Chemosphere 132 (2015) 40–46

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Comparative study on the sensitivity of turions and active fronds of giant duckweed (*Spirodela polyrhiza* (L.) Schleiden) to heavy metal treatments

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ARTICLE INFO

Article history:
Received 14 October 2014
Received in revised form 19 January 2015
Accepted 20 January 2015
Available online 13 March 2015

Handling Editor: A. Gies

Keywords:
Spirodela polyrhiza
Turion
Vegetative propagule
Duckweed test
Growth inhibition
Heavy metal

ABSTRACT

Standard ecotoxicological test procedures use only active forms of aquatic plants. The potential effects of toxicants on vegetative propagules, which play an important role in the survival of several aquatic plant species, is not well understood. Because turion-like resting propagules overwinter on the water bottom in temperate regions, they could be exposed to contaminants for longer periods than active plants. Due to its turion producing capability, giant duckweed (*Spirodela polyrhiza*) is widely used in studying morphogenesis, dormancy, and activation mechanisms in plants. It is also suitable for ecotoxicological purposes. The present work aims to compare the growth inhibition sensitivity of active (normal frond) and overwintering (turion) forms of *S. polyrhiza* to concentrations of nickel (Ni), cadmium (Cd) and hexavalent chromium (Cr) ranging from 0 to 100 mg L^{-1}. The results indicated that in general, resting turions have higher heavy metal tolerance than active fronds. Cd proved to be the most toxic heavy metal to *S. polyrhiza* active frond cultures because it induced rapid turion formation. In contrast, the toxicity of Ni and Cr were found to be similar but lower than the effects of Cd. Cr treatments up to 10 mg L^{-1} did not result in any future negative effects on turion activation. Turions did not survive heavy metal treatments at higher concentrations of Cr. Cd and Ni treatments affected both the floating-up and germination of turions but did not significantly affect the vigor of sprouts. Higher concentrations (of 100 mg L^{-1}) Cd completely inhibited germination.

1. Introduction

The duckweed species (Lemnoideae subfamily) are globally occurring, free-floating monocots. Due to their highly reduced anatomy, small size and rapid growth, they are representatives of aquatic macrophytes in ecotoxicology research (Environment Canada, 2007). These tiny plants normally reproduce and survive unfavorable environmental conditions by producing new fronds or vegetative propagules by their meristems (Jacobs, 1947). Propagules (also known as turions) of giant duckweed (*Spirodela polyrhiza* (L.) Schleiden) are widely used for assessing morphogenesis, the dormant state, and the activation of vegetative buds (Newton et al., 1978; Chaloupková and Smart, 1994; Appenroth, 2002). Mature *S. polyrhiza* turions have a higher density than water and, therefore, sink to the bottom after separation from the mother frond. The dormant turions are not ready to germinate immediately even if the environmental conditions are favorable. They require cold conditions after the ripening period to break their dormancy (Ley et al., 1997). After becoming non-dormant, external signals initiate the phytochrome-mediated germination process. Due to enhanced respiration, a CO_2 bubble is formed and the buoyant turions rise to the water surface (Newton et al., 1978). Activated meristems start to differentiate vegetative fronds within a few days, and newly formed duckweed plants re-colonize the habitat.

Formation of turions, or other vegetative propagules, is a common strategy amongst hydrophytes to survive in unfavorable conditions or to colonize new habitats (Barrett et al., 1993; Santamaría, 2002; Adamec and Kučerová, 2013).

Heavy metals are considered to be among the most harmful anthropogenic threats to natural surface waters. In addition to specific physiological effects, they generally induce oxidative stress, degrade macromolecules, and disturb metabolic processes by competing for uptake or displacing essential metal cofactors in enzymes and signaling pathways (DalCorso, 2012).

Vegetative propagules can stay in a dormant state for several months and may be exposed to heavy metal contaminated sediments for long periods of time. Despite this assumption, very

Abbreviations: Cd, cadmium; Cr, hexavalent chromium in this paper; Ni, nickel; RGR, relative growth rate; IC_{50}, the estimated toxicant concentration that induces 50% inhibition of the measured parameter.

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http://dx.doi.org/10.1016/j.chemosphere.2015.01.050
0045-6535/

limited and contradictory information is available on the tolerance of resting propagules to toxic substances. Standard ecotoxicological test methods are based on the responses of higher aquatic plants in the active stage of the life cycle (Environment Canada, 2007). However, these results are hard to extrapolate to resting propagules. According to Srivastava and Jaiswal (1989), Cd induced the formation of turions but inhibited their germination. Turion formation of *S. polyrhiza* plants was also induced by Cr treatments (Susplugas et al., 2000). Contrary to these findings, Xyländer et al. (1993) reported the inhibition of turion formation by Ni and Cd. Xyländer et al. (1993) also observed that Ni broke the dormancy of turions and strongly inhibited their germination. The results for Cd were similar to those for Cr.

The present study focuses on comparing the sensitivity of active fronds and resting turions of *S. polyrhiza* to heavy metal treatments. Standard growth inhibition tests were performed and the tolerance of turions to heavy metal was assessed by simulating a cold period in a heavy metal-contaminated environment and their subsequent germination in a pure growth medium.

Three heavy metals were applied: nickel (Ni), cadmium (Cd) and hexavalent chromium. Because only the hexavalent form of chromium was used, it will be referred to as 'Cr' throughout the text. All metals are strong toxicants and are released into the environment in large amounts (Moore and Ramamoorthy, 1984). Two of them, Ni and hexavalent Cr, are used as reference toxicants in duckweed tests (Environment Canada, 2007).

