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Biochemical responses of the golden mussel *Limnoperna fortunei* under dietary glyphosate exposure

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ABSTRACT

The aim of this study was to analyze the biochemical alterations in the golden mussel *Limnoperna fortunei* under dietary glyphosate exposure. Mussels were fed during 4 weeks with the green algae *Scenedesmus vacuolatus* previously exposed to a commercial formulation of glyphosate (6 mg L^{-1} active principle) with the addition of alkyl aryl polyglycol ether surfactant. After 1, 7, 14, 21 and 28 days of dietary exposure, glutathione-S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), acetylcholinesterase (AChE), carboxylesterases (CES) and alkaline phosphatase (ALP) activities, glutathione (GSH) content and damage to lipids and proteins levels were analyzed. A significant increase (72%) in the GST activity and a significant decrease (26%) in the CES activity in the mussels fed on glyphosate exposed algae for 28 days were observed. The ALP activity was significantly increased at 21 and 28 days of dietary exposure (48% and 72%, respectively). GSH content and CAT, SOD and AchE activities did not show any differences between the exposed and non exposed bivalves. No oxidative damage to lipids and proteins, measured as TBARS and carbonyl content respectively, was observed in response to glyphosate dietary exposure. The decrease in the CES activity and the increases in GST and ALP activities observed in *L. fortunei* indicate that dietary exposure to glyphosate provokes metabolic alterations, related with detoxification mechanisms.

1. Introduction

The water bodies in Argentina are increasingly exposed to several agrochemicals. This occurs as a consequence of the expansion of the agricultural frontier and a growing trend in the use of different agrochemicals in current agricultural practices (Altieri and Pengue, 2006). Agrochemicals can be distributed in different aquatic compartments (water column, suspended particles, sediments, biota) (Warren et al., 2003) and they interact with the biota of all trophic levels, producing adverse effects over short and long term. According to their chemical nature, agrochemicals can also be bioaccumulated and transferred through the trophic chains (Nfon et al., 2008).

The use of glyphosate (N-phosphonomethyl glycine) for crop production is world-wide spread, both in industrialized and developing countries (Benbrook, 2016). For example, the global use of this herbicide reached about 127,000 t in USA and 700,000 t worldwide in 2012 (Van Bruggen et al., 2018). Similarly, glyphosate is the most widely used herbicide in Argentina. During 2013 its consumption was 182,484,206 L, representing 65% of the total pesticides used in the country (Aparicio et al., 2015). Glyphosate is highly soluble in water (12 g L^{-1}, log K_{ow}: −3,2) with a half-life ranging from 4 to 5 days to 60 days in water and from a few days to several months in the soil,

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depending on the environmental conditions (Székács and Darvas, 2012; Vereecken, 2005).

Glyphosate has a powerful herbicidal action and its primary mechanism of action is the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Sergiev et al., 2006), which is only present in plants and microorganisms. This inhibition results in the arrest of protein synthesis and the death of the target organisms. Glyphosate is an organophosphonate, therefore it has a C-P bond which makes it thermally and chemically stable (Kertesz et al., 1994). Its microbiological degradation occurs mainly in the soil, whereas in water column and aquatic sediments it occurs to a lesser extent (Lipok et al., 2010).

In general, the regulation of pesticide use is based on the effects of the active principles that exert herbicidal, insecticidal or fungicidal actions (Séguin et al., 2017). Nevertheless, in the field, glyphosate is applied as formulations containing adjuvants and inerts, some of which also have toxic effects on the organisms. In particular, in the central Pampean region of Argentina, one of the most applied formulations is Glifosato Atanor® (48% glyphosate as isopropylamine salt) with 2.5% of the surfactant Impacto® (alkyl aryl polyglycol ether) (Romero et al., 2011).

Microalgae can accumulate and bioconcentrate xenobiotics, biotransform them (sometimes generating more toxic metabolites) and/or adsorb them on their cell walls (Okay et al., 2000). Algae are the basis of food webs of aquatic ecosystems, so they act as a point of entry of pollutants into aquatic organisms that feed directly or indirectly on them (Okay et al., 2000).

Bivalves are organisms that filter large amounts of water for nutritional and respiratory requirements. They are placed in the first levels of many aquatic food webs and play an important ecological role, regulating the turbidity of the water column, recycling nutrients and organic matter and controlling phytoplankton biomass (Binelli and Provini, 2003). Mussels are considered excellent biomonitors of pollution due to their feeding habits, sedentary lifestyle, abundance, wide distribution in different water bodies, tolerance to a wide range of environmental conditions, and tendency to accumulate contaminants in their tissues (Belaich et al., 2006). Then, as filter feeding organisms, bivalves could be exposed to pollutants directly or by dietary exposure. Although glyphosate has a high water solubility and low octanol/water partition coefficient, it could potentially bioccumulate in bivalves, as it has already been reported for other aquatic animals (CCME, 2012; Contardo-Jara et al., 2009).

