



Research Article

Understanding the chemistry of manganese fertilizers and glyphosate mixtures by using synchrotron X-ray spectrometry



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Received: 27 May 2020 / Accepted: 5 October 2020 / Published online: 17 October 2020
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Abstract

The spraying of tank mixtures with manganese (Mn) and glyphosate is a practical alternative to alleviate nutritional deficiency while controlling weeds. Thereby, this study investigates the chemical interactions between glyphosate and commercial sources of Mn, such as MnSO_4 , Mn-phosphite, Mn-EDTA, Mn-glycine, and MnCO_3 . Nearly 30% of the Mn supplied as MnSO_4 and Mn-glycine precipitated with glyphosate, yielding a Mn:glyphosate solid complex with molar ratio of nearly 2:1, both presenting similar chemical environment. XANES analysis of the supernatants indicate no formation of Mn-glyphosate soluble complexes. The use of Mn-EDTA as well as the maintenance of the mixture pH below 2.5 prevented precipitation, while pH above 7 caused the formation of $\text{MnO}(\text{OH})$. In conclusion, the Mn source and the pH of the mixtures matter. The absence of Mn-glyphosate soluble complexes suggests that dissolved Mn and glyphosate are still able to accomplish their functions, however, the precipitation significantly decreases their active availability.

Keywords Tank mixture · Mn-glyphosate complex · Soybean · Weed control · XAS

1 Introduction

In the 2019/2020 crop season, Brazil cultivated 36 million of hectares of soybean (*Glycine max* L.), harvesting more than 125 million tons of grains. Such achievement made Brazil the world's largest soybean producer, a title previously belonged to the USA [1]. The increasing cultivation under no-tillage systems has caused the widespread use of lime applications in Brazil. This practice raise the topsoil pH, decreasing availability and consequent root uptake of transition metal micronutrients, such as manganese (Mn) [2]. Manganese is required by many plant enzymes, and plays an important role in photosynthesis, nitrogen

metabolism, nodulation, and respiration. It is also a component of aromatic amino acids, auxins, phenols and lignin [3]. The importance of Mn for plant nutrition and the current evidence of Mn deficiency in soybean trials increased the usage of Mn by soybean growers all over Brazil.

Meanwhile, the transgenic glyphosate-resistant soybean was introduced and rapidly adopted in Brazil, since it highly facilitates the weed management. Indeed, glyphosate (*N*-(phosphonomethyl)glycine) is a non-selective herbicide which mechanism of action consists of competitive inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, essential for the biosynthesis of the aromatic amino acids phenylalanine, tyrosine and

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s42452-020-03632-y>) contains supplementary material, which is available to authorized users.

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SN Applied Sciences (2020) 2:1837 | <https://doi.org/10.1007/s42452-020-03632-y>

tryptophan [4]. Besides the introduction of transgenic crops, glyphosate's success is also due to its highly effective broad spectrum and low toxicity for animals [5], being also a very cheap herbicide [6]. Glyphosate is considered a once in a century herbicide [5], being the best-selling active ingredient for herbicides worldwide [7]. In 2018, more than 190 thousands of tons of glyphosate's active ingredient and its salts were sold within the Brazilian territory, four times more than the second best-selling herbicide, the 2,4-D [8, 9].

In Brazilian soybean fields, Mn foliar fertilization [10] is often performed in conjunction with glyphosate at least twice during the crop cycle, generally around the V3 and V7 phenological stages. The tank mixing is as an alternative to reduce the number of fields sprayings, farmers costs, soil compaction, and mechanical damage to the crop [11]. This practice can, however, compromise not the sole efficacy of weed control by glyphosate, but also the nutrition potential of the dissolved ions. In that respect, Bernards et al. [12] demonstrated that glyphosate interactions with Mn increased at higher pH values of the mixture similar to those found in plant symplast. It was also noticed a significant decrease of leaf Mn content in soybean plants, after foliar applications of glyphosate [13].

Face to the wide adoption of tank mixtures with Mn and glyphosate, and considering their antagonist potential on both weed control and plant nutrition, this survey targeted the chemical reactions between this herbicide and five of the most common Mn sources (MnSO_4 , Mn-EDTA, Mn-phosphite, Mn-glycine, and MnCO_3 nanopowder). The effect of the pH on MnSO_4 + glyphosate interactions was also evaluated. Therefore, the electric conductivity (EC) and pH of the mixtures were monitored over 72 h, while X-ray fluorescence spectroscopy (XRF) quantified the amount of glyphosate and Mn found in the solution and in the precipitates. Chemical speciation analysis using X-ray absorption near edge structure (XANES) determined the Mn chemical environment for both supernatants and precipitates. The chemistry of MnSO_4 + glyphosate supernatants at different pH was evaluated through XANES. Thermogravimetric analysis revealed the composition of precipitates at pH 7, and zeta potential and dynamic light scattering (DLS) analysis showed the charge and diameter of the precipitates' particles in a varying pH.

2 Materials and methods

2.1 Reagents and experimental design

The following Mn sources were employed: $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (31.85 wt.% Mn, Synth, Brazil), MnCO_3 nanopowder 80–100 nm (47.78 wt.% Mn, Nanoshel LLC, USA),

Mn-ethylenediaminetetraacetic acid (Mn-EDTA) (