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Radiometric approaches with carbon-14-labeled molecules for determining herbicide fate in plant systems

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ABSTRACT

Weeds cause economic losses in cropping systems, leading to the use of 1.7 million tons of herbicides worldwide for weed control annually. Once in the environment, herbicides can reach non-target organisms, causing negative impacts on the ecosystem. Herbicide retention, transport, and degradation processes determine their environmental fate and are essential to assure the safety of these molecules. Radiometric strategies using carbon-14 herbicides (^{14}C) are suitable approaches for determining herbicide absorption, translocation, degradation, retention, and transport in soil, plants, and water. In this work, we demonstrate how ^{14}C-herbicides can be used from different perspectives. Our work focused on herbicide-plant-environment interactions when the herbicide is applied (a) through the leaf, (b) in the soil, and (c) in the water. We also quantified the mass balance in each experiment. ^{14}C-mesotrione foliar absorption increased with oil and adjuvant addition (5–6 % to 25–46 %), and translocation increased only with adjuvant. More than 80 % of ^{14}C-quinclorac and ^{14}C-indaziflam remained in the soil and cover crops species absorbed less than 20 % of the total herbicides applied. In water systems, *Salvinia* spp. plants removed 10–18 % of atrazine from the water. Atrazine metabolism was not influenced by the presence of the plants. The radiometric strategies used were able to quantify the fate of the herbicide in different plant systems and the mass balance varied from 70 % to 130 %. Importantly, we highlight a critical and practical view of tracking herbicides in different matrices. This technique can aid scientists to explore other pesticides as environmental contaminants.

Important definitions

- *DPM* - disintegrations per minute = represents the activity of a radioactive sample through the measurement of the radioactive decays occurring in one minute;
- *Bq* - becquerel = official international system of units of activity, defined as the activity of a radionuclide decaying at the rate, on average, of one spontaneous nuclear transition per second (1 Bq = 1 s^{-1}), kBq = 1000 Bq, MBq = 1000 kBq;
- *Ci* - curie = also a unit of radioactive activity, but not of the official international system (1 Ci = 3.7×10^{10} Bq), mCi - millicurie = 1/1000 Ci;
- *Specific activity* = the radioactivity present in the unit of mass of the product, commonly expressed as Bq $mmol^{-1}$, Bq mg^{-1}, mCi $mmol^{-1}$, or mCi mg^{-1};
- *Radiochemical purity* = the proportion of the radioactive pesticide in a solution concerning the other compounds. A radiochemical purity higher than 95 % is required for studies;
- *Parent compound* = refers to the non-modified herbicide, in its original form after the experimental procedures;
- *Metabolite* = a compound derived from the original herbicide, resulting through a degradation step (like hydroxylation, dichlorination) or pathway (biodegradation);
- *Total applied* = the final quantity of radioactivity applied to a plant;

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- *Total absorbed* = the total amount of product that enters the plant (the sum of radioactivity in all plant sections);
- *Total non-absorbed* = the radioactivity recovered from the water, soil, and washing solution of the leaves;
- *Total translocated* = the total absorbed product that has moved from the application site to other tissues of the plant, through the plant vascular system.

1. Introduction

Pesticides are used to reduce biological losses in food production worldwide, such that there would be an estimated 54 % reduction in crop production without the use of these inputs (Wang et al., 2023; Zuo et al., 2024). These losses harm society, as the food produced is used for human and animal consumption, seeds, industry, and biofuels (Hasnaki; Ziaee; Mahdavi, 2023). Since 2016, the world has been using around 3.3–3.5 million tons of pesticides annually, with herbicides accounting for approximately 48–50 % of the total (1.5–1.7 million tons per year), followed by fungicides (0.8 million tons per year) and insecticides (0.76 million tons per year) (FAO, 2024). Effective herbicide application modes are crucial for ensuring food and environmental safety. Applications in cropping systems can occur through spraying the active ingredient evenly over the field, the plant leaves, or soil surface (Rao, 2000; Yang et al., 2003; Li et al., 2021; Polli et al., 2022).

Post-emergent herbicides, such as mesotrione, are applied directly to plant leaves in order to control weeds after they emerge. Pre-emergent herbicides, such as indaziflam, are applied to the soil to control the weed seed bank (Table 1). Pre- and post-emergent herbicides, such as atrazine and quinclorac, can be applied on plant leaves or the soil surface, depending on the cropping system and target weed (Table 1). In aquatic systems, herbicides can be applied on the plant leaves or in the water. They can also be transported to water from other environmental compartments, such as atrazine (Bachetti et al., 2021), acting as a contaminant. It is clear that herbicides can reach non-target organisms and other environmental matrices, evidenced by numerous studies (Gentil et al., 2020; Tang et al., 2021; Rico et al., 2022; Liu et al., 2021; Brochado et al., 2022; Gentil-Sergent et al., 2021). Transport processes, such as drift, Volatilization, thermal inversion, leaching, and runoff (Kyung et al., 2015; Kumar and Shing, 2020; Vieira et al., 2020; Trovato et al., 2020; Bish et al., 2021) are the main ways in which herbicides are lost to the environment. These losses depend on the application method (Tudi et al., 2022). In addition, the environmental fate of herbicides is determined by a complex interaction with environmental characteristics.

The efficacy of mesotrione depends on the amount absorbed by plant, with a reduction up to 52 % if the product is removed from the leaves before absorption (Sun et al., 2022). In leaf applications, any herbicide that is not absorbed by the plant remains on the surface and can be lost to the environment (Nandula and Vencill, 2015; Takeshita et al., 2021; Mendes et al., 2022). Similarly, when herbicides are applied to reach the soil, any amount that is not absorbed by plants or sorbed by soil particles is also susceptible to losses (Cabral et al., 2023; Guimarães et al., 2022; Ju et al., 2020). For example, the high half-life time of indaziflam and quinclorac (Table 1), contributes to high persistence in soil, causing carryover to sequential crops (Torres et al., 2018; Mendes et al., 2021). This results in a lower amount of herbicide available in its toxic form to the sequential crop or organisms. In aquatic systems, herbicide losses occur when the product is not absorbed by plants leaves/roots or is sorbed in the sediment (Sperry et al., 2021; Haug et al., 2021; Hu et al., 2021; Droz et al., 2021). However, due to the limited use of chemical weed control in aquatic weeds, herbicides like atrazine, detected in water bodies (Correia et al., 2020), are prevented from reaching other environmental compartments. In all these cases, herbicides move from an essential input to an environment contaminant. This necessitates strategies for reducing this contamination, through monitoring their usage on crops and evaluating the associated environmental risks (Bhandari et al., 2020; Cech et al., 2022; Knapp et al., 2023).

The fate of herbicides in plant systems is a complex process, influenced by the intrinsic physicochemical factors of the compounds, plant characteristics, and environmental matrix. Advanced techniques, such as radiation-based methods, are essential for uncovering these complex interactions. Carbon-14 labeled herbicides are invaluable in this regard. Carbon-14 in herbicide molecules allows precise quantification with a liquid scintillation spectrometer (Hou and Roos, 2008; Mendes et al., 2017; Takeshita et al., 2023). This method is expensive, requires a meticulous experimental procedure due the use or radioactive compounds, the institution needs a license from the regulatory agency and the professionals need to be trained concerning radiological protection.

Using radiometric technique in plant systems is an approach with potential to be used to determine the herbicide fate in different environments. It has been successfully applied to regulatory studies in soil matrices, with protocols developed by Organisation for Economic Cooperation and Development (OECD, 2000, 2002, 2004). When applied in plant systems, it is possible to quantify amounts of less than 0.01 % of the total radioactivity initially applied to the samples (Viti et al., 2019; Takeshita et al., 2023). However, there is a need for guiding protocols in using radiotracers in plant experiments, to prevent misinterpreted results and avoid wasting resources.

Herein, we explored different plant experimental models for radiolabeled pesticide applications using a tracer approach. We evaluated the fate of the herbicide in three scenarios: (a) when applied as a necessary input to weed control, measuring the effect of the adjuvant on absorption and translocation of ^{14}C-Mesotrione in *Euphorbia heterophylla* leaves; (b) in the soil profile when the herbicide acts as a contaminant, evaluating the extraction potential of different cover crop species for removing persistent herbicides (^{14}C-Indaziflam and ^{14}C-Quinclorac) from soil; and (c) as a water contaminant, studying ^{14}C-Atrazine uptake and dissipation in different environments. The studies were performed with detailing of the protocols, number of radiolabeled molecules, radioactivity quantification, types of application, ways to explore the

Table 1
Characters of herbicides studied, including physicochemical properties, groups, mode of action, and practical uses.

Parameters	Herbicides			
	Mesotrione	Indaziflam	Quinclorac	Atrazine
Solubility (mg L^{-1})[a]	1500	2.8	0.065	35
Log Kow[a]	0.11	2.8	−1.15	2.7
pKa[a]	3.12	3.5	4.34	1.7
GUS[a]	1.45	2.18	6.29	2.57
Group[a]	Tryketone	Fluoroalkyltriazine	Quinolinecarboxylic acid	Chlorotriazine
Mode of action[a]	Inhibits HPPD	Inhibits cellulose biosynthesis	Inhibition of cell wall synthesis	Inhibits photosynthesis
Practical uses	Post emergent	Pre-emergent	Pre and post emergent	Pre and post emergent
Main crop systems	Mayze and sugarcane	Sugarcane and perennial trees	Rice	Mayze and sugarcane
Environmental contamination[b]	Transport from leaves	Persistence in soil	Persistence in soil	Transport from leaves and soil

Source: [a]Lewis et al. (2016). [b] Based on Torres et al. (2018), Correa et al. (2020), Bachetti et al. (2021), Mendes et al. (2021) and Brankov et al. (2023).

results, and any highlights of the methods. Our results contribute to the scientific community by tracking pesticides in plant samples, to aid understanding of the modes of action of the molecules, their dissipation in different compartments, and their potential as a contaminant.

2. Material and methods

2.1. Chemicals

Radiochemical molecules targeted with carbon-14 (^{14}C) and with a purity greater than 95 % ^{14}C-mesotrione (specific activity 3.2 MBq mg^{-1}), ^{14}C-atrazine (specific activity of 25.7 MBq mg^{-1}), ^{14}C-indaziflam (specific activity 3.96 MBq mg^{-1}), and ^{14}C-quinclorac (specific activity 1.5 MBq mg^{-1}) were purchased from American Radiolabeled Chemicals (Inc., St. Louis, MO, USA). Technical grade non-radiolabeled pesticides (purity > 95 %) were purchased from Sigma Aldrich (Sigma-Aldrich, Chem. Co., San Loius, MO, USA). Commercial mesotrione (Callisto® 480 SC) and solvents, including acetone, acetonitrile, methanol, chloroform, and hexane (99 % purity), were also used.

2.2. Absorption and translocation through the foliar pathway

Radiolabeled ^{14}C molecules were used to investigate the uptake and translocation of mesotrione herbicide applied to plant leaves with and without oil and adjuvant.

2.2.1. Plant cultivation and experimental design

Euphorbia heterophylla plants were cultivated in 300 cm^3 pots filled with a soil:substrate mixture (1:2, *m/m*) until three completely expanded leaf pairs. The soil properties can be found in Table S1. The plants were maintained in a growing chamber with a controlled environment (12 h light/dark, temperature 21 – 27 °C, and 60 % air humidity) and moisture adjusted daily. The experimental design was completely randomized with 3 treatments and 4 evaluation times, with 3 replicates. The treatments consisted of mesotrione, mesotrione + oil (coconut amino amide), and mesotrione + commercial adjuvant (Surfom®). Absorption and translocation were evaluated 4, 8, 24, and 48 hours after application (HAA). One plant per pot was used as the experimental unit.

2.2.2. Non-radiolabeled herbicide application

The non-radiolabeled solution was prepared by adding 1 mL (equivalent to the field recommended dose of 192 g a.i. ha^{-1}) of commercial mesotrione (Callisto® 480 SC) in 500 mL of water (field volume of 200 L ha^{-1}) plus 5 mL of oil or adjuvant (1 % of the total solution), in the respective treatments. The second fully expanded pair of leaves was covered with a plastic bag (to avoid herbicide overdosage) and the non-radiolabeled solution was applied using a CO_2 pressure sprayer, calibrated to 200 L ha^{-1} and 250 kPa, equipped with a flat fan spray nozzle (XR 11002) located 50 cm from the plants. After the solution had dried the plastic bags were removed, and the plants were moved to the laboratory to receive the radiolabeled solution with ^{14}C-mesotrione.