Several biochemical markers are used to evaluate the toxicity of pesticides on bivalves. These biomarkers include parameters related to the oxidation of biomolecules, and to changes in the levels of antioxidant, detoxification and general metabolic enzymes. Thus, the lipid and protein oxidation markers (thiobarbituric reactive substances –TBARS-, and carbonyl contents), the levels of the antioxidant metabolite reduced glutathione (GSH), and the activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) are commonly evaluated in pesticide exposed bivalves (Mottier et al., 2015). Other enzymes that could be altered in bivalves exposed to pesticides are the detoxification enzymes glutathione-S-transferase (GST) and carboxylesterases (CES), the general metabolism enzyme alkaline phosphatase (ALP) and the organophosphate sensitive enzyme acetylcholinesterase (AChE) (Castro et al., 2017; Galloway et al., 2002).

The golden mussel *Limnoperna fortunei* (Bivalvia, Mytilidae) is a freshwater bivalve from rivers and streams in China and Southeast Asia, and it was first detected in Argentina in 1991 (Darrigran and Ezcurra, 2000). This species has a high reproductive potential and adaptability and it has no natural predators or pathogens in the aquatic systems of Argentina (Darrigran and Damborenea, 2006; Darrigran and Ezcurra, 2000). Thus, L. *fortunei* has expanded to the main river basins, reaching densities higher than 200,000 individuals/m^2 (Boltovskoy et al., 2006), and occurring in wetland areas impacted by glyphosate.

Although glyphosate is promoted as harmless for animals, several ecotoxicological studies show that glyphosate could be toxic to bivalves and other aquatic organisms (Modesto and Martinez, 2010; Mottier et al., 2015). Inhibition of CES and AChE activity and alteration of oxidative stress parameters have been reported in bivalves exposed to glyphosate acid or their formulations (Abdel-Nabi et al., 2007; Iummato et al., 2013; Mottier et al., 2015).

Aquatic organisms, mainly those that filter large amounts of phytoplankton, face the impact of the contaminants that enter through their food. In general, few researches have addressed the study on the effects of this via of pesticide exposure. Additionally, there are no studies on enzymatic and oxidative stress responses of a freshwater mussel with dietary exposure to glyphosate. Therefore, the aim of this study was to evaluate biochemical alterations, mainly related to oxidative stress and detoxification responses, in the golden mussel L. *fortunei* after dietary exposure to glyphosate.

2. Materials and methods

2.1. Chemicals

The commercially available herbicide used in this study was Glifosato Atanor® (48% p/v isopropylamine salt of N-phosphonomethyl glycine, Atanor, Munro, Buenos Aires province, Argentina) and the surfactant was alkyl aryl polyglycol ether 50% (p/v) Impacto® (Agroasist S.R.L., Argentina).

2.2. Organisms

The BAFC CA4 strain of *Scenedesmus vacuolatus* (Chlorophyceae, Chlorophyta) is currently kept in the Culture Collection of the Laboratorio de Biología de Protistas, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

Specimens of L. *fortunei,* of 25.7 ± 2.2 mm shell length, were collected manually on the banks of the Río de la Plata (34° 29' 5.55"S, 58° 28' 49.67" W, Buenos Aires, Argentina), and were transported to the laboratory. Specimens of this size would correspond to adult animals of approximately 1–1.5 years old (Boltovskoy and Cataldo, 1999). Prior to the experiments, the specimens of *L. fortunei* were acclimatized for 4 weeks in 20 L tanks (200 individuals per tank). Tanks were filled with aerated dechlorinated tap water and the mussels were maintained at 20 ± 1 °C and 12:12 photoperiod. During the acclimation period, mussels were fed twice a week with 2×10^5 *S. vacuolatus* cells mL^{-1} for 24 h.

2.3. Algal cultures

S. vacuolatus cultures were prepared in 500 mL flasks containing 300 mL of Bold´s basal medium (BBM, Bischoff and Bold, 1963) with Glifosato Atanor® (6 mg L^{-1} active principle) and 2.5% surfactant Impacto® (treated cultures) or without glyphosate nor surfactant (control cultures). Cells from an exponential phase culture were used as inoculum to achieve an initial cell density of 30,000 cells mL^{-1}. The flasks were incubated at 23 ± 1 °C, under continuous agitation and illumination (80 μmol photons m^{-2} s^{-1}). After 96 h, the cells from both culture groups (control and treated) were harvested, washed and suspended in dechlorinated water to obtain a concentrated cell suspension. The cell density from the different suspensions was determined by cell counting in a Neubauer's chamber, using a Leica light microscope at 400×.

The initial glyphosate concentration in the BBM medium solution was analytically determined at INQUIMAE–CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. The determination of glyphosate was performed by HPLC-UV chromatography after a derivatization step with FMOC-Cl (Sancho et al., 1996; Stalikas and Konidari, 2001). The analysis was performed using a HPLC-UV