μM Na, 0.01 μM Ni, 2 μM P, 151 μM S, 0.05 μM Zn. The pH was adjusted to 7.8 with KOH. This nutrient solution is, in principle, a simulation of conditions in oligotrophic freshwater habitats with low water hardness, where submerged macrophytes thrive. Experimental treatments were, therefore, performed in SMNS as control, in water from the epi- and hypolimnion of Lake Ammelshain and in different simulations of lake water investigating which of the differences between SMNS and the lake water caused inhibition of plant growth in the lake water. The different simulations are displayed in Table 1. Further, for investigating the effect of water hardness, the experiments using SMNS with Cd, Ni and reduced phosphate which caused the greatest inhibitory effects were repeated with increased water hardness (CdNi-P (hard water)). Finally, a lake simulation as accurately as possible (Lakesimulation) and the same simulation without extra Cd, Ni and P-stress (Lakesimulation – CdNi-P) were performed. The respective concentrations can be found in Table 2. Elevated Na and Cl levels were also tested, but the samples did not show any effect (not shown).