2.2.3. ^{14}C-herbicide application

Working solutions were prepared with the addition of ~180 μL of ^{14}C-mesotrione solution at 1.97 × 10^{-1} kBq μL^{-1}, and 100 μL of non-radiolabeled solution (See Section 2.2.2) for each treatment, into glass vials. Mesotrione treatment reached a total activity of 33.4 kBq (2004,686.7 dpm), mesotrione+oil 40.3 kBq (2420,860 dpm), and mesotrione+adjuvant 36 kBq (2163,546.7 dpm). Ten 1-μL drops of the respective working solution were applied to each leaf of *E. heterophylla* with an automatic microsyringe (Hamilton PB6000 Dispenser, Hamilton Co., USA), totaling 20 μL plant^{-1} (2.3 – 2.8 kBq plant^{-1}) After application, three aliquots of 1 μL of each working solution were applied to scintillation flasks containing 5 mL of a scintillation cocktail and submitted to a Liquid Scintillation Spectrometer (LSS) for 5 min to quantify the radioactivity applied to each plant in each treatment (considering the total applied per experimental unit).

2.2.4. ^{14}C-herbicide uptake and distribution evaluation

The non-absorbed ^{14}C-mesotrione was determined 4, 8, 24, and 48 h after application by washing the leaf with 6 mL (3 mL per foliole) of washing solution (methanol:water - 50:50, *v/v*). The solution was weighed and three aliquots of 500 μL were transferred to scintillation flasks and submitted to LSS, for 5 min. The plants were pressed on paper and dried in a drying oven under 45ºC for 24 h. Phosphorescent films were sensitized in one plant of each treatment, for 24 h, to evaluate the herbicide translocation. The plant dry tissue was combusted by a biological oxidizer, for 3 min, and the samples were submitted to LSS to determine the radioactivity in each plant part (considered the total absorbed by the plant). The supplementary information includes the equations used to calculate the total applied, absorbed, non-absorbed, and translocated.

2.3. Absorption and translocation through the soil-root pathway

This experiment used radiotracers to assess the efficacy of cover crop plants in remediating indaziflam and quinclorac herbicides in soil.

2.3.1. Experimental design, soil collection, and plant growth

The experiment was carried out in a completely randomized design, in a 5 ×2 factorial scheme, with 4 replicates. The treatments consisted of five cover crop species (sunn hemp – *Crotalaria spectabilis*, common vetch – *Vicia cracca*, white lupin – *Lupinus albus*, black oat – *Avena strigosa*, and forage turnip – *Raphanus sativus)* and two herbicides (indaziflam and quinclorac). Samples of the soil surface layer (0 – 20 cm) from Oxisol (USDA, 1999), located in Piracicaba (São Paulo, Brazil), were collected and sieved at 2 mm. Pots of 300 cm^3 were filled with soil and five seeds of each species were sown 1 – 2 cm deep. After emergence, only one plant was kept in each pot. The plants were cultivated in a growing chamber with a controlled environment (See Section 2.2.1), and irrigated with 5 – 8 mL water day^{-1} directly on the soil surface. The herbicide application occurred when plants reached the stage of two fully expanded leaves.

2.3.2. ^{14}C-herbicide application

The dose used for both herbicides was 10 % of the field recommended dose (indaziflam – 7.5 g i.a. ha^{-1}, quinclorac – 37.5 g i.a. ha^{-1}) to simulate a pesticide residue in the soil from previous applications. In total, 20.7 μL of a solution containing non-radiolabeled indaziflam (technical grade), at 1.72 mg mL^{-1}, were mixed with 650 μL of ^{14}C-indaziflam solution (21.6 Bq μL^{-1}), and 41.328 mL of water. The working solution of quinclorac was prepared with 50.2 μL of solution with non-radiolabeled quinclorac (technical grade), at 5 mg mL^{-1}, 1078 μL of ^{14}C-quinclorac solution (113.3 Bq μL^{-1}), and 40.871 mL of water. Both working solutions were prepared in a 42 mL final volume (20 pots + 1 extra) and 2 mL were applied on the soil surface with an automatic pipette. Three 500 μL aliquots of each working solution were transferred to scintillation flasks, with 5 mL of scintillation cocktail, to determine the total applied (Equation I, Supplementary material).

2.3.3. ^{14}C-herbicide uptake and distribution through the roots

Root absorption was determined at 21 DAA. The plant was removed from the soil and the root was washed with water. The plants were pressed and dried after autoradiographs had been taken, as mentioned in Section 2.2.4. The plants were then divided into root and aerial parts, burned in a biological oxidizer for 3 min, and the radioactivity in the samples was quantified by LSS. The total values absorbed and translocated from the roots were determined using equations III and IV, respectively (Supplementary material). Both water and soil were kept in aluminum plates and dried at 35 ºC, for 96 h. The soil was homogenized and ground (Marconi MA330, Piracicaba, SP, Brazil), before being weighed, and three samples of 200 mg were combusted in the biological oxidizer. Samples were submitted to LSS for 5 min to determine the amount of herbicide retained in the soil (Equation VII, supplementary material).

2.3.4. Mass balance components

To determine the mass balance, the plant root needs to be washed with a low water volume and this liquid needs to be kept with the soil. In our study, we washed the plant roots inside aluminum plates, using a pecker, to reduce radioactivity loss during the experimental procedures. To verify the radioactivity recovery at the end of the experiment, the components of mass balance were the total ^{14}C-herbicide remaining in the soil and the total uptake by the plants, calculated in relation to the total C-herbicide initially applied (See supplementary material).

2.4. Absorption, translocation, and dissipation through the water-root pathway

The experiment aimed to assess the phytoremediation capacity of *Salvinia spp.* to remove atrazine from water. Radiotracers were utilized to aid the study, and mineralization experiments were conducted to effectively evaluate atrazine degradation during the experiment.

2.4.1. Toxicity test

Aquatic plants of *Salvinia* spp. were acquired from the Department of Biological Sciences, ESALQ/USP. They were acclimatized in a 20 L capacity aquarium for 7 days, in a controlled environment (Section 2.2.1). The experimental design of the toxicity test was completely randomized, with 6 treatments and 4 replicates. The treatments consisted of five concentrations of atrazine (5 μg L^{-1}, 15 μg L^{-1}, 45 μg L^{-1}, 135 μg L^{-1}, and 405 μg L^{-1}), based on the environmental concentration (Bachetti et al., 2021), and a treatment without atrazine.

The experiment was performed in beakers with 800 mL of water (pH ~7.0) and 5 plants per beaker^{-1}. No changes in water pH were observed after herbicide addition due to the low concentration used. The test was carried out for 21 days in the same controlled environment as previously mentioned. The plants were removed from the water, drained, and weighed to obtain the fresh mass, and then dried at 45°C in an oven, and weighed to obtain the dry mass.

2.4.2. Water phytoremediation assay

The experimental design was completely randomized, with three repetitions. The treatments were made up of two concentrations of ^{14}C-atrazine (15 μg L^{-1} - 10-times the environmental concentration and 135 μg L^{-1} - 90-times the environmental concentration) according to previously conducted toxicity tests (Fig. S2), and three water pH levels (3.5, 5.5, and 7.5). The experimental units consisted of a 1-L beaker filled with 800 mL of water and five *Salvinia* spp. plants (~18–20 g of fresh weight). The water pH was adjusted with HCl 0.1 M or NaOH 0.1 M before the addition of *Salvinia* spp. plants and atrazine. The experiment was conducted under the same environmental conditions as described above (Section 2.4.1).

2.4.3. ^{14}C-atrazine solution

For the treatments with 15 μg L^{-1} of atrazine, a working solution was made with 1.2 mL of non-radiolabeled atrazine (concentration of 0.1 mg mL^{-1}) and 0.2 mL of ^{14}C-atrazine, with 36 kBq total activity (2160,000 dpm). Then, 140 μL of the working solution were added to each experimental unit (4 kBq beaker^{-1}). In treatments with 135 μg L^{-1}, a working solution was made with 1.08 mL of non-radiolabeled atrazine (1 mg mL^{-1}) and 0.2 mL of ^{14}C-atrazine, with 41.6 kBq total activity (2490,00 dpm), after which 128 μL were added to each experimental unit (4.6 kBq beaker^{-1})

2.4.4. ^{14}C-atrazine dissipation in water-plant system

The herbicide solutions were added to beakers with 800 mL of deionized water and 5 plants of *Salvinia* spp. The test was carried out over 21 days in the same controlled environment as previously mentioned (Section 2.4.1), with water loss compensation through time. To analyze atrazine dissipation, 5 mL of water from each beaker were collected, in duplicate, 0, 4, 8, 16, and 21 days after application. Aliquots were transferred to scintillation flasks with 5 mL of Insta-Gel Plus scintillation solution, submitted to LSS for 5 min, and the atrazine remaining in water was calculated according to Equation VIII (Supplementary material). After 21 days, the plants were removed from the water, dried, and pressed for 48 hours, in an oven at 45 °C. Phosphorescent films were sensitized to plants for 48 h, for qualitative analysis of ^{14}C-atrazine uptake. Each plant was weighed and combusted in a biological oxidizer, for 3 min. The samples were then submitted to LSS for 5 min to determine the radioactivity in each plant. The total atrazine absorbed by the plants was calculated concerning the total applied (Equation III, Supplementary material).

2.4.5. Degradation of ^{14}C-atrazine in water in the presence of Salvinia spp.

To study ^{14}C-atrazine metabolization, 150 mL of the residual water from the beakers (with the plants) were concentrated in a rotary evaporator, at 60°C and 90 rpm. The volumetric flask from the rotary evaporator was washed with 20 mL of acetone and sonicated for 30 seconds. The samples were concentrated in nitrogen flux until 1 mL and filtered through a 0.45 μm filter. Next, two aliquots of 25 μL were collected to measure the extracted radioactivity, and the amount extracted was calculated with equation IX (Supplementary material). Then, due to low radioactivity per volume, the samples were dried again until 100 μL. From this amount, 80 μL from each sample were applied to a Thin Layer Chromatography (TLC) plate with an automatic applicator at a 0.25 μL seg^{-1} flow rate and 3 mm bandwidth. TLC plates were submitted to elution in 100 mL of isopropanol/ammonium hydroxide/water (80:10:10 *v/v*) solution. Metabolite formation was identified by radioactivity in the function of the retention factor in TLC plates and quantified using equation X (Supplementary material).

2.4.6. Mineralization of ^{14}C-atrazine in water

Experiments on mineralization and metabolization were carried out at the same time as ^{14}C-atrazine dissipation by aquatic plants. They were used to verify incoherent mass balance results according to the guideline "Test number 307: Aerobic and Anaerobic Transformation in Soil" from OECD (2002). In total, 100 mL of water (135 μg L^{-1} of atrazine) were added to biometric flasks with NaOH (0.2 M) as a CO_2 trap (Mendes et al., 2017). Then, 1.8–2 kBq (100–120,000 dpm) of ^{14}C-atrazine were applied to each flask. At the end of the experiment (21 days after application), the ^{14}C-atrazine mineralized to $^{14}C\text{-}CO_2$, the amount in residual water (Equation XI, Supplementary material), and the ^{14}C-atrazine metabolized were also evaluated. To study metabolism, 100 mL of water were submitted to the extraction process (see Section 2.4.5), the radioactivity extracted was quantified according to equation XII (Supplementary material), and the samples were applied to TLC plates in the same way as described in Section 2.4.5.

2.4.7. Mass balance components

To verify the radioactivity remaining in the glassware from metabolization and mineralization studies, it was washed with 10 mL of acetone. The acetone was evaporated in an oven at 45°C for 24 hours and 5 mL of scintillation cocktail were added. To finalize the mass balance, we considered the total of ^{14}C-atrazine absorbed by plants, the total remaining in water, the total remaining in beakers or flasks, and the total mineralized (Equation XIII supplementary material).

2.5. Statistical analysis

Statistical tests were performed to verify homogeneity, homoscedasticity, and normality in each dataset. When the variance presents a normal and homogeneous distribution, an analysis of variance (ANOVA) was performed to identify the treatment effect and the means were compared by Tukey's HSD test. When assumptions were not met, the data were transformed using the Yeo-Jonhson transformation or submitted to non-parametric analysis (Kruskal-Wallis test) with mean comparisons by Dunn's test. Results contrasted with a quantitative variable (days or hours) were submitted to regression analysis. A level of 5 % of significance ($p < 0.05$) was considered for all statistical analyses performed. The graphs and analysis were performed using Origin 2024 (Version 10.100178, OriginLab Corporation, Northampton, MA).