Ni is an essential micronutrient for both plants and animals, but in high doses, it results in severe growth reduction of roots and disorders in nutrient uptake, nitrogen metabolism, water balance and photosynthesis (DalCorso, 2012). Its prevalent oxidation form is Ni^{2+} in aqueous environments. Cd is not essential and is strongly toxic even in trace concentrations (Das et al., 1997). The Cd^{2+} ion, which is the most stable form in aqueous solutions, is highly mobile in aquatic environments. In general, Cd induces imbalances in plants' mineral nutrition, water relations, photosynthesis and respiration. These issues result in chlorosis, stunting and senescence (Das et al., 1997; Solti et al., 2008). The trivalent form of chromium is essential for animals, but the hexavalent form has no physiological role in living cells (Cervantes et al., 2001). In well aerated waters, hexavalent chromium forms CrO_4^- and $Cr_2O_7^-$ oxyanions (Shanker et al., 2005). In living cells Cr(VI) is readily reduced to Cr(III) by reacting with macromolecules, triggering oxidative stress and consequent malfunction of cells (Appenroth et al., 2000; Oláh et al., 2010).

2. Materials and methods

2.1. Culturing conditions

Axenic cultures of a local *S. polyrhiza* (L.) Schleiden clone (Lake Kis-Balaton, W. Hungary) were used for the experiments in this work. Cultures were maintained in 300 cm^3 Erlenmeyer flasks, under continuous illumination by white light (GE Polylux F30W/830, PPFD: 60 ± 10 $\mu mol\ m^{-2}\ s^{-1}$) at a constant temperature (22 ± 2 °C) and in a sterile Steinberg medium (pH 5.5 ± 0.2, Environment Canada, 2007). Subcultures that were 7–10 d old were used for test purposes.

2.2. Tests with active fronds of S. polyrhiza

Crystallizing dishes (80 mm diameter) were used in these tests. Each contained 100 cm^3 of freshly prepared Steinberg medium and was covered with plastic Petri dishes during tests. At the beginning of the experiments, 10 ± 2 randomly selected fronds, corresponding to 2.3 ± 0.4 cm^2 total frond area, were used to inoculate each test vessel. The heavy metals Ni, Cd and Cr were added as solutions of $NiSO_4 \times 7H_2O$, $3(CdSO_4) \times 8H_2O$ and $K_2Cr_2O_7$ salts, respectively. The concentrations of heavy metals in the growth medium ranged between 0.001 and 10 mg metal L^{-1}, that is, 0.017–170.4 μM for Ni^{2+}, 0.009–88.95 μM for Cd^{2+} and 0.019–192.3 μM for chromate. This concentration range application of Cd and Ni increased the SO_4^{-2} content in the Steinberg medium (39 mg L^{-1}) to 0.004–42% and 0.002–22% respectively. Such an elevation in the sulfate level was not considered to be a growth modifying factor because Appenroth et al. (2008) reported culture growth rates using sulfate concentrations up to 10000 μM (~1000 mg L^{-1}). The application of $K_2Cr_2O_7$ caused a negligible increase of potassium concentration in the medium (0.005–5% in range of 0.001–10 mg Cr L^{-1}), which was assumed not to influence plant growth.

The irradiation and temperature conditions during the active frond tests were identical to those of stock-culturing described in Section 2.1.

The duration of the tests with heavy metals was 7 d. A digital image of the plants in each vessel was taken on the first and last day of the testing period. The images were processed using the ImageJ image analysis software (Abramoff et al., 2004). The total number and area of fronds were used as test endpoints. All visible fronds were counted. The relative growth rates with respect to change in assimilating area and frond number (referred below as RGR_{area} and RGR_{frond}, respectively) were calculated according to the Environment Canada (2007) guidelines as follows:

$$RGR_X = \ln(X_j) - \ln(X_i)/(t_j - t_i);$$

where RGR_X is the calculated average relative growth rate and X_i and X_j are the measured values of the respective parameter at day t_i and t_j, respectively.

2.3. Experiments with turions

2.3.1. Production and store of turions

Turions were produced by allowing untreated axenic cultures to senesce in 50 cm^3 growth medium in Erlenmeyer flasks. The depletion of nutrients induced the production of turions, which detached from the mother fronds and sank to the bottom (Susplugas et al., 2000). Sunken turions were collected aseptically from the bottom of flasks and transferred to fresh medium. To break their dormancy, the turions were stored in pure Steinberg medium in a cold room (6 °C, in dark) for an additional 4 weeks before being used in experiments (Ley et al., 1997).

2.3.2. Heavy metal treatments of turions

For heavy metal treatments, inoculums of 30 ± 10 turions were transferred to test tubes containing 20 cm^3 of cold Steinberg medium supplemented with heavy metals. The final concentrations in the medium were 0.01, 0.1, 1, 10 and 100 mg metal L^{-1}, which corresponded to 0.170–1704 μM of Ni^{2+}, 0.089–889.6 μM of Cd^{2+} and 0.192–1923 μM of Cr. The turions were exposed to the medium in a dark and cold room for 7 d. These conditions prevented the induction of turion germination. After 7 d, long exposition turions were removed from the medium and washed thoroughly with distilled water. These were transferred to 100 cm^3 of pure Steinberg medium in crystallizing dishes (80 mm diameter) and covered with plastic Petri dishes. The dishes were transferred to germination-inducing conditions as explained in Section 2.1. Germination and the subsequent growth of new fronds was monitored for 7 d.

The vigor of germinating turions was assessed by the following parameters:

Floating-up (%): the number of buoyant turions was visually counted 24, 48 and 96 h after starting the germination–induction test. Activation was expressed as the percent ratio of turions