Chemosphere 185 (2017) 975–982

Contents lists available at ScienceDirect

Chemosphere

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

Can an aquatic macrophyte bioaccumulate glyphosate? Development of a new method of glyphosate extraction in *Ludwigia peploides* and watershed scale validation

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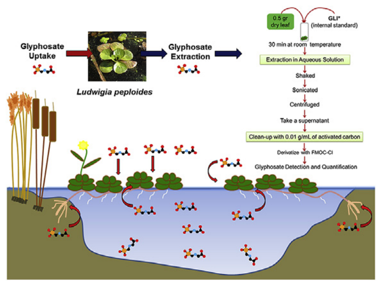
HIGHLIGHTS

- A glyphosate extraction method in the hydrophyte *Ludwigia peploides* was developed.
- Environmental levels of glyphosate in *Ludwigia peploides* were measured.
- Glyphosate bioconcentration and bioaccumulation in *Ludwigia peploides* was calculated.
- *Ludwigia peploides* accumulates glyphosate in its leaves mainly from surface water.
- *Ludwigia peploides* can be used as a biomonitor of glyphosate levels in stream water.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 January 2017
Received in revised form
11 July 2017
Accepted 17 July 2017
Available online 19 July 2017

Handling Editor: Klaus Kümmerer

Keywords:
Ludwigia peploides
Hydrophyte
Glyphosate extraction
Bioconcentration factor
Biota-sediment accumulation factor
Agricultural watershed

ABSTRACT

Glyphosate is intensively used in agricultural fields and it is frequently detected in non-target wetland ecosystems. The floating hydrophyte *Ludwigia peploides* is widely distributed in American streams and it is an abundant species. Therefore, our objectives were (1) to establish and validate an extraction and quantification methodology for glyphosate in *L. peploides* and (2) to evaluate the role of this species as a potential glyphosate biomonitor in an agricultural watershed. We developed a new method of glyphosate extraction from leaves of *L. peploides*. The method recovery was 117± 20% and the matrix effect 20%. To validate the method using environmental samples, plants of *L. peploides* were collected in March 2016 from eight monitoring sites of El Crespo stream. Surface water and sediment samples were collected at the same time to measure glyphosate and to calculate bioconcentration factors (BCFs) and biota-sediment accumulation factors (BSAFs). Glyphosate was detected in 94.11% in leaves, the concentrations ranging between 4 and 108 μg/kg. Glyphosate was detected in surface water and sediments at 75% and 100% of the samples, at concentrations that varied between 0 and 1.7 μg/L and 5–10.50 μg/kg dry weight, respectively. The mean BCFs and BSAFs were 88.10 L/Kg and 7.61, respectively. These results indicate that *L. peploides* bioaccumulates glyphosate mainly bioavailable in the surface water. In this sense, *L. peploides* could be used as a biomonitor organism to evaluate glyphosate levels in freshwater

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http://dx.doi.org/10.1016/j.chemosphere.2017.07.093
0045-6535/

aquatic ecosystems because, in addition to its capacity to bioconcentrate glyphosate, it is easy to sample and it has a restricted mobility.

1. Introduction

Glyphosate [N-(phosphonomethyl) glycine] is an effective and non-selective post-emergence herbicide used worldwide for the control of many grasses, broadleaf weeds, aquatic grasses and brush (Zhang et al., 2011). Due to its massive application within agro-ecosystems it is frequently detected, as well as its metabolite aminomethylphosphonic acid (AMPA). The reported concentrations of glyphosate and AMPA in USA surface waters range between 0.08 and 450 μg/L (Coupé et al., 2012; Battaglin and Meyer, 2014), while the concentrations in sediments reach 470 μg/kg (Battaglin and Meyer, 2014). In Switzerland, the reported glyphosate concentrations range from 0.024 to 3.3 μg/L (Hanke et al., 2010); while in Argentina, levels in surface water are within 0.5–7.6 μg/L and from 5 to 200 μg/kg in sediments (Aparicio et al., 2013).

In particular, in El Crespo watershed, which is focus the of the present study, the glyphosate and AMPA levels in surface water ranged from 2.00 to 2.90 μg/L, and in sediments from 18.50 to 47.50 μg/kg (Pérez et al., 2017). The spatial variations are mainly dependent on the proximity of the agricultural fields, in the upper basin, where there are extensive crops, glyphosate and AMPA levels increase in surface water and in the lower basin, where the main farming activity is the extensive livestock, the levels decrease (Pérez et al., 2017).