3. Results and discussion

3.1. Foliar absorption

^{14}C-mesotrione absorption by *E. heterophylla* over time was adjusted to the linear and Michaelis-Menten equation model ($R^2 = 0.59–0.89$), according to the Akaike information criterion (AICc) (Tab. S2, Fig. 1a–c). The maximum absorption (A_{max}) estimated for ^{14}C-mesotrione applied alone was 6.2 % (Fig. 1a). The oil and commercial adjuvant increased A_{max} to 30.5 % and 55.9 %, respectively (Fig. 1b and c, data can be consulted in Tab. S2). The total absorption and translocation at the initial (4 h) and final (48 h) evaluation times were compared. When the herbicide was applied alone, only 5.7–6.6 % of ^{14}C-mesotrione was absorbed, regardless of time (Fig. 1d). The oil in the herbicide solution showed absorption of 22.2 % and 24.9 %, at 4 and 48 h, respectively (Fig. 1d). For the adjuvant, absorption at 4 h did not differ from the other treatments, however, at 48 h the adjuvant proportioned the highest absorption (46.7 %) (Fig. 1d). This same absorption pattern was observed in autoradiograph images (Fig. 2), in which the plants treated with oil and adjuvant presented more radioactivity, compared to the control. The oil and adjuvants can increase the maximum retention of the herbicide on the leaf surface, contributing to higher penetration and control of the target (Izadi-Darbandi et al., 2013; Ma et al., 2021; Palma-Bautista et al., 2020), as observed herein.

^{14}C-mesotrione translocation can be evaluated in two perspectives. Considering the total initially applied (Fig. 1e) or the total absorbed by the plants (Fig. 1f). In both cases it was time-dependent (Fig. S1 a-c). Considering the translocation in relation the total applied, only adjuvant proportioned an increase in ^{14}C-mesotrione translocation (9.5 %), at 48 h after application, compared to the other treatments (1 – 2.8 %) (Fig. 1e). When considering the translocation in relation to the total absorbed, it was 21.9 % in the control and reduced in the presence of oil (8.4 %) and adjuvant (15.4 %) (Fig. 1f). This difference occurs because the equation IV (Supplementary information) uses the total absorbed as the denominator, so the translocation rate should decrease. However, the total ^{14}C-Mesotrione translocated is higher for oil and adjuvant than for the herbicide alone (Fig. S1). We recommend that scientist look to the results in the two forms and, if the objective is to compare application solutions, the total applied should be used. If the objective is to show how the translocation depends on absorption or to compare translocation rates in different environments, the total absorbed should be used.

In autoradiography we observed low radioactivity intensity in the control treatment (Fig. 2) because the absorption was lower than the other treatments. Adding oil to the solution resulted in necrosis of *E. heterophylla* leaves (Fig. 2); although necrosis is considered favorable for weed control, it makes herbicide translocation difficult, compromising weed control (Rolando et al., 2020; Queiroz et al., 2022; Santos et al., 2023). Oil or adjuvants can change the physicochemical properties of the solution, leading to a more hydrophobic medium and allowing facilitated diffusion of the herbicide through the plant cuticle (Brankov et al., 2023) in an extracellular compartment. Importantly, the formulation or pesticide medium should be carefully considered because it can change the dynamic in plants and the study approach. When herbicide interacts with plant cells, barriers such as cell walls and subcellular fractions (like cell membranes and organelles) reduces herbicide translocation (Ju et al., 2020). So, the increase in translocation observed in this work is mostly related to the higher absorption rate than to changes in physiological interactions inside plant cells.

Moreover, when applied to plant leaves, radiolabeled pesticides allow the quantification of the non-absorbed (available to environmental losses), absorbed, and translocated herbicide in plants. Following the steps described in our work allows the adaptation of experimental conditions (such as contact time, temperature, light availability, and application solution content), contributing to the use of new approaches by the scientific community and better understanding of the pesticide's environmental fate, and thus enhancing the critical and safe positioning of these tools in the field.

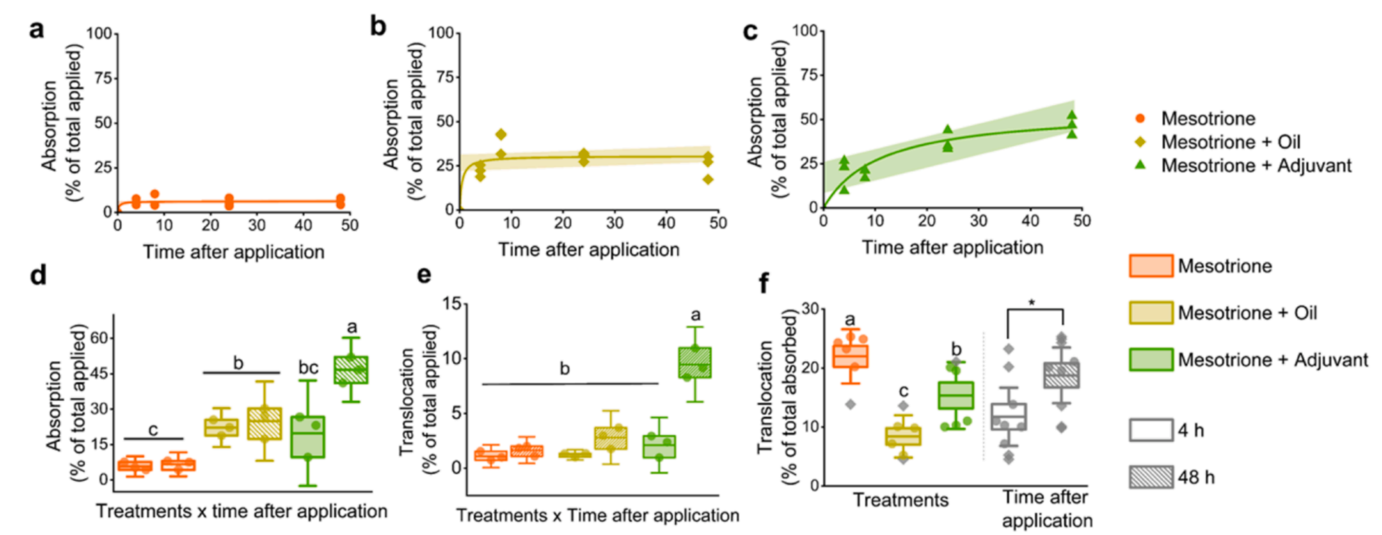

Fig. 1. Absorption (a - d) and translocation (e - f) of ^{14}C-mesotrione in *E. heterophylla* plants. In a, b, and c the line represents the absorption rate as a function of time, adjusted to the Michaelis-Menten model (p <0.05), and the shaded band around the line represents the 95 % prediction band. In e, the lines represent a linear regression. In d and f, the box represents the mean ± SE and the bars represent 95 % of the confidence interval. Symbols represent the data and may cover the bars. Outliers are represented by gray symbols. Treatments followed by the same lowercase letter are not significantly different according to Tukey's test ($p < 0.05$). *Significant ($p < 0.05$).

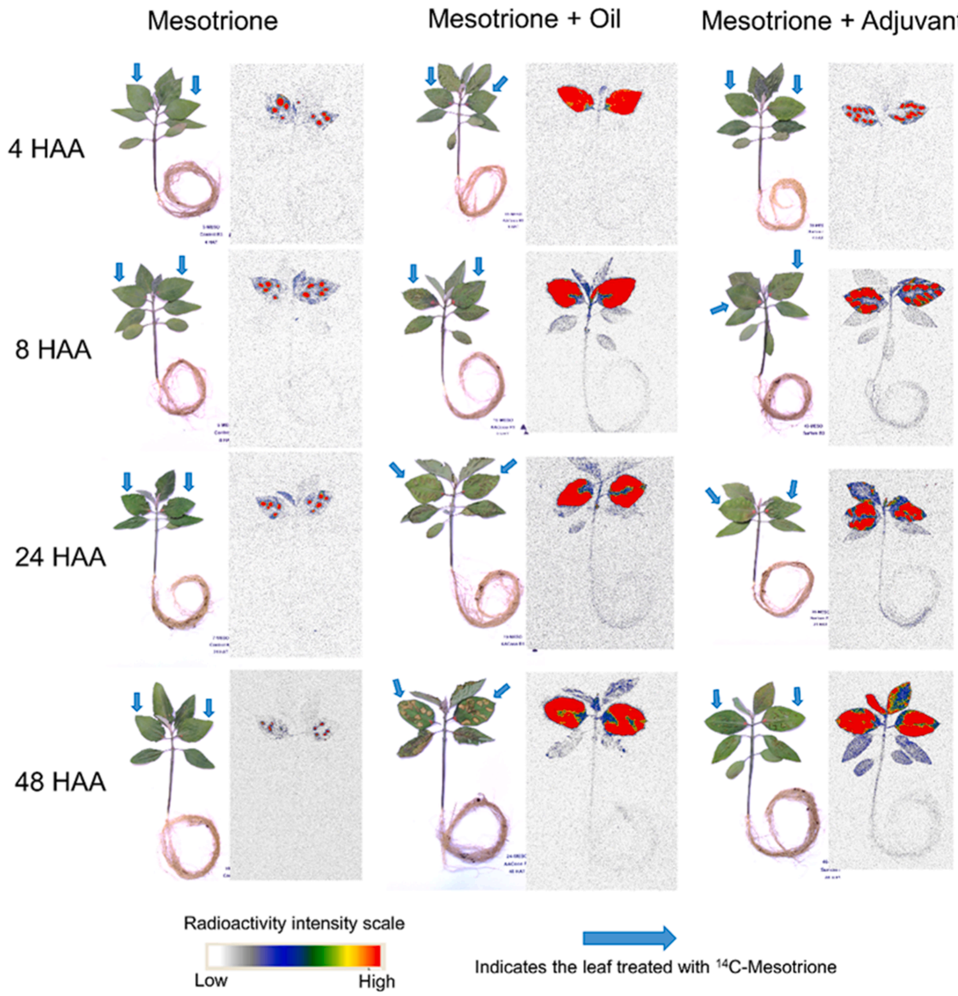

Fig. 2. Images (left) and autoradiography (right) of *E. heterophylla* plants submitted to ^{14} C-mesotrione application, alone or in combination with oil and adjuvant, as a function of time. The blue arrows indicate leaves treated with ^{14}C-mesotrione, the red color indicates a higher intensity of radioactivity, and the blue color indicates a lower intensity. HAA - hours after application.

3.2. Soil-root absorption

Low concentrations of ^{14}C-indaziflam (0.6 – 5.1 %) and ^{14}C-quinclorac (0.8 – 12.4 %) were extracted from soil by plant species (Fig. 3a and b). The white lupin (*Lupinus albus*) absorbed a higher amount of both herbicides (5.1 % of ^{14}C-indaziflam and 12.4 % of ^{14}C-quinclorac) among the species evaluated, reducing the herbicide concentration in soil (Fig. 3c). The translocation of ^{14}C-indaziflam from the root to aerial part was 8-fold higher in white lupin (4.3 %) compared to sunn hemp (0.5 %) (Fig. 3d). For ^{14}C-quinclorac, white lupin translocated 19-fold more herbicide (7.9 ± 1.8 %) than sunn hemp and common vetch (~0.4 %) (Fig. 3d). The indaziflam caused death to forage turnip plants and reduced the height of black oat (Fig. 3e and f) according to the ratio between plant height in the control treatment *versus* the plants that received herbicide. No toxic effect was observed in treatments with quinclorac application, demonstrating the tolerance of the species tested to this herbicide (Fig. 3f).

Radiolabeled herbicides can be effectively used to investigate the ability of weeds or crops to absorb different herbicides from the soil, since the complexity of the soil-root interaction does not fit in an experimental set under hydroponic conditions (root in direct contact with contaminated water). This was used to determine the potential of plants to remove herbicides from media (Bailey et al., 2003; Yu et al., 2013; Khrunyk et al., 2016); however, an upper estimate value of herbicide removal from soil is observed, as the root-herbicide interaction is influenced by the amount of herbicide available in the soil solution (Wang et al., 2021), governed by sorption-desorption from soil particles (Mendes et al., 2021), thus demonstrating the importance of using realistic experimental sets in phytoremediation studies, like that proposed in the current study.

Successful phytoremediation depends on the absorption, translocation, and toxicity of herbicides to plant species. Root absorption of herbicides is limited by the herbicide-soil interaction. The amount of herbicide available in the soil solution is regulated by sorption-desorption processes (Guimarães et al., 2022; Takeshita et al., 2022). Indaziflam (Log Kow = 2.8) with moderate sorption (rates of up to 40 % after 24 hours) (Mendes et al., 2021a), should be less available for plant uptake (Alonso et al., 2011; Mendes et al., 2021b) than quinclorac (Log Kow = −1.15) with low retention (Lewis et al., 2016; Mendes et al., 2019). In addition, the herbicide extraction depends on cover crops *(Crotalaria spp., Lupinus spp., Raphanus spp., Vicia spp., Raphanus spp.)*, varying among species that need a certain level of tolerance for an efficient phytoremediation process (Alves et al., 2019; Mendes et al., 2019; Bian et al., 2020; Teófilo et al., 2020; Conciani et al., 2023; Kafle et al., 2022; Ogura et al., 2023; Barroso et al., 2023). The mechanisms behind the differences in absorption and translocation patterns between the species studied were not evaluated in the current work. In this sense, we have no solid evidence of the reasons for these differences between species.

