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Italian ryegrass for the phytoremediation of solutions polluted with terbuthylazine

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HIGHLIGHTS

- Italian ryegrass removed high amounts of TBA from contaminated solutions.
- RHIZOtest was found to be adequate in testing herbicides removal from aqueous/soil solutions.
- Inductions of glutathione S-transferase with TBA-dose dependent trends were found.
- General inductions of ascorbate peroxidase (APX) activities were found.

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ABSTRACT

The phytoextraction capacity of Italian ryegrass (*Lolium multiflorum* L.) to remove terbuthylazine (TBA) from aqueous solution has been assessed using a plant-based biotest (RHIZOtest). Three TBA concentrations (0.5, 1.0 and 2.0 mg L^{-1}) were chosen to evaluate the tolerance capacity of the ryegrass. Even though the treatments negatively affected plants, they were able to remove up to 30–40% of TBA. In addition, some enzymatic activities involved in the response to TBA-induced stress were determined. Glutathione S-transferase (GST) has been activated with a TBA-dose dependent trend; ascorbate peroxidase (APX) activities have been induced within the first hours after the treatments, followed by decreases or disappearance in plants exposed to two higher dosages. In conclusion, this case-study highlights that the combination of ryegrass and RHIZOtest resulted to be effective in the remediation of aqueous solutions polluted by TBA.

1. Introduction

Herbicides are agrochemicals used worldwide for crop protection against weeds, with the objective to increase the yields of cultivated fields. Among them, triazines are a very widely-used class of herbicides which kills the infesting weeds, by interrupting the photosynthetic electron transport at level of photosystem II *via* inhibition of the activity of D1 protein (Cañero et al., 2011). The triazines are mainly absorbed by roots and, to a lesser extent, by leaves (Ibrahim et al., 2012; Olette et al., 2008). However, some general concerns are linked to the risk that many of these chemicals can drift off target reaching non-target crops and other organisms. Furthermore, it has been demonstrated that some herbicides (including also some compounds belonging to the class of triazines) can be very persistent in soils representing a risk of pollution for both surface and ground waters (Delin and Landon, 2002; Gerard and Poullain, 2005). Therefore, water bodies are constantly exposed to a wide variety of toxic chemicals; during the last years, a particular emphasis has been paid to the problems of water pollution by herbicides and some studies have evidenced frequent contaminations by these chemicals, triazines included (Botta et al., 2012). As the environmental pollution caused by toxic compounds is becoming one of the main concerns, phytoremediation technologies are being considered, among all the techniques available, the more promising for remediating polluted environments (Ibrahim et al., 2012). These technologies take advantage from the ability of some plants, tolerating high amounts of contaminants (including herbicides) in soils, to take up (*removal*) and/or metabolize (*degradation*) these pollutants (Pilon-Smits, 2005). This ability has been demonstrated for several plant species and toward many different polluting agents (Pilon-Smits, 2005). In particular, some woody, bushy and herbaceous plant species have shown potentiality in decontaminating soils polluted with

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triazines. Hybrid poplar trees have been ascertained to take up and, once inside, transform atrazine into less toxic metabolites (Burken and Schnoor, 1997), while common reed (*Phragmites australis*) and switchgrass (*Panicum virgatum*) have been demonstrated to be efficient in the detoxification of terbuthylazine and atrazine, respectively (Schröder et al., 2005; Albright et al., 2013). Differently, in parrotfeather (*Myriophyllum aquaticum*), stems and leaves are the main tissues involved in the uptake of simazine being the roots unable to accumulate the herbicide (Wilson et al., 2001). Concerning riparian buffer strips, it has been clearly demonstrated their capacity to absorb significant amount of atrazine favouring its degradation (Reungsang et al., 2001). Also corn (*Zea mays*) has been clearly demonstrated to be an efficient crop in remediating contaminated soils by atrazine (Ibrahim et al., 2012). In addition, the remediation of water bodies has been conducted also by using wetland plants, like *Typha Latifolia*, which were capable to reduce significantly the amount of terbuthylazine in polluted environments (Papadopoulos et al., 2009).

