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Copper and zinc in *Elodea canadensis* from rivers with various pollution levels

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ABSTRACT

The anthropogenic impact of xenobiotics contributes to environmental risk for the aquatic environment and thus, must be controlled. *Elodea canadensis*, a cosmopolitan aquatic macrophyte with an important role in the ecology of many littoral zones, may provide an integrated record of pollution. Therefore, it was interesting to investigate the accumulation of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in this species and in water and bottom sediments collected from rivers with various levels of contamination. Of these rivers one control and one polluted was selected for the collection of *E. canadensis* for an experiment to compare the ability of this species to accumulate Cu and Zn. These elements were supplemented at concentrations (mg L^{-1}) of 0.01, 0.02, 0.03, 0.05, 0.08 and 0.14 as $CuSO_4 \cdot 5H_2O$, and 0.4, 0.6, 0.9, 1.4, 2.03 and 3.04 as $ZnSO_4 \cdot 7H_2O$ and in a mixture containing (mg L^{-1}) 0.01Cu + 0.4Zn, 0.02Cu + 0.6Zn, 0.03Cu + 0.9Zn, 0.05Cu + 1.4Zn, 0.08Cu + 2.03Zn and 0.14Cu + 3.04Zn. After the experiment, *E. canadensis* from the polluted river contained significantly higher Cu and Zn concentrations when applied separately and also significantly higher Cu and Zn concentrations when applied as a mixture compared to the control river. These higher concentrations in *E. canadensis* from the polluted river were found in all combinations in the experiment. Thus, *E. canadensis* habituated in polluted sites to the exposure, and long-term influence of elevated metal levels appeared to be better adapted, and it also exhibited a higher increase in biomass than plants from the control river in all the experimental Cu and Zn solutions. Younger leaves of *E. canadensis* were more resistant to the effects of Cu and Zn than older leaves. Both Cu and Zn negatively affected the cell structure of older leaves, although the influence of Cu on plasma membrane integrity and chloroplast distribution was stronger than that of Zn. The influence of the Cu + Zn mixture on *E. canadensis* resulted in less pronounced cell disintegration than the influence of Cu added separately.

The explanation of differences in the *E. canadensis* biomass increase and metal concentrations under the binary Cu and Zn impact needs further examination.

1. Introduction

The pollution of aquatic ecosystems is an important problem of ecological concern because of its negative effects on the food chain, biodiversity, purity of drinking water and public health (Mountouris et al., 2002; Xue et al., 2010). The ecological status of many surface waters is strongly affected by anthropogenic activities which produce various types of pollution with environmental risk for the aquatic environment (Nábělková et al., 2004). Studies of vegetation in running waters may provide information on the temporal and spatial quality of these systems (Grasmuck et al., 1995; Martinez and Shu-Nyamboli, 2011). In addition, macrohydrophytes could be used in bioremediation of xenobiotics in contaminated waters (Hansen et al., 2011; Harguinteguy et al., 2013). These plants can serve as a sink for these substances, removing and metabolizing