Herein, we highlight the potential of white lupin as a suitable species

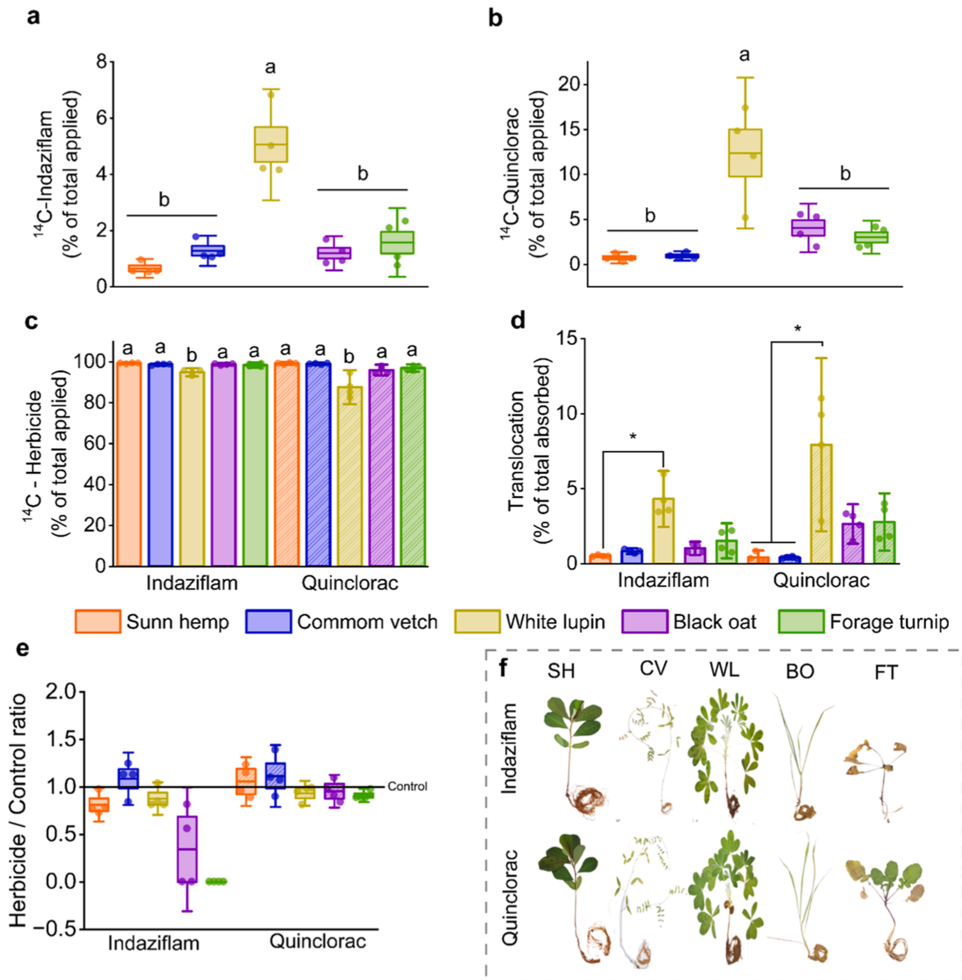

Fig. 3. Uptake of ^{14}C-indaziflam (a) and ^{14}C-quinclorac (b) from soil; herbicide remaining in the soil after cover crop growing (c); ^{14}C-herbicide translocation by cover crops (d); herbicide effect on plant height compared to the control (e) and cover crop images (f). The symbols represent the data, and the bars represent 95 % of the confidence interval. Symbols can cover the bars. In a, b, and e the box represents the mean ± SE. Boxes or bars followed by the same lowercase letter are not significantly different according to Tukey's test ($p < 0.05$). Asterisks indicate a significant difference according to Dunn's test ($p < 0.05$). SH - sunn hemp, CV - common vetch, WL - white lupin, BO - black oat, and FT - forage turnip.

to remove indaziflam and quinclorac from contaminated soils, even at low uptake rates. Overall, considering sorption-desorption, leaching studies, and soil-root absorption studies, we can determine the effective dose to be applied in the field that will be taken up by the plants. This perspective can be used to design new formulations or application modes based on data from herbicide uptake *versus* toxicity, thus improving technological development in pesticide science.

3.3. Water-root herbicide absorption and translocation

The atrazine concentration and water pH influenced ^{14}C-atrazine dissipation through absorption by *Salvinia* spp. plants, independently ($p < 0.05$) (Fig. 4). Higher amounts of ^{14}C-atrazine were found in plants submitted to 15 μg L^{-1} of ^{14}C-atrazine (17.5 %) compared to the treatment with 135 μg L^{-1} (10.5 %) (Fig. 4a). In water pH 7.5, plants absorbed less ^{14}C-atrazine (11.7 %) compared to those in pH 3.5 (15.2 %) and 5.5 (15.1 %) (Fig. 4b). Different concentrations of atrazine can be found in the environment (Graymore et al., 2001; Nödler et al., 2013; Triassi et al., 2022). Recent monitoring quantified atrazine in surface water (up to 5 μg L^{-1}) (Bachetti et al., 2021), in groundwater (up to 1.4 μg L^{-1}), and through the food chain (Urseler et al., 2022), leading to the need for practices to reduce the herbicide concentration in water, in order to decrease the anthropogenic impact on the environment.

Although differences were observed in atrazine dissipation, we normalized the total of ^{14}C-atrazine absorbed concerning the plant biomass (dry weight), to determine the real capacity of ^{14}C-atrazine absorption by *Salvinia* spp. plants. Normalizing the data to plant biomass reduces the effect of lack of uniformity within plants and environmental variables. The normalized results show no influence of atrazine concentration or water pH on ^{14}C-atrazine absorption by *Salvinia* spp. (Fig. 4c and d). In this sense, *Salvinia* spp. can uptake ^{14}C-atrazine from water in amounts of approximately 15 % of the total applied (Fig. 4c and d). This represents 2.2 μg L^{-1} and 20.2 μg L^{-1} considering the two concentrations tested (15 and 135 ug L^{-1}, respectively). Aquatic plants presented varied rates of pesticide uptake from water. *Lemma minor* removed 4.6 times (11.5 %) more dimethomorph than *Cabomba aquatica* (2.5 %) (Olette et al., 2008), *Sesbania grandifolia* only removed 3 % of DDT (Mouhamad et al., 2012), *Hydrocotyle vulgaris* removed 94 % of prometryn (Ni et al., 2018), *Pistia stratiotes* took up 75 % of mesotrione (Barchanska et al., 2019), and *Salvinia* spp. removed 100 % of glyphosate from water (Santos et al., 2020). However, *Salvinia* spp. did not demonstrate the ability to remove atrazine from water at concentrations of 5–20 mg L^{-1}, as the herbicide was shown to be highly toxic to the plants (Guimarães et al., 2011; Loureiro et al., 2023). In addition, our study tested higher concentrations of atrazine (15 and 135 μg L^{-1}) compared to the concentrations found in Brazilian water (1.4–4.95 μg L^{-1}) (Riquinho et al., 2020; Barizon et al., 2020; Vizioli et al., 2023), and *Salvinia* spp. was demonstrated to be an effective

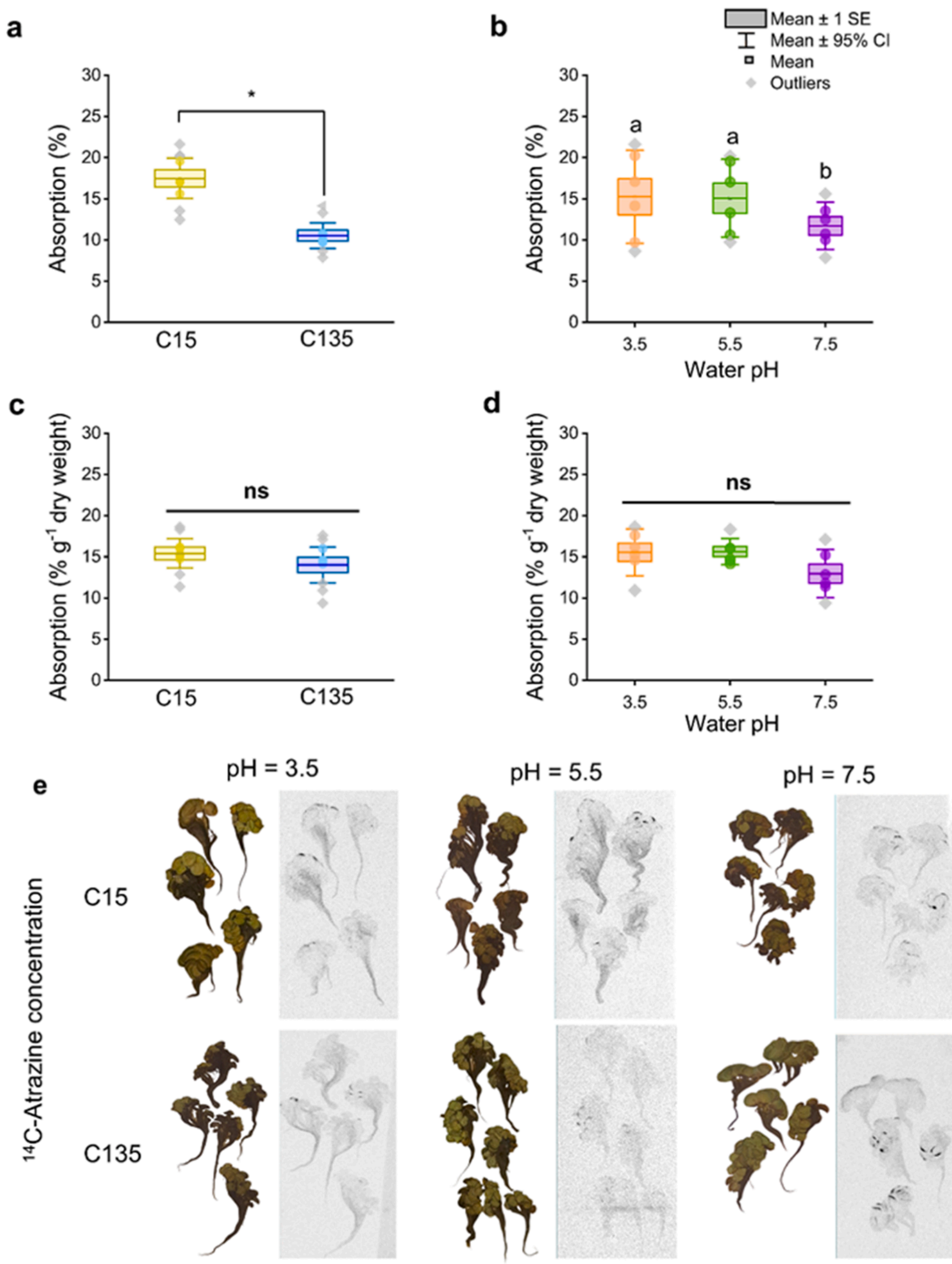

Fig. 4. Absorption of ^{14}C-atrazine from water under different concentrations (a) and water pH (b), concerning the total initially applied. The absorption data were normalized to plant dry weight (c and d). Images (left) and autoradiography (right) of *Salvinia* spp. plants 21 days after contact with ^{14}C-atrazine in water (e); the higher intensity of radioactivity is represented by the black color in autoradiography images. The symbols represent the data, and the bars represent 95 % of the confidence interval. Symbols can cover the bars. Outliers are represented by gray symbols. Boxes followed by the same lowercase letter are not significantly different according to Tukey's test ($p < 0.05$). Ns - non-significant ($p > 0.05$). C15 - 15 μg L^{-1}, C135 - 135 μg L^{-1}.

alternative for removing atrazine from water at rates of up to 135 μg L^{-1}.

No differences were observed in the translocation patterns of ^{14}C-atrazine in *Salvinia* spp., in which the herbicide was absorbed by roots and transported to the leaf margins in all treatments (Fig. 4e). PSII inhibitors like atrazine enter the root by the apoplastic pathway and are translocated through xylem tissue from roots to leaves, with the water flux (Takeshita et al., 2021; Jachetta et al., 1986). Atrazine is a weak base (pKb = 1.7), in environments with pH > pKb and is in neutral form, acting similarly to non-ionic herbicides (Bromilow et al., 1990). Lipophilic herbicides (Log Kow > 4) have difficulty crossing cell walls and lipids in the plant's roots, being accumulated in the root tissue; while hydrophilic compounds can be acropetally translocated through the xylem (Bromilow et al., 1990; Wang et al., 2021). This movement can aid the translocation and extraction of atrazine from water by plants such as *Salvinia* spp., as observed herein.