Floating, submerged and emergent macrophytes can be used as *in situ* bioindicators of water quality because of their ability to accumulate agrochemicals, and because wetlands and agricultural fields are strongly associated (Lewis, 1995; Carvahlo et al., 2007; Turgut, 2005; Pérez et al., 2013). They comprise an important component of benthic primary production in wetlands that must be protected from adverse chemical effects in order to maintain ecosystem structures and functions. Macrophytes fulfil several critical functions in aquatic ecosystems such as the conversion of solar energy and carbon dioxide into organic matter, oxygen providers, nutrient cycling, sediment stabilization, and habitat and shelter for aquatic life (Freemark and Boutin, 1994; Arts et al., 2010). Also, they provide natural habitats for pollinators and beneficial insects that can act as biological pests control in nearby agricultural fields. However, these plant resources may be at significant eco-toxicological risk from herbicides applied in crop fields.

The genus *Ludwigia* (Fam. Onagraceae) has been extensively studied because it belongs to a native aquatic group of macrophytes of North and South America (Bedoya and Madriñán, 2014). Nowadays, this genus has become important due to its expansion as an alien species in some European countries (Dandelot et al., 2005; Bou Manobens and Font Garcia, 2016).

Ludwigia peploides (H.B.K.) or floating primrose willow is a native perennial dicotyledonous hydrophyte, extensively distributed from USA and Mexico to South America (Lahitte and Hurrell, 1997). *L. peploides* commonly grows in natural wetlands and fresh marshes (Lahitte and Hurrell, 1997), and it is frequently found in Austral Pampas streams (Menone et al., 2015). This riparian hydrophyte is a postrate amphibious plant anchored in water-logged soils (Ellmore, 1981). It commonly grows forming abundant clumps of large floating shoots, which are easy to sample from the wetlands. In addition, *L. peploides* has been demonstrated to be a biomonitor of organochlorine pesticide residues in Argentinean streams (Gonzalez et al., 2013). Therefore, we have chosen *L. peploides* as a potential aquatic macrophyte biomonitor.

Over the past decades, the use of persistent highly lipophilic organic pesticides has resulted in a wide range of adverse ecological effects due to their high bioaccumulation capacity. For this reason, nowadays there is an increase in the use of less persistent and more water-soluble (hydrophilic) pesticides, which generally have low bioconcentration factors (Alvarez et al., 2008). The physicochemical properties of glyphosate, such as its high water solubility (Log Kow $= -3.57$) and high adsorption to different soil/sediment components, as organic matter and clay minerals (Okada et al., 2016), suggest that this compound would have low bioconcentration (BCFs) and biota-sediment accumulation factors (BSAFs) in the aquatic biota. However, the environmental fate of glyphosate in plant tissues of aquatic macrophytes is a topic scarcely studied.

There are several extraction protocols for glyphosate extraction in plant tissues (Koskinen et al., 2016). Due to the complex and diverse composition of this type of material, in relation to photosynthetic pigments, lipids and proteins, there is not a consensus about the use of a standardized protocol. In this sense, it is necessary to determine an optimal glyphosate extraction protocol for the plant model to be used.

The objectives of this study were (1) to establish and to validate a methodology of glyphosate extraction and quantification in the hydrophyte *Ludwigia peploides* and (2) to evaluate the role of this species as a potential glyphosate biomonitor in aquatic ecosystems.

2. Materials and methods

2.1. Study area

El Crespo is a third-order stream located in the southeast of Buenos Aires Province - Argentina with the catchment area of 489.42 Km^2 and flows from south-west to north-east through 65 Km (Fig. 1A) and with a mean discharge of 0.85 m^3/s (Pérez et al., 2017). The headwaters are located in the Tandilia hills System in the southern upper part; while the mouth is located at the northern end into the floodplains (Fig. 1A). This watershed is only influenced by farming activities without urban or industrial impact; also without significant inputs from other streams or surface water channels, being an optimal site to study processes like pollution, transport and dynamic of pesticides. The watershed can be divided in two areas: the southern upper basin mainly composed of agricultural lands and the northern lower basin, with native grassland coverage, used only for extensive livestock, without history of pesticide applications (Fig. 1B). The sampling sites were enumerated from the headwater (S1) to the mouth (S8), which have been previously characterized concerning glyphosate pollution. Sites S1-S7 are surrounded by agricultural lands, mainly transgenic crops, as soybean and maize, where the occurrence and input of glyphosate is increased, and S8 belongs to an area of natural grassland without agricultural activities, where the levels of glyphosate are lower than the upper sampling sites (Pérez et al., 2017).