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The treatment of duckweed with a plant biostimulant or a safener improves the plant capacity to clean water polluted by terbuthylazine

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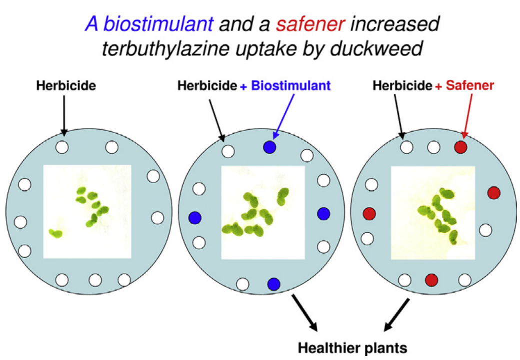
HIGHLIGHTS

- Terbuthylazine (TBA) toxicity to duckweed was assessed.
- TBA severely affected duckweed especially at the higher concentrations.
- The effects of a biostimulant and a safener on duckweed were investigated.
- Biostimulant and safener reduced TBA toxicity to the species.
- Biostimulated and safened plants adsorbed higher amounts of TBA from polluted water.

GRAPHICAL ABSTRACT



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ABSTRACT

Water pollution is becoming alarming since thousands contaminants are dispersed in the aquatic environments, and agricultural practices, for the massive use of pesticides, are contributing to exacerbating this problem.
In this context, a research aimed at investigating the ability of duckweed (*Lemna minor*), a free-floating aquatic species widespread throughout the world, to remediate water polluted with five different concentrations of a herbicide - terbuthylazine (TBA) - was carried out. In addition, duckweed was treated with a plant biostimulant and a safener with the aim of increasing the plant's capacity to tolerate and remove the TBA from the water. The results evidenced that the herbicide affected the duckweed already at the lower concentrations, reducing its capacity to proliferate and the area of its fronds. On the contrary, when the TBA treatments were performed in combination with the biostimulant or the safener the average area of the fronds was affected of lesser extents, compared to the plants treated with the herbicide only. Antioxidant enzymes, namely ascorbate peroxidases (APX) and catalases (CAT), were investigated and it was found that the biostimulated and safened duckweed showed increased activities of these enzymes, compared to the plants treated with TBA only. At last, some phytofiltration experiments were planned. The biostimulated and safened duckweed removed more TBA from polluted water than the plants treated with the herbicide alone. In conclusion, this research showed that duckweed is suitable for cleaning water polluted with TBA and this potential can be successfully improved by treating the species with a biostimulant or a safener.

1. Introduction

Several million toxic substances are continuously dispersed in the environment, causing serious problems of environmental pollution

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(Brumovský et al., 2016). The case of water pollution is of particular concern since thousands of contaminants are emitted into the aquatic environments (Dachs and Méjanelle, 2010). With regard to agriculture, cultivated fields require large quantities of pesticides in order to protect crops from many different pests (Gaba et al., 2017). However, pesticides can easily pass through the various environments and contaminate water, soil and atmosphere (Laini et al., 2012; Melo et al., 2012; Palma et al., 2014). Focusing attention on herbicides, a very important subclass of the larger family of pesticides, their use in agriculture is crucial for the management of weed species, which otherwise could be very competitive with crops for space, nutrients and light (Bartucca et al., 2018). Benefits notwithstanding, weed control based on the use of herbicides is causing pollution of freshwater and marine ecosystems (Brumovský et al., 2016). In fact, the active compounds or their metabolites have frequently been found in the aquatic environments (Brumovský et al., 2016).

There are several chemical and physical methods which can be used to clean up polluted water/soil (Olguín and Sánchez-Galván, 2012). However, these techniques can be very expensive, and, in some cases, they can have a negative impact on the environment and the cleaning may not be entirely satisfactory (Sakakibara et al., 2011). There is another recent technique, which is gaining attention, called phytoremediation, which can be applied in order to remediate polluted environments. This technology is based on the use of plants, and possibly their associate microbes, to perform environmental remediation or to prevent water/soil pollution (Pilon-Smits, 2005). Phytoremediation can be used to clean water and soil contaminated by a variety of organic and inorganic pollutants (Pilon-Smits, 2005). Regarding the decontamination of polluted water, phytoremediation can be practiced for the treatment of sewage and municipal wastewater, agricultural drainage/runoff water, groundwater plumes, etc. (Pilon-Smits, 2005). In this regard, aquatic plant species have recently gained special attention for their potential to remediate freshwater polluted by a series of organic and inorganic substances (Panfili et al., 2017; Rezania et al., 2016). These plants function as an onsite bio-filter that can easily reach and absorb the pollutants present in the water (phytofiltration) (Olette et al., 2008). The effectiveness of aquatic plants, especially if free-floating on the water surface, is due to the fact that they are in direct contact with the polluted media from which the contaminants are taken up (Maine et al., 2004). For instance, some studies showed that *Lemna minor* (duckweed), *Elodea canadensis*, *Cabomba aquatica* and *Salvinia auriculata* are species suitable for the decontamination of water polluted by heavy metals, excessive amounts of nutrients and organic compounds (Olette et al., 2008; Vymazal, 2016). In addition, some authors have documented that the ability of duckweed to remove heavy metals from polluted water can be significantly enhanced by treating the floating species with compounds that can increase the plant's resistance to abiotic stresses (Panfili et al., 2017). Finally, it should be noted that free-floating aquatic species are widespread, easy to cultivate and grow rapidly. For all these reasons, some of these species are attracting the attention of the scientific community. For example, it is possible to create wetlands using these plants, with the aim of remedying or preventing the contamination of water bodies (Valipour and Ahn, 2016).