3.3.1. Atrazine dissipation in water

More than 95 % of atrazine remained in the water (Fig. 5a) and the amount mineralized to $^{14}CO_2$ was from 0.5 % to 3.5 %, regarding the treatments ($p > 0.05$) (Fig. 5b). Metabolites derived from atrazine were found in quantities lower than 10 %, except in water with pH = 7.5, where metabolites were formed in amounts of 7–27 % (Fig. 5c). In the treatments with *Salvinia* spp. plants, 67–100 % of atrazine was quantified in parental form (Fig. 5d) and tree metabolites were distinguished in TLC tests (Fig. 5e - g), with a retention factor lower (0–0.6) than atrazine

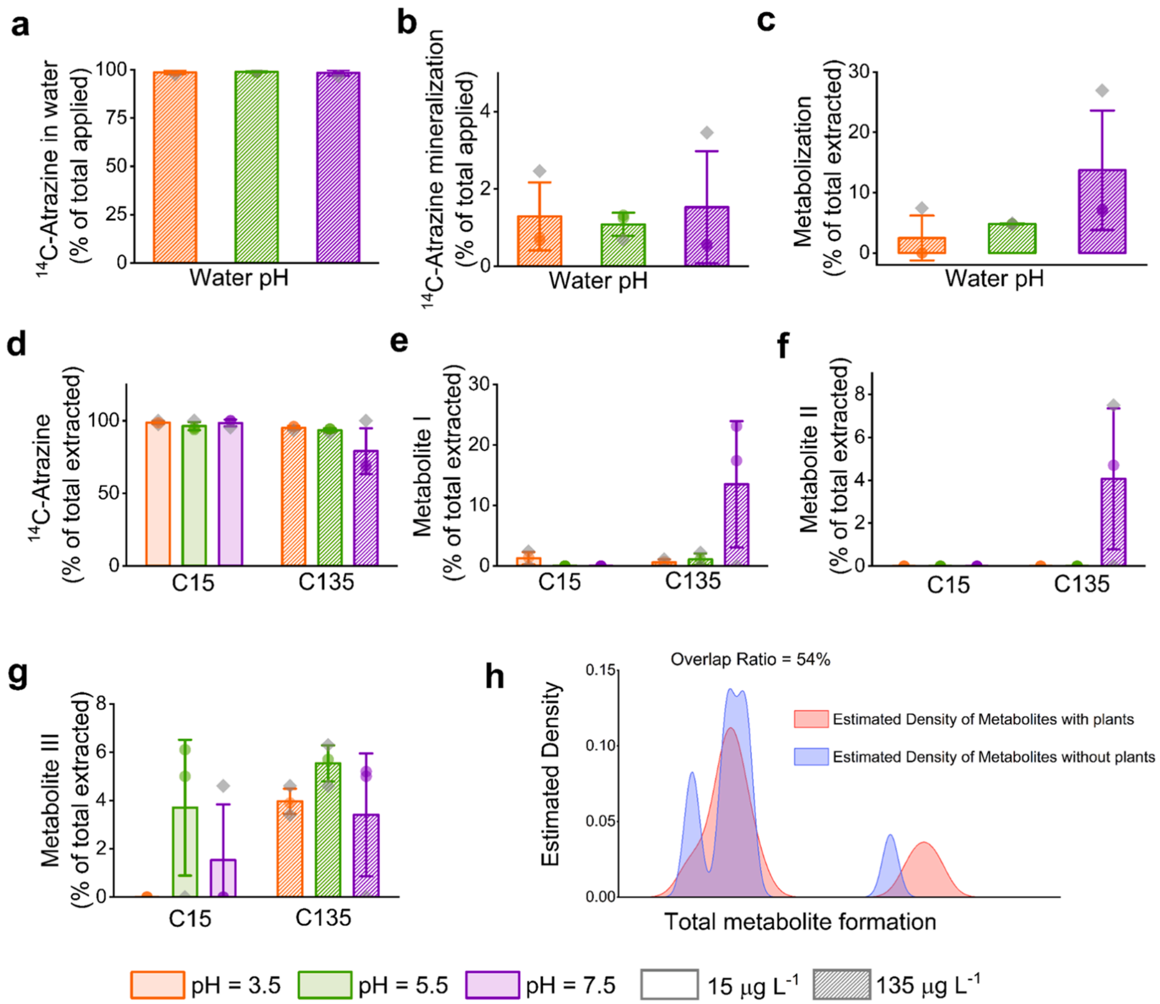

Fig. 5. Mineralization and degradation study of ^{14}C-atrazine in water. ^{14}C-atrazine quantified in water (a), total mineralized to $^{14}CO_2$ (b), and metabolized in biometric flasks (c), without the plants. Total atrazine remaining in water (d) and the amount of each metabolite formed in water in the presence of the *Salvinia* spp. plants (e - g). Similarity between the atrazine degradation in the presence and absence of plants (h) according to Kernel's density estimation. The symbols represent the data and the bars represent 95 % of the confidence interval. Symbols can cover the bars. Outliers are represented by gray symbols.

(0.8). No significant differences within treatments were found and metabolite III was found in all treatments except one (15 µg at pH = 3.3) (Fig. 5g). The kernel density overlapping data identified 54 % similarity in atrazine metabolization considering treatments with and without plants (Fig. 5h).

The low degradation rates of atrazine in water observed in our study are consistent with previous research indicating the high persistence of atrazine in the environment, which is thought to be due to a complex degradation mechanism involving the interaction of biotic and abiotic factors (Roberts et al., 1998; Mersie et al., 2000; Singh et al., 2018; Hong et al., 2022). The major degradation pathway for atrazine in the environment is N-dealkylation and hydroxylation, resulting in a range of metabolites (desethylatrazine - DEA, desisopropylatrazine - DIA, hydroxyatrazine - HA and desethylhydroxyatrazine - DEHA) and desisopropylhydroxyatrazine - DIHA) (Bhatti et al., 2022). In the current study, metabolites were not identified because the synthesis of ^{14}C-metabolites is limited and few suppliers are available in the market. Overall, atrazine degradation was not quantitatively or qualitatively influenced by the presence of the plants in water. Further research is needed to unveil the impact of *Salvinia* spp. in the degradation of atrazine.

Using radiometric techniques integrated with ^{14}C-metabolites can be useful to determine the metabolite identity and amount in water and plants, explore the complex interactions between aquatic plants and water in the phytoremediation process, investigate non-target site resistance mechanisms in aquatic species (Ortiz et al., 2021), and unveil the interactions between herbicide mixtures for controlling aquatic weed species (Ortiz et al., 2022). Nevertheless, radiometric techniques can also be used from an agronomic perspective to help unravel the herbicide-water-root interaction in aquatic environments, where herbicides are proposed to be applied and are needed to impact weed species.

3.4. *Potential applications in experimental sets*

Further studies can be conducted using the protocols described here (Table 2). The approach suggested in the leaf pathway makes it possible to obtain data to answer questions such as; (I) How much time does the herbicide need to be in contact with the leaves to achieve maximum absorption? (II) Do adjuvants (surfactants, oils, other substances) increase herbicide absorption/translocation? (III) Can uptake and translocation pathways be involved in herbicide resistance? and (IV) Is the plant metabolism involved in the resistance/degradation of herbicides? The responses can provide precise information about herbicide losses and interactions with complex environments.

Using the soil-root pathway approach, it is possible to explore the interactions between pesticides applied to plants through drenching or directly on the soil, and their translocation from roots to shoots, flowers,

Table 2
Summary of main parameters for protocols using ^{14}C-herbicides in experiments with different plant systems.

Parameters	Application mode			Observations
	Plant leaves	Soil	Water	
Experimental unity	Pots filled with soil or soil + substrate. One plant per pot	Pots filled with soil (do not use substrate). The size of the pot should be minimal to reduce the amount of contaminated soil with carbon−14. The amount of soil within the pots should be homogeneous (*e.g.,* 100 g per pot). No water can be lost by leaching from the pot and the irrigation needs to be done in soil surface to promote a realistic approach. The number of plants can vary based on the objective of the study.	Pots filled with water. If sediments, sorbents, or organisms are present in the water, the herbicide retention of its materials must be evaluated. The water in the pots must be kept homogeneous, and a daily control of water loss is necessary. This can be done by monitoring the weight loss and repositioning the water daily. Water from rivers or lakes can be used in a more realistic approach. The previous presence of the herbicide needs to be quantified. More than one plant can be used, as well the integration with other types of organisms, like fishes.	It is essential to maintain high levels of homogeneity within the plants, which necessitates the implementation of a controlled environment. At a minimum, three replicates per treatment ($n = 3$) should be used.
Washing solution	3 – 6 mL of water:methanol (50/50, v/v) depending on the size of leaves. For plants with trifoliums, is recommended to use 3 – 5 mL of solution in each foliole. The non-absorbed^{14}C-herbicide is measured using the washing solution from each experimental unity.	Water in a minimal volume, kept with soil, and further dried in an oven or room temperature. The non-absorbed^{14}C-herbicide is measured using the soil remaining in the pots.	No washing is needed. Use a funnel to remove the excess water and return the water into the pot. The non-absorbed^{14}C-herbicide is measured using the water remaining in the pots.	It is crucial to ensure that the washing solutions remain refrigerated (4ºC) at all times until the quantification.
Quantification of^{14}C-herbicide	Only in plants. Plant parts can be evaluated separetelly to translocation rate measurements.	In plants, if necessary, short and roots would be quantified separately In soil, it needs to be weighted and homogenized before quantification.	In plants and water. Plant parts can be evaluated separetelly to translocation rate measurements. In water, the herbicide can be extracted and degradation can be evaluated.	It is suggested that the dry weight of the plants be measured prior to the onset of biological oxidation.
Data Acquisition	^{14}C-herbicide absorbed, non-absorbed, and translocated in function of time and/or specific treatments (like adjuvants, light conditions, temperature).	^{14}C-herbicide absorbed, retained in soil, and translocated, in function of time and/or specific treatments (environmental conditions, soil type or modifications).	^{14}C-herbicide absorbed, retained in soil, and translocated, in function of time and/or specific treatments (environmental conditions, water characteristics, or mesocosms approach with different organisms).	It is recommended that the results be reported as a percentage of the total applied or absorbed, in order to facilitate comparison with other studies and enhance comprehension by the scientific community.
Perspective for using in other studies	Herbicide efficacy, non-target resistance, plant metabolism, bioaccumulation, herbicide degradation in leaf surface and discovering herbicide mode of action.	Herbicide efficacy, weed competition, plant metabolism, degradation and persistence in soil, and discovering herbicide mode of action.	Herbicide efficacy, plant metabolism, degradation and persistence in water, interaction with other organisms, bioaccumulation, and discovering herbicide mode of action.	These studies can be directly performed using^{14}C-herbicides or they can be combined with other techniques (such as microscopy, chromatography, spectroscopy, and biometry) to unveil the environmental fate.

and fruits. If a mesocosm is used in this scenario, it is possible to assess the herbicide risk of transference to pollinator agents and other non-target organisms. Bioaccumulation in soil organisms, such as earthworms can also be assessed in this scenario. Generally, the root absorption assay is applied to investigate pesticide uptake by plants (target and non-target), for example foliar uptake, so we can focus on questions similar to those mentioned above. Importantly, the soil properties must be considered for complete understanding of pesticide dynamics.

In water, radiometric techniques can be used, adopting the same perspective as plant uptake, and are effective in determining bioaccumulation and depletion of contaminants in aquatic organisms (Vilca et al., 2020; Evangelista et al., 2023). Determining the amount of herbicides that reach target and non-target organisms is crucial for realistic toxicity studies, making it possible to perform an effective risk assessment of these molecules, and providing more information, security, and knowledge about their risk to people and the environment.

3.5. Highlights for ^{14}C-herbicide protocols

The use of a radiometric strategy enables calculation of the mass balance; the direct quantification of the herbicide absorbed by the plant through different application modes; and the amount remaining in the soil, water, or environment, relative to the total radioactivity initially applied to each experimental unit (Fig. 6). The mass balance is crucial for accurately quantifying the total herbicide recovered and verifying the quality of the experimental procedures. OECD guidelines recommend a mass balance between 90 % and 110 % for soil studies under closed environments (OECD, 2002, 2004). However, studies conducted in open environments (such as plant system assays) may result in lower recovery rates (Fig. S3) due to environmental influences. The main challenges in using ^{14}C-herbicides are related to the need of license to work with radioactive materials, expansive equipment to detect radioactivity, a specialized and trained team, the waste management, the need of use reduced replicates (n = 2 or 3), the cost of labeled herbicides and the need of a homogeneous and controlled environment to perform the experiments.