The plant abilities to decontaminate polluted environments rely on their capability to overcome the presence of contaminants thanks to the presence of some specific enzymatic activities which are involved in the response to the herbicide-induced oxidative stress and in the inactivation/detoxification of these chemicals (Vàzquez et al., 2009). In fact, it is well known that herbicides frequently cause the overproduction of reactive oxygen species (ROS: i.e. superoxide radicals [O^{-2}], hydroxyl radical [OH^{-}] and hydrogen peroxide [H_2O_2]; (Del Buono et al., 2011) causing serious problems to the plants (degradation of nucleic acids, pigments and proteins, membranes and in turn, the cell death; Gomes et al., 2013). Among antioxidant enzymes and molecules involved in facing these injuries, ascorbate peroxidases (APX; EC 1.11.1.11), which catalyse the removal of H_2O_2 by using ascorbic acid as a specific electron donor (Asada, 1992; Shigeoka et al., 2002), are well known for their important role. Concerning the detoxification process, glutathione S-transferases (GST; EC 2.5.1.18) are responsible for the herbicide conjugation with the natural, endogenous tripeptide glutathione (GSH) causing the toxicity loss of the molecule. Once produced, the conjugates, which usually are not only less toxic but also less mobile than the parent compounds, are then transferred into the vacuole (Del Buono et al., 2007; Puglisi et al., 2012). It is interesting to note that plant species tolerant to a wide spectrum of herbicides are characterized by high constitutionally expression of GST activity (Marrs, 1996), which can be further induced in response to the treatments with agrochemicals (Del Buono et al., 2007). Furthermore, the glutathione S-transferases have been found to be particularly active in the detoxification of chloroacetanilides and triazines herbicides (Rossini et al., 1996). However, to date little is known about the advantage in possessing these enzymatic activities and the possibility to activate them to cope with high agrochemical concentrations in soil by plant species used in programs of herbicide remediation.

Therefore, this work is aimed at ascertain the possibility to use Italian ryegrass in the remediation of aqueous and soil solutions polluted with terbuthylazine, a herbicide belonging to the triazines class and widely used in agriculture for crop protection. To this purpose, the resistance of Italian ryegrass to high TBA concentrations in relation also to the activity level of detoxifying enzymes like GST and antioxidant enzymes APX, has been evaluated. The phytoextraction potential of this plant species has been assessed using a plant-based biotest, i.e. the RHIZOtest system, verifying its possible use in studies focused on the agrochemical cycle in the soil–water–plant interface.

2. Materials and methods

2.1. Plant material and growth conditions

Italian ryegrass (*Lolium multiflorum* L.) seeds were placed into a RHIZOtest as described by Chaignon and Hinsinger (2003) and Bravin et al. (2010). Plant pots were inserted on a floating platform (Fig. 1) in 5 L tanks and ryegrass germination (0.75 g pot^{-1}, 12 pots $tank^{-1}$) was directly performed in plant pots covered with aluminium foil to prevent light influence for 48 h at 22 °C (relative humidity 90%) with continuous aeration of the aqueous medium. Plant roots were physically separated from the hydroponic solution by a 30 μm nylon membrane. On the third day after sowing, seedlings were transferred in a growth chamber in day-night conditions with 14 h of light at 25 °C, light intensity 150 μmol m^{-2} s^{-1}, and 10 h of darkness at 22 °C (relative humidity 80%). The nutrient solution was composed as follow: 2 mM $Ca(NO_3)_2 \times 4H_2O$, 0.5 mM $MgSO_4 \times 7H_2O$, 0.7 mM K_2SO_4, 0.1 mM KCl, 0.1 mM KH_2PO_4, 1 μM H_3BO_3, 0.5 μM $MnSO_4 \times H_2O$, 0.5 μM $CuSO_4$, 0.5 μM $ZnSO_4 \times 7H_2O$, 0.01 μM $(NH_4)_6Mo_7O_{24} \times 4H_2O$, 100 μM Fe-EDTA. The growth medium was renewed weekly.

In order to assess the effects of terbuthylazine on ryegrass, on the seventh day after sowing three different tanks (the experiments were run in triplicate) were added with solutions containing

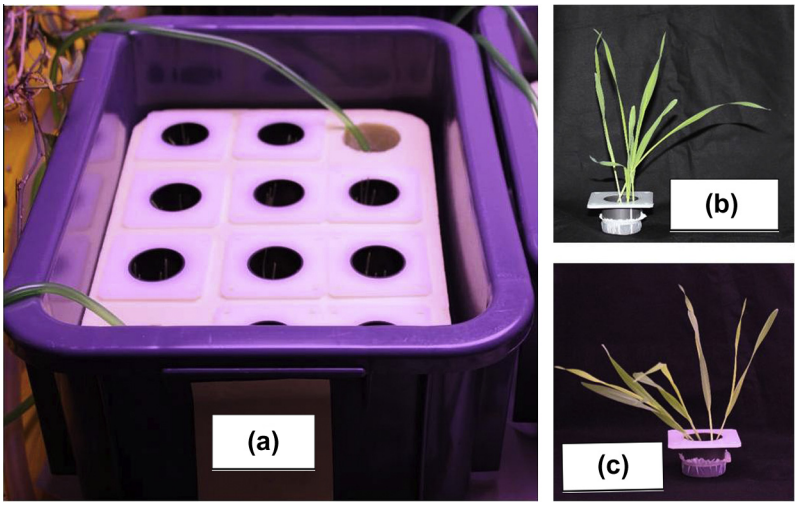

Fig. 1. (a) RHIZOtest device with young seedlings of Italian ryegrass, (b) control plants without TBA and (c) plants treated with 2.0 mg L^{-1} TBA.