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them before xenobiotics can impact the ecosystem (Dosnon-Olette et al., 2011). Metals that cannot be degraded are one of the threats to aquatic environments because they can migrate and, accumulate in abiotic and biotic compartments (Miretzky et al., 2004; Khazheeva et al., 2005). Aquatic macrophytes similarly to algae can reduce metal concentrations and serve as indicators of metal contamination in water bodies (Thiébaut et al., 2010; Martinez and Shu-Nyamboli, 2011; Bácsi et al., 2015; Novák et al., 2014). Final metal concentrations in plants are usually significantly higher than in the environment (Miretzky et al., 2004). Investigations of macrohydrophytes are also valuable for the identification of pollution sources (Berg and Steinnes, 1997; Viskari et al., 1997). Plants are stationary, constantly exposed to contaminants and integrate variation of the trophic level over time (Thiébaut et al., 2010). The quantity of metals accumulated by plants is a major importance in assessing the risk of toxicity (Kabata-Pendias, 2001; Roy and Gunjan, 2010). Quite much information is available on the contamination of plants by single metals, but aquatic plants are usually exposed to the combined influence of these elements with mutually mitigating or amplifying effects (Ince et al., 1999; Pivetz, 2001; Haiyan, 2003). *Elodea canadensis* is a cosmopolitan submerged macrophyte rooted in the sediment. This species has wide trophic amplitude, and it plays an important role in the ecology of many littoral zones (Simpson, 1986; Carbiener et al., 1990; Kahkonen and Manninen, 1998; Nyquist and Greger, 2009). It has very thin submerged leaves almost lacking the cuticle arranged around the stem, which results in a high surface-to-volume ratio (Fritioff et al., 2005). *E. canadensis* has been successfully used as an accumulator of metals and certain organic pollutants in the remediation of contaminated sites (Maleva et al., 2004; Malec et al., 2009; Xue et al., 2010; Thiébaut et al., 2010; Dosnon-Olette et al., 2011; Hansen et al., 2011; Martinez and Shu-Nyamboli, 2011). It is easy to culture and handle, tolerant to a broad range of environmental conditions and has a fast growth rate (Mielecki and Pieczyńska, 2005). As a sessile organism, it may provide an integrated pollution record within a particular part of the river. Plants have an ability to adapt to local conditions (Fernándèz et al., 2000), so *E. canadensis* from less contaminated sites should acquire significantly lower metal concentrations than the same species in habitats with a surplus of these elements. The objective of this survey was (i) to evaluate metal concentrations in water, sediments and *E. canadensis* collected from small rivers with different pollution levels and (ii) in laboratory experiments to compare the impact of combined or separately added Cu and Zn (as dominating metals in the selected waters). To evaluate the individual or combined influence of Cu and Zn on the cellular organization of *E. canadensis* leaves microscopic observations were conducted to visualize the influence of metals on the vitality of leaf cells. This is an interdisciplinary approach to the research problem. We hypothesized the followings: (1) combined Cu and Zn concentrations supplied to *E. canadensis* may cause mitigating effects on the concentration of both toxicants in plants; (2) *E. canadensis* from more polluted sites accumulates significantly more Cu and Zn than the same species from less polluted sites because of the adaptation to habitats with elevated concentration of these metals; (3) younger leaves are more resistant to the Cu and Zn effect than older leaves; (4) both Cu and Zn adversely affect the cell structure of older leaves, and the influence of Cu on plasma membrane integrity and chloroplast distribution is stronger than that of Zn; (5) Cu + Zn influence on *E. canadensis* results in less pronounced cell disintegration than the influence of Cu added separately.

The investigation into the impact of metal mixtures is useful for understanding and explaining metal interactions which affect their distribution and bioavailability in nature (Stewart and Malley, 1999). This study constitutes combined research of the ecology and cell biology of the bioindicator *E. canadensis* plant.

2. Materials and methods

2.1. Sampling design

Based on a preliminary study of 206 rivers in which *E. canadensis* occurred naturally (Jusik, personal communication), seventeen rivers in western Poland were selected with relatively clean sections indicated as A or potentially polluted sections indicated as B. The sections A and B are added to the river numbers 1–17 as indicated in Fig. 1. In each of fourteen rivers, three random sampling sites in the relatively clean section and three random sampling sites in the potentially polluted section were selected. Samples of water, sediment and *E. canadensis* (all in three replicates) were collected in these sections of the rivers. In three additional rivers (11, 13 and 15) it was possible to collect samples in no more than three random sampling sites in the clean section because of the absence of plants in the polluted section. Plants were washed in river water to remove sediment, detritus and attached invertebrates. The total number of sampling sites was 14 rivers × 2 sections × 3 sites + 3 rivers × 1 section × 3 sites = 93. The total number of water, bottom sediments and plant samples was 93 sites × 3 replicates = 279.

2.2. Water, bottom sediment and plant analysis

Prior to the analysis, water samples were acidified to pH ≤ 2 with ultra pure HNO_3 and filtered through 0.45 μm glass microfibre filters to determine total metal concentrations (Ladislas et al., 2012). Three replicates were analyzed separately. Fresh sediment was used for potentiometric pH_{H_2O} determinations. Before analysis, the plants were washed carefully for a 2 min in distilled water. *E. canadensis* and bottom sediments were dried at 50 °C to constant weight. Plant samples were homogenized in an IKA Labortechnik M20 laboratory mill. Bottom sediments were homogenized with an agate mortar and pestle after the coarse material had been removed using a 2 mm sieve. Subsequently, 300 mg of dry weight, in triplicate was digested with 3 mL of nitric acid (ultra pure, 65%) and 2 mL of perchloric acid (ultra pure, 70%) in a CEM Mars 5 microwave oven. After dilution to 50 mL, the digests together with water samples were analyzed for Fe, Mn and Zn using FAAS (Avanta PM from GBC) and Cd, Co, Cr, Cu, Ni and Pb using GFAAS (PinAAcle 900Z from Perkin-Elmer). All the elements were determined against standards (Atomic Absorption Standard Solution from Sigma Chemical Co.), and blanks containing the same matrix as the samples and were subjected to the same procedure. All results for plants were calculated on a dry weight basis. The accuracy of the methods applied for the determination of the elements in the samples was checked by the analysis of the *Phragmites communis* IPE 176 standard (Wageningen Evaluating Programs for Analytical Laboratories, WEPAL) (Electronic Supplementary Material ESM 1).

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