Although several studies have been conducted using radiometric techniques, standardization is needed concerning the methods and results. Some important points to be observed in studies with ^{14}C-pesticides in plants are listed below:

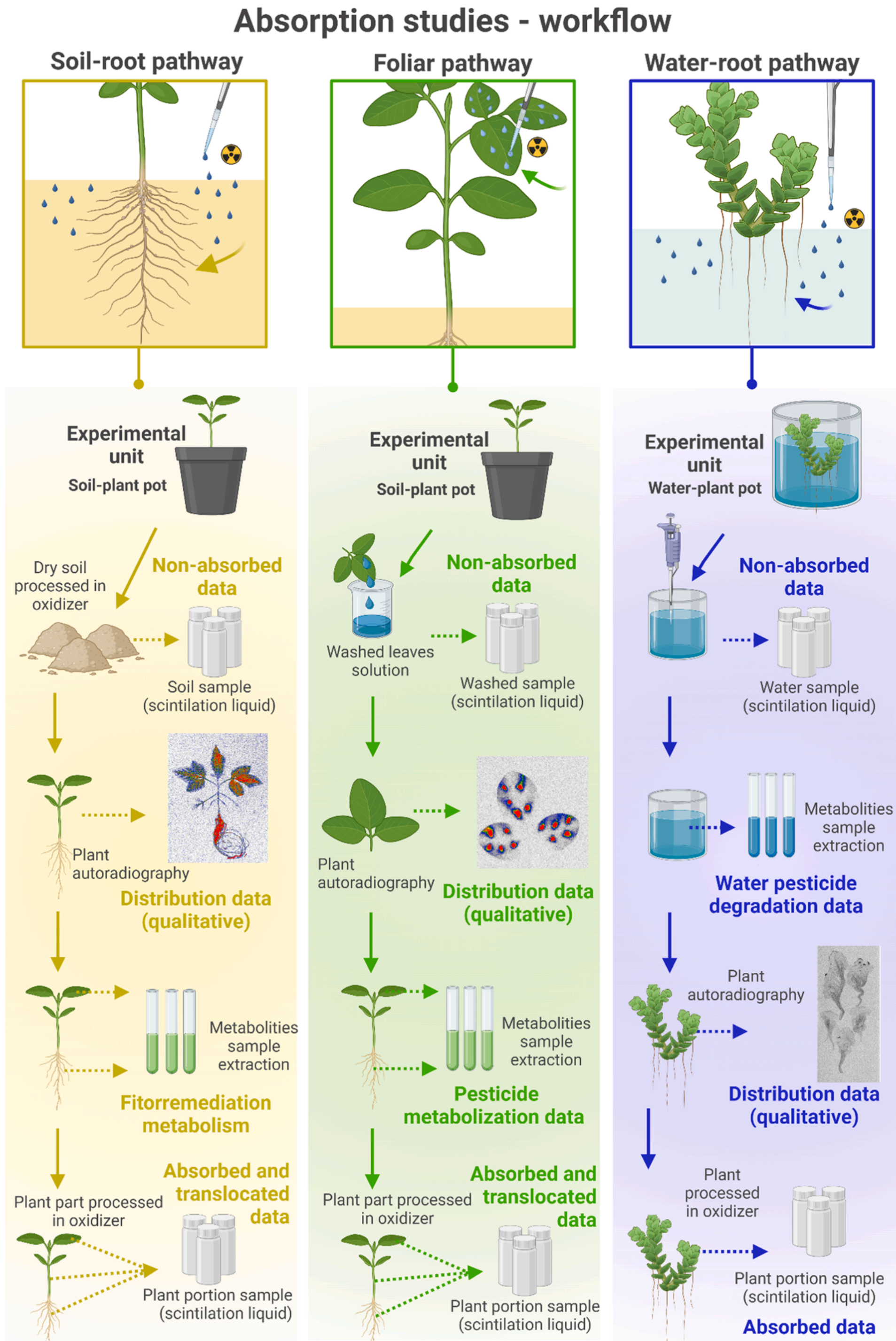

Fig. 6. General overview of absorption and translocation studies using radiometric techniques.

- The use of small droplets of ^{14}C-herbicide solution (0.5–2 μL) is preferred, because the use of only one drop or drops with a high volume (5–10 μL) can change the herbicide concentration in the leaf area and uptake patterns;
- After ^{14}C-pesticide application on the leaves, it is important to pay attention to the washing solution used; do not consider only the pesticide affinity but also the leaf surface composition, since the use of pure compounds could remove unrealistic amounts of pesticides from leaves.
- The radioactivity recovery used in the results (Pereira et al., 2019) rather than the percentage concerning the total applied (as used in the current work) can lead to a mistaken interpretation, since the total radioactivity varies between treatments and needs to be quantified in each working solution, resulting in a total applied for each treatment (See Section 2.2.3).
- The residues generated by carbon-14-labeled studies must be minimal because they need to be carefully discharged, as recommended by the International Atomic Energy Agency for radioactive materials.
- An accreditation laboratory and qualified human resources are crucial for the development of these studies. Safety is essential to manipulate radioactive materials.

4. Conclusion

Radiometric strategies using ^{14}C-herbicides were demonstrated as viable tools to understand the environmental fate of herbicides from different perspectives. Through the (a) leaf pathway, more ^{14}C-mesotrione was absorbed in the presence of oil and adjuvant in the application solution; however, the translocation was increased only with adjuvant. In the soil-root pathway (b), ^{14}C-indaziflam and ^{14}C-quinclorac were absorbed by cover crop plants at low rates. In the aquatic environment (c), ^{14}C-atrazine was absorbed by *Salvinia spp.* plants, causing no toxicity to the plants in doses of 10 and 90-times higher than the environmental concentration, which also demonstrates no effect of *Salvinia spp.* in ^{14}C-atrazine metabolism in water.

Herein we explored dynamic aspects of herbicides regarding application in plants and as a contaminant in the environment, in order to elicit the differences in the experimental models using radiolabeled pesticides. Following the method steps and caution points highlighted it is possible to establish reliable and robust data that provide information regarding the tracking of pesticides in the environment.

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CRediT authorship contribution statement

Gustavo Vinícios Munhoz-Garcia: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vanessa Takeshita:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Camila de Werk Pinácio:** Writing – original draft, Methodology, Investigation. **Brian Cintra Cardoso:** Writing – original draft, Methodology, Investigation. **Bruno Dalla Vecchia:** Writing – original draft, Methodology, Investigation. **Daniel Nalin:** Writing – original draft, Methodology, Investigation, Formal analysis. **Ana Laura Camachos de Oliveira:** Writing – original draft, Methodology, Investigation. **Leandro Fernando Felix:** Methodology, Investigation. **Valdemar Luiz Tornisielo:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the writing process.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The dataset can be consulted at <Munhoz Garcia, Gustavo Vinícios (2024), "Supplementary Information for "Radiometric approaches with carbon-14-labeled molecules for determining herbicide fate in plant systems"", Mendeley Data, V1, doi: 10.17632/drbx9gyn32.1>

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.117003.

References

Alonso, D., Koskinen, W., Oliveira, R., Constantin, J., Mislankar, S., 2011. Sorption-desorption of indaziflam in selected agricultural soils. J. Agric. Food Chem. *59* (24), 13096–13101. https://doi.org/10.1021/jf203014g.

Alves, C., Galon, L., Winter, F.L., Basso, F.J.M., Holz, C.M., Kaizer, R.R., Perin, G.F., 2019. Winter species promote phytoremediation of soil contaminated with protox-inhibiting herbicides. Planta Daninha 37. https://doi.org/10.1590/s0100-83582019370100020.

Bachetti, R.A., Urseler, N., Morgante, V., Damilano, G., Porporatto, C., Agostini, E., Morgante, C., 2021. Monitoring of atrazine pollution and its spatial-seasonal variation on surface water sources of an agricultural river basin. Bull. Environ. Contam. Toxicol. *106* (6), 929–935. https://doi.org/10.1007/s00128-021-03264-x.

Bailey, W.A., et al., 2003. Absorption, translocation, and metabolism of sulfentrazone in potato and selected weed species. Weed Sci. 51 (1), 32–36. https://doi.org/10.1614/0043-1745(2003)051[0032:ATAMOS]2.0.CO;2.

Barchanska, H., Plonka, J., Jaros, A., Ostrowska, A., 2019. Potential application of *Pistia stratiotes* for the phytoremediation of mesotrione and its degradation products from water. Int. J. Phytoremediat. *21* (11), 1090–1097. https://doi.org/10.1080/15226514.2019.1606780.

Barizon, R.R.M., Figueiredo, R., de, O., de Souza Dutra, D.R.C., Regitano, J.B., Ferracini, V.L., 2020. Pesticides in the surface waters of the Camanducaia River watershed, Brazil. J. Environ. Sci. Health, Part B *55* (3), 283–292. https://doi.org/10.1080/03601234.2019.1693835.

Barroso, G.M., dos Santos, E.A., Pires, F.R., Galon, L., Cabral, C.M., dos Santos, J.B., 2023. Phytoremediation: a green and low-cost technology to remediate herbicides in the environment. Chemosphere 334, 138943. https://doi.org/10.1016/j.chemosphere.2023.138943.

Bhandari, G., Atreya, K., Scheepers, P.T.J., Geissen, V., 2020. Concentration and distribution of pesticide residues in soil: non-dietary human health risk assessment. Chemosphere *253*. https://doi.org/10.1016/j.chemosphere.2020.126594.

Bhatti, P., Duhan, A., Pal, A., Monika, Beniwal, R.K., Kumawat, P., Yadav, D.B., 2022. Ultimate fate and possible ecological risks associated with atrazine and its principal metabolites (DIA and DEA) in soil and water environment. Ecotoxicol. Environ. Saf. *248* https://doi.org/10.1016/j.ecoenv.2022.114299.

Bian, F., Zhong, Z., Zhang, X., Yang, C., Gai, X., 2020. Bamboo – an untapped plant resource for the phytoremediation of heavy metal contaminated soils. In: Chemosphere, Vol. 246. Elsevier Ltd. https://doi.org/10.1016/j.chemosphere.2019.125750.

Bish, M., Oseland, E., Bradley, K., 2021. Off-target pesticide movement: A review of our current understanding of drift due to inversions and secondary movement. In: Weed Technology, Vol. 35. Cambridge University Press, pp. 345–356. https://doi.org/10.1017/wet.2020.138.

Brankov, M., Vieira, B.C., Alves, G.S., Zaric, M., Vukoja, B., Houston, T., Kruger, G.R., 2023. Adjuvant and nozzle effects on weed control using mesotrione and rimsulfuron plus thifensulfuron-methyl. Crop Prot. 167, 106209 https://doi.org/10.1016/j.cropro.2023.106209.

Brankov, M., Vieira, B.C., Alves, G.S., Zaric, M., Vukoja, B., Houston, T., Kruger, G.R., 2023. Adjuvant and nozzle effects on weed control using mesotrione and rimsulfuron plus thifensulfuron-methyl. Crop Prot. 167, 106209 https://doi.org/10.1016/j.cropro.2023.106209.

Brochado, M.G. da S., Mielke, K.C., de Paula, D.F., Laube, A.F.S., Alcántara-de la Cruz, R., Gonzatto, M.P., Mendes, K.F., 2022. Impacts of dicamba and 2,4-D drift on 'Ponkan' mandarin seedlings, soil microbiota and Amaranthus retroflexus. J. Hazard. Mater. Adv. *6* https://doi.org/10.1016/j.hazadv.2022.100084.

Bromilow, R.H., Chamberlain, K., Evans, A.A., 1990. Physicochemical aspects of phloem translocation of herbicides. Weed Sci. *38* (3), 305–314. https://doi.org/10.1017/S0043174500056575.

Cabral, C.M., Souza, M. de F., Alencar, B.T.B., Ferreira, E.A., Silva, D.V., Reginaldo, L.T. R.T., dos Santos, J.B., 2023. Sensibility, multiple tolerance and degradation capacity of forest species to sequential contamination of herbicides in groundwaters. J. Hazard. Mater. *448* https://doi.org/10.1016/j.jhazmat.2023.130914.

Cech, R.M., Jovanovic, S., Kegley, S., Hertoge, K., Leisch, F., Zaller, J.G., 2022. Reducing overall herbicide use may reduce risks to humans but increase toxic loads to honeybees, earthworms and birds. Environ. Sci. Eur. *34* (1) https://doi.org/10.1186/s12302-022-00622-2.