For all the aforesaid reasons, the research has been planned for investigating duckweed tolerance and its capacity to remediate solutions polluted with terbuthylazine (TBA). TBA is a graminicide herbicide used worldwide for the control of maize weeds; it acts by targeting the photosynthesis of the weed species at the level of the photosystem II (Bartucca et al., 2017b). Nonetheless, this chemical and its metabolites are considered compounds of concern for their frequent detection in surface and groundwater (Bottoni et al., 2013). At last, it should also be indicated that the environmental hazard of TBA is due to its mobility and persistence, which in certain environmental conditions may be considerable (Del Buono et al., 2016). We then treated duckweed plants with various substances with the aim of increasing their capacity to tolerate TBA and, possibly, to improve their ability to phytofiltrate the water polluted with the herbicide. To this purpose, a herbicide safener (benoxacor - Ben) and a plant-biostimulant (Megafol - Meg) were selected. The safener was chosen for its documented ability to increase plant tolerance to herbicides (Hatzios and Burgos, 2004) and to some other abiotic stresses (Bartucca et al., 2017a). In addition, a very recent study conducted on aquatic species showed how safeners can also increase the ability of plants to remove copper from polluted water (Panfili et al., 2017). The plant biostimulant was tested because it has recently been shown how these substances not only stimulate the plant efficiency in the use of nutrients, but they can also improve the ability of some species to tolerate biotic or abiotic stresses (Yakhin et al., 2017).

2. Materials and methods

2.1. Plants growth conditions

Duckweed (*Lemna minor*) was collected in spring from a natural freshwater basin located in Perugia (Italy). The plants were initially disinfected by immersion into a solution of 0.5% sodium hypochlorite for 1–2 min and then gently rinsed with distilled water for 1 min twice. Successively, the plants were transferred into polyethylene trays (35 × 28 × 14 cm) containing a sterilized nutrient solution (pH 6.5) composed as follows: 3.46 mmol L^{-1} KNO_3, 1.25 mmol L^{-1} $Ca(NO_3)_2 \cdot 4H_20$, 0.66 mmol L^{-1} KH_2PO_4, 0.071 mmol L^{-1} K_2HPO_4, 0.41 mmol L^{-1} $MgSO_4 \cdot 7H_2O$, 0.28 mmol L^{-1} K_2SO_4, 1.94 μmol L^{-1} H_3BO_3, 0.63 μmol L^{-1} $ZnSO \cdot 7H_2O$, 0.18 μmol L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 1 μmol L^{-1} $MnSO_4 \cdot H_2O$, 21.80 μmol L^{-1} FeEDTA and 1 μmol L^{-1} $CuSO_4$. Trays were then positioned into a growth chamber at 24 ± 2 °C and at a light intensity of 100 μmol m^{-2} s^{-1} (light/dark photoperiod: 12/12 h). Plants were maintained in these conditions for a minimum of eight weeks prior to use them and to carry out the experiments. Growth mediums were renewed once a week.

2.2. TBA, Meg and Ben toxicity tests to duckweed

Toxicity tests were carried out on duckweed (4 plants, in the same growth stage), placing the species in 9 cm Petri dishes, containing 40 mL of static ultrapure water and maintained at the light intensity of 100 μmol m^{-2} s^{-1} (light/dark photoperiod: 12/12 h – temperature: 24 ± 2 °C). Under these conditions, without a nutrient solution, plants did not show symptoms of stress for at least three weeks. Therefore, duckweed samples were exposed to the concentrations of terbuthylazine (TBA) (Sigma Aldrich - St Louis, MO) of 0.031, 0.062, 0.125, 0.250 and 0.500 mg L^{-1}. These concentrations were selected on the basis of data published in the literature, concerning the range of TBA found in polluted water basins/sediments (Melo et al., 2012; Palma et al., 2014; Silva et al., 2012). After one week, the number of new fronds produced by the plants (proliferation capacity) and the area of the fronds (Khellaf and Zerdaoui, 2010) were recorded. In particular, the frond area was estimated by image analysis. To this purpose, a camera was used to acquire an image of each Petri dish, then the images were processed using the software ImageJ 1.50i (Wayne Rasband, National Institute of Health, USA).

From this study, it was obtained, by interpolation (linear or polynomial) the $\%I_{50}$ evaluated as the TBA concentration causing a 50% growth inhibition to duckweed in one week of TBA treatment or expressed as the amount of the herbicide capable to reduce the average frond area of the 50% (Khellaf and Zerdaoui, 2010).

The same parameters were also determined for duckweed treated with Megafol (Valagro – Atessa, Chieti, Italy) or Benoxacor (Sigma Aldrich - St Louis, MO). Megafol was applied to duckweed at 5 different dosages corresponding to 0.25, 0.50, 1.00, 2.00 and 4.00 fold the field rate suggested by the manufacturer (1 L ha^{-1}). Benoxacor was applied at the following concentrations: 0.031, 0.062, 0.125, 0.250 and 0.500 mg L^{-1} which cover the range of the safener application rates suggested by the manufacturer (0.125 mg L^{-1}). Since Megafol was