Conciani, P.A., Mendes, K.F., de Sousa, R.N., Ribeiro, A., de, P., Pimpinato, R.F., Tornisielo, V.L., 2023. Peanut and sorghum are excellent phytoremediators of 14C-tebuthiuron in herbicide-contaminated soil. Adv. Weed Sci. *41* https://doi.org/10.51694/AdvWeedSci/2023;41:00002.

Correia, N.M., Carbonari, C.A., Velini, E.D., 2020. Detection of herbicides in water bodies of the Samambaia River sub-basin in the Federal District and eastern Goiás. J. Environ. Sci. Health, Part B 55 (6), 574–582. https://doi.org/10.1080/03601234.2020.1742000.

Droz, B., Drouin, G., Maurer, L., Villette, C., Payraudeau, S., Imfeld, G., 2021. Phase transfer and biodegradation of pesticides in water-sediment systems explored by compound-specific isotope analysis and conceptual modeling. Environ. Sci. Technol. *55* (8), 4720–4728. https://doi.org/10.1021/acs.est.0c06283.

Evangelista, P.A., Lourenço, F.M. de O., Chakma, D., Shaha, C.K., Konate, A., Pimpinato, R.F., Louvandini, H., Tornisielo, V.L., 2023. Bioaccumulation and depletion of the antibiotic sulfadiazine 14C in Lambari (Astyanax bimaculatus). Animals *13* (15). https://doi.org/10.3390/ani13152464.

Gentil, C., Basset-Mens, C., Manteaux, S., Mottes, C., Maillard, E., Biard, Y., Fantke, P., 2020. Coupling pesticide emission and toxicity characterization models for LCA: application to open-field tomato production in Martinique. J. Clean. Prod. *277* https://doi.org/10.1016/j.jclepro.2020.124099.

Gentil-Sergent, C., Basset-Mens, C., Gaab, J., Mottes, C., Melero, C., Fantke, P., 2021. Quantifying pesticide emission fractions for tropical conditions. Chemosphere *275*. https://doi.org/10.1016/j.chemosphere.2021.130014.

Graymore, M., Stagnitti, F., Allinson, G., 2001. Impacts of atrazine in aquatic ecosystems. Environ. Int. *26*, 483–495. ⟨www.elsevier.com/locate/envint⟩.

Guimarães, F.P., Aguiar, R., Karam, D., Oliveira, J.A., Silva, J.A.A., Santos, C.L., Sant'anna-Santos, B.F., Lizieri-Santos, C., 2011. Potential of macrophytes for removing atrazine from aqueous solution. Planta Daninha *29* (spe), 1137–1147. https://doi.org/10.1590/S0100-83582011000500022.

Guimarães, A.C.D., de Paula, D.F., Mendes, K.F., de Sousa, R.N., Araújo, G.R., Inoue, M. H., Tornisielo, V.L., 2022. Can soil type interfere in sorption-desorption, mobility, leaching, degradation, and microbial activity of the 14C-tebuthiuron herbicide. J. Hazard. Mater. Adv. *6* https://doi.org/10.1016/j.hazadv.2022.100074.

Hasnaki, R., Ziaee, M., Mahdavi, V., 2023. Pesticide residues in corn and soil of corn fields of Khuzestan, Iran, and potential health risk assessment. J. Food Compos. Anal. *115* https://doi.org/10.1016/j.jfca.2022.104972.

Haug, E.J., Ahmed, K.A., Gannon, T.W., Richardson, R.J., 2021. Absorption and translocation of florpyrauxifen-benzyl in ten aquatic plant species. Weed Sci. *69* (6), 624–630. https://doi.org/10.1017/wsc.2021.38.

Hong, J., Boussetta, N., Enderlin, G., Merlier, F., Grimi, N., 2022. Degradation of Residual Herbicide Atrazine in Agri-Food and Washing Water. In: Foods, Vol. 11. MDPI. https://doi.org/10.3390/foods11162416.

Hou, X., Roos, P., 2008. Critical comparison of radiometric and mass spectrometric methods for the determination of radionuclides in environmental, biological and nuclear waste samples. *Anal. Chim. Acta* Vol. 608 (Issue 2), 105–139. https://doi.org/10.1016/j.aca.2007.12.012.

Hu, M., Liu, L., Hou, N., Li, X., Zeng, D., Tan, H., 2021. Insight into the adsorption mechanisms of ionizable imidazolinone herbicides in sediments: kinetics, adsorption model, and influencing factors. Chemosphere *274*. https://doi.org/10.1016/j.chemosphere.2021.129655.

Izadi-Darbandi, E., Aliverdi, A., Hammami, H., 2013. Behavior of vegetable oils in relation to their influence on herbicides' effectiveness. Ind. Crops Prod. *44*, 712–717. https://doi.org/10.1016/j.indcrop.2012.08.023.

Jachetta, J.J., Appleby, A.P., Boersma, L., 1986. Apoplastic and symplastic pathways of atrazine and glyphosate transport in shoots of seedling sunflower. Plant Physiol. Vol. 82. ⟨https://academic.oup.com/plphys/article/82/4/1000/6082066⟩.

Ju, C., Zhang, H., Wu, R., Dong, S., Yao, S., Wang, F., Cao, D., Xu, S., Fang, H., Yu, Y., 2020. Upward translocation of acetochlor and atrazine in wheat plants depends on their distribution in roots. Sci. Total Environ. *703* https://doi.org/10.1016/j.scitotenv.2019.135636.

Kafle, A., Timilsina, A., Gautam, A., Adhikari, K., Bhattarai, A., Aryal, N., 2022. Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. In: Environmental Advances, Vol. 8. Elsevier Ltd. https://doi.org/10.1016/j.envadv.2022.100203.

Khrunyk, Y., Schiewer, S., Carstens, K.L., Hu, D., Coats, J.R., 2016. Uptake of C14-atrazine by prairie grasses in a phytoremediation setting. Int. J. Phytoremediat. *19* (2), 104–112. https://doi.org/10.1080/15226514.2016.1193465.

Knapp, J.L., Nicholson, C.C., Jonsson, O., de Miranda, J.R., Rundlöf, M., 2023. Ecological traits interact with landscape context to determine bees' pesticide risk. Nat. Ecol. Evol. *7* (4), 547–556. https://doi.org/10.1038/s41559-023-01990-5.

Kumar, A., Singh, N., 2020. Crop residue ashes reduce leaching, persistence and bioavailability of sulfosulfuron and pretilachlor used in the succeeding crop. Soil Res. 58, 551–560. https://doi.org/10.1071/SR20142.

Kyung, K.S., Ahn, K.C., Kwon, J.W., Lee, Y.P., Lee, E.Y., Kim, Y.J., Führ, F., Lee, J.K., 2015. Long-term fate of the herbicide mefenacet in a rice-grown lysimeter over a period of 6 consecutive years. J. Korean Soc. Appl. Biol. Chem. *58* (1), 35–43. https://doi.org/10.1007/s13765-015-0048-4.

Lewis, K.A., Tzilivakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk assessments and management. Hum. Ecol. risk Assess.: Int. J. 22 (4), 1050–1064. https://doi.org/10.1080/10807039.2015.1133242.

Li, X., Giles, D.K., Niederholzer, F.J., Andaloro, J.T., Lang, E.B., Watson, L.J., 2021. Evaluation of an unmanned aerial vehicle as a new method of pesticide application for almond crop protection. Pest Manag. Sci. *77* (1), 527–537. https://doi.org/10.1002/ps.6052.

Liu, X., Li, W., Kümmel, S., Merbach, I., Sood, U., Gupta, V., Lal, R., Richnow, H.H., 2021. Soil from a Hexachlorocyclohexane Contaminated Field Site Inoculates Wheat in a Pot Experiment to Facilitate the Microbial Transformation of β-Hexachlorocyclohexane Examined by Compound-Specific Isotope Analysis. Environ. Sci. Technol. *55* (20), 13812–13821. https://doi.org/10.1021/acs.est.1c03322.

Loureiro, D.B., Lario, L.D., Herrero, M.S., Salvatierra, L.M., Novo, L.A.B., Pérez, L.M., 2023. Potential of Salvinia biloba Raddi for removing atrazine and carbendazim from aquatic environments. Environ. Sci. Pollut. Res. *30* (8), 22089–22099. https://doi.org/10.1007/s11356-022-23725-y.

Ma, S., Jia, R., Liu, L., Zhu, Z., Qiao, X., Zhang, W., Zhang, L., Dong, J., 2021. The adjuvant effects of rosin and coconut oil on nicosulfuron and mesotrione to control weeds. Ecotoxicol. Environ. Saf. *225* https://doi.org/10.1016/j.ecoenv.2021.112766.

Mendes, K.F., Furtado, I.F., Sousa, R.N. de, Lima, A. da C., Mielke, K.C., Brochado, M.G. da S., 2021b. Cow bonechar decreases indaziflam pre-emergence herbicidal activity in tropical soil. J. Environ. Sci. Health, Part B *56* (6), 532–539. https://doi.org/10.1080/03601234.2021.1916302.

Mendes, K.F., Martins, B.A.B., Reis, F.C., Dias, A.C.R., Tornisielo, V.L., 2017. Metodologias para estudos de comportamento de herbicidas na planta e no solo utilizando radioisótopos. Planta Daninha *35*. https://doi.org/10.1590/s0100-83582017350100049.

Mendes, K.F., Mielke, K.C., D'Antonino, L., Alberto da Silva, A., 2022. Retention, Absorption, Translocation, and Metabolism of Herbicides in Plants. Applied Weed and Herbicide Science. Springer International Publishing, pp. 157–186. https://doi.org/10.1007/978-3-031-01938-8_5.

Mendes, K.F., Soares, M.B., Sousa, R.N. de, Mielke, K.C., Brochado, M.G. da S., Tornisielo, V.L., 2021a. Indaziflam sorption–desorption and its three metabolites from biochars- and their raw feedstock-amended agricultural soils using radiometric technique. J. Environ. Sci. Health, Part B *56* (8), 731–740. https://doi.org/10.1080/03601234.2021.1941559.

Mendes, K.F., Soares, M.B., Sousa, R.N., Mielke, K.C., Brochado, M.G. da S., Tornisielo, V. L., 2021. Indaziflam sorption–desorption and its three metabolites from biochars- and their raw feedstock-amended agricultural soils using radiometric technique. J. Environ. Sci. Health, Part B 56 (8), 731–740. https://doi.org/10.1080/03601234.2021.1941559.

Mendes, K.F., de Sousa, R.N., Takeshita, V., Alonso, F.G., Régo, A.P.J., Tornisielo, V.L., 2019. Cow bone char as a sorbent to increase sorption and decrease mobility of hexazinone, metribuzin, and quinclorac in soil. Geoderma *343*, 40–49. https://doi.org/10.1016/j.geoderma.2019.02.009.

Mersie, W., Mcnamee, C., Seybold, C.A., Tierney, D.P., 2000. Diffusion and degradation of atrazine in a water/sediment system. Environ. Toxicol. Chem. *19* (8), 2008–2014. https://doi.org/10.1002/etc.5620190808.

Mouhamad, R., Ghanem, I., AlOrfi, M., Ibrahim, K., Ali, N., Al-Daoude, A., 2012. Phytoremediation of Trichloroethylene And Dichlorodiphenyltrichloroethane-Polluted Water Using Transgenic Sesbania grandiflora and Arabidopsis thaliana Plants Harboring Rabbit Cytochrome P450 2E1. Int. J. Phytoremediat. *14* (7), 656–668. https://doi.org/10.1080/15226514.2011.619232.

Nandula, V.K., Vencill, W.K., 2015. Herbicide absorption and translocation in plants using radioisotopes. Weed Sci. *63* (SP1), 140–151. https://doi.org/10.1614/ws-d-13-00107.1.

Ni, J., Sun, S.X., Zheng, Y., Datta, R., Sarkar, D., Li, Y.M., 2018. Removal of prometryn from hydroponic media using marsh pennywort (*Hydrocotyle vulgaris* L.). Int. J. Phytoremediat. *20* (9), 909–913. https://doi.org/10.1080/15226514.2018.1448359.

Nödler, K., Licha, T., Voutsa, D., 2013. Twenty years later - Atrazine concentrations in selected coastal waters of the Mediterranean and the Baltic Sea. Mar. Pollut. Bull. *70* (1–2), 112–118. https://doi.org/10.1016/j.marpolbul.2013.02.018.

Ogura, A.P., Lima, J.Z., Silva, L.C.M. da, Dias, M.A., Rodrigues, V.G.S., Montagner, C.C., Espíndola, E.L.G., 2023. Phytotoxicity of 2,4-D and fipronil mixtures to three green manure species. J. Environ. Sci. Health, Part B *58* (3), 262–272. https://doi.org/10.1080/03601234.2023.2178789.

Olette, R., Couderchet, M., Biagianti, S., Eullaffroy, P., 2008. Toxicity and removal of pesticides by selected aquatic plants. Chemosphere *70* (8), 1414–1421. https://doi.org/10.1016/j.chemosphere.2007.09.016.

Organization for Economic Co-operation and Development (OECD) (2000). Test number 106: Adsorption-Desorption Using a Batch Equilibrium Method. In: Guidelines for the Testing of Chemicals, 2000. Paris, OECD Publishing, 1 edn. https://doi.org/10.1787/20745753.

Organization for Economic Co-operation and Development (OECD) (2002). Test number 307: Aerobic and Anaerobic Transformation in Soil, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, 1 edn. https://doi.org/10.1787/9789264070509-en.

Organization for Economic Co-operation and Development (OECD) (2004). Test number. 312: Leaching in Soil Columns, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris, https://doi.org/10.1787/9789264070561-en.

Ortiz, M.F., Figueiredo, M.R.A., Nissen, S.J., Wersal, R.M., Ratajczyk, W.A., Dayan, F.E., 2021. 2,4-D and 2,4-D butoxyethyl ester behavior in Eurasian and hybrid watermilfoil (Myriophyllum spp. Pest Manag. Sci. *78* (2), 626–632. https://doi.org/10.1002/ps.6671.

Ortiz, M.F., Nissen, S.J., Dayan, F.E., 2022. Endothall and florpyrauxifen-benzyl behavior in hydrilla (Hydrilla verticillata) when applied in combination. Weed Sci. *70* (5), 537–542. https://doi.org/10.1017/wsc.2022.39.

Palma-Bautista, C., Vazquez-Garcia, J.G., Travlos, I., Tataridas, A., Kanatas, P., Domínguez-Valenzuela, J.A., De Prado, R., 2020. Effect of adjuvant on glyphosate effectiveness, retention, absorption and translocation in Lolium rigidum and Conyza canadensis. Plants *9* (3). https://doi.org/10.3390/plants9030297.

Pereira, F.C.M., Tayengwa, R., Alves, P.L. Da C.A., Peer, W.A., 2019. Phosphate Status Affects Phosphate Transporter Expression and Glyphosate Uptake and Transport in Grand Eucalyptus (Eucalyptus grandis). Weed Sci. 67 (1), 29–40. https://doi.org/10.1017/wsc.2018.58.

Polli, E.G., Alves, G.S., Gizotti de Moraes, J., Kruger, G.R., 2022. Influence of surfactant-humectant adjuvants on physical properties, droplet size, and efficacy of glufosinate formulations. Agrosystems, Geosci. Environ. *5* (1) https://doi.org/10.1002/agg2.20230.

Queiroz, A.R.S., Delatorre, C.A., Markus, C., Lucio, F.R., Angonese, P.S., Merotto, A., 2022. Rapid necrosis II: physiological and molecular analysis of 2,4-D resistance in Sumatran fleabane (*Conyza sumatrensis*). Weed Sci. *70* (1), 36–45. https://doi.org/10.1017/wsc.2021.71.

Rao, V.S., 2000. Principles of Weed Science. Science Publishers Inc., New Hampshire, USA, p. 555.

Rico, A., de Oliveira, R., Silva de Souza Nunes, G., Rizzi, C., Villa, S., De Caroli Vizioli, B., Montagner, C.C., Waichman, A.V., 2022. Ecological risk assessment of pesticides in urban streams of the Brazilian Amazon. Chemosphere *291*. https://doi.org/10.1016/j.chemosphere.2021.132821.

Roberts, T.R., Hutson, D.H., Lee, P.W. and Nicholls, P.H. Plimmer, J.R. (ed.) 1998. Metabolic pathways of agrochemicals. Part 1: herbicides and plant growth regulators. Royal Society of Chemistry (RSC), Cambridge.

Rolando, C.A., Gaskin, R.E., Horgan, D.B., Richardson, B., 2020. Effect of dose and adjuvant on uptake of triclopyr and dicamba into Pinus contorta needles. Plant-Environ. Interact. *1* (1), 57–66. https://doi.org/10.1002/pei3.10012.

Santos, A., Leal, J.F.L., Montgomery, J.S., Ortiz, M.F., Simões Araujo, A.L., Morran, S., de Figueiredo, M.R.A., Langaro, A.C., Zobiole, L.H.S., Nissen, S.J., Gaines, T.A., Pinho, C.F., 2023. Nontarget-site resistance due to rapid physiological response in 2,4-D resistant *Conyza sumatrensis*: reduced 2,4-D translocation and auxin-induced gene expression. Pest Manag. Sci. 79 (10), 3581–3592. https://doi.org/10.1002/ps.7541.

Santos, J., da Silva Pontes, M., Grillo, R., Fiorucci, A.R., José de Arruda, G., Santiago, E. F., 2020. Physiological mechanisms and phytoremediation potential of the macrophyte Salvinia biloba towards a commercial formulation and an analytical standard of glyphosate. Chemosphere 259, 127417. https://doi.org/10.1016/j.chemosphere.2020.127417.

Singh, S., Kumar, V., Chauhan, A., Datta, S., Wani, A.B., Singh, N., Singh, J., 2018. Toxicity, degradation and analysis of the herbicide atrazine. In: Environmental Chemistry Letters, Vol. 16. Springer Verlag, pp. 211–237. https://doi.org/10.1007/s10311-017-0665-8.

Sperry, B.P., Mudge, C.R., Getsinger, K.D., 2021. Simulated herbicide spray retention on floating aquatic plants as affected by carrier volume and adjuvant type. Weed Technol. *36* (1), 56–63. https://doi.org/10.1017/wet.2021.85.

Sun, M., Guo, M., Guo, S., Li, Y., Dong, S., Song, X., Shi, X., Yuan, X., 2022. Effects of mesotrione on the control efficiency and chlorophyll fluorescence parameters of Chenopodium album under simulated rainfall conditions. PLoS One 17 (6), e0267649. https://doi.org/10.1371/journal.pone.0267649.

Takeshita, V., Munhoz-Garcia, G.V., Pereira, A.E.S., Tornisielo, V.L., Fraceto, L.F., 2023. Radiometric strategy to track nanopesticides: An important approach to understand the fate, mechanisms of action and toxicity. In: TrAC - Trends in Analytical Chemistry, Vol. 165. Elsevier B.V. https://doi.org/10.1016/j.trac.2023.117156.

Takeshita, V., Munhoz-Garcia, G.V., Werk Pinácio, C., Cardoso, B.C., Nalin, D., Tornisielo, V.L., Fraceto, L.F., 2022. Availability of metribuzin-loaded polymeric nanoparticles in different soil systems: an important study on the development of safe nanoherbicides. Plants *11* (23). https://doi.org/10.3390/plants11233366.

Takeshita, V., de Sousa, B.T., Preisler, A.C., Carvalho, L.B., Pereira, A. do E.S., Tornisielo, V.L., Dalazen, G., Oliveira, H.C., Fraceto, L.F., 2021. Foliar absorption and field herbicidal studies of atrazine-loaded polymeric nanoparticles. J. Hazard. Mater. *418* https://doi.org/10.1016/j.jhazmat.2021.126350.

Tang, F.H.M., Lenzen, M., McBratney, A., Maggi, F., 2021. Risk of pesticide pollution at the global scale. Nat. Geosci. *14* (4), 206–210. https://doi.org/10.1038/s41561-021-00712-5.

Teófilo, T.M. da S., Mendes, K.F., Fernandes, B.C.C., Oliveira, F.S. de, Silva, T.S., Takeshita, V., Souza, M. de F., Tornisielo, V.L., Silva, D.V., 2020. Phytoextraction of diuron, hexazinone, and sulfometuron-methyl from the soil by green manure species. Chemosphere *256*. https://doi.org/10.1016/j.chemosphere.2020.127059.

Torres, A.B., Meneghin, S.P., Ribeiro, N.M., Santos, H.V., Schedenffeldt, B.F., Monquero, P.A., 2018. Saflufenacil and indaziflam herbicide effects on agricultural crops and microorganisms. Afr. J. Agric. Res. 13 (16), 872–885. https://doi.org/10.5897/AJAR2018.13067.

Triassi, M., Montuori, P., Provvisiero, D.P., De Rosa, E., Di Duca, F., Sarnacchiaro, P., Díez, S., 2022. Occurrence and spatial-temporal distribution of atrazine and its metabolites in the aquatic environment of the Volturno River estuary, southern Italy. Sci. Total Environ. *803* https://doi.org/10.1016/j.scitotenv.2021.149972.

Trovato, V.W., Portilho, I.I.R., Barizon, R.R.M., Scorza Júnior, R.P., 2020. Herbicide runoff from a soil with different levels of sugarcane straw coverage in Brazil. Ecotoxicol. Environ. Contam. *15* (1), 25–35. https://doi.org/10.5132/eec.2020.01.04.

Tudi, M., Li, H., Li, H., Wang, L., Lyu, J., Yang, L., Tong, S., Yu, Q.J., Ruan, H.D., Atabila, A., Phung, D.T., Sadler, R., Connell, D., 2022. Exposure Routes and Health Risks Associated with Pesticide Application. In: Toxics, Vol. 10. MDPI. https://doi.org/10.3390/toxics10060335.

Urseler, N., Bachetti, R., Biolé, F., Morgante, V., Morgante, C., 2022. Atrazine pollution in groundwater and raw bovine milk: Water quality, bioaccumulation and human risk assessment. Sci. Total Environ. *852* https://doi.org/10.1016/j.scitotenv.2022.158498.

Vieira, B.C., Luck, J.D., Amundsen, K.L., Werle, R., Gaines, T.A., Kruger, G.R., 2020. Herbicide drift exposure leads to reduced herbicide sensitivity in Amaranthus spp. Sci. Rep. *10* (1) https://doi.org/10.1038/s41598-020-59126-9.

Vilca, F.Z., Vilca, O.M.L., Silveira, R.F., Tornisielo, V.L., 2020. Uptake and depletion of the antibiotic sulfadiazine 14C in rainbow trout (Oncorhynchus mykiss). J. Radioanal. Nucl. Chem. *323* (3), 1033–1039. https://doi.org/10.1007/s10967-020-07026-7.

Viti, M.L., Alves, P.A.T., Mendes, K.F., Pimpinato, R.F., Guimarães, A.C.D., Tornisielo, V. L., 2019. Translocation and Root Exudation of Glyphosate by Urochloa brizantha and its Transport on Sugarcane and Citrus Seedlings. Planta Daninha *37*. https://doi.org/10.1590/s0100-83582019370100030.

Vizioli, B., Silva da Silva, G., Ferreira de Medeiros, J., Montagner, C.C., 2023. Atrazine and its degradation products in drinking water source and supply: risk assessment for environmental and human health in Campinas, Brazil. Chemosphere *336*. https://doi.org/10.1016/j.chemosphere.2023.139289.

Wang, F., Li, X., Yu, S., He, S., Cao, D., Yao, S., Fang, H., Yu, Y., 2021. Chemical factors affecting uptake and translocation of six pesticides in soil by maize (Zea mays L.). J. Hazard. Mater. *405* https://doi.org/10.1016/j.jhazmat.2020.124269.

Wang, L., Zhang, Z.F., Liu, L.Y., Zhu, F.J., Ma, W.L., 2023. National-scale monitoring of historic used organochlorine pesticides (OCPs) and current used pesticides (CUPs) in Chinese surface soil: old topic and new story. J. Hazard. Mater. *443* https://doi.org/10.1016/j.jhazmat.2022.130285.

Yang, C., Prasher, S.O., Landry, J., Ramaswamy, H.S., 2003. Development of an image processing system and a fuzzy algorithm for site-specific herbicide applications, 5 –M8 Precis. Agric. *4* (1). https://doi.org/10.1023/A:1021847103560.

Yu, J., McCullough, P.E., Vencill, W.K., 2013. Absorption, Translocation, and Metabolism of Amicarbazone in Annual Bluegrass (Poa annua), Creeping Bentgrass (Agrostis stolonifera), and Tall Fescue (Festuca arundinacea. Weed Sci. 61 (2), 217–221. https://doi.org/10.1614/WS-D-12-00136.1.

Riquinho, D.L., Souto, L.H.D., Carlotto, F.D., Pinto, V.L., 2020. Mortality rate and water contamination by atrazine in Rio Grande do Sul State: na ecological study. International Journal of Development Research 10 (7), 38235–38240.

Zuo, W., Zhao, Y., Qi, P., Zhang, C., Zhao, X., Wu, S., An, X., Liu, X., Cheng, X., Yu, Y., Tang, T., 2024. Current-use pesticides monitoring and ecological risk assessment in vegetable soils at the provincial scale. Environ. Res. *246* https://doi.org/10.1016/j.envres.2023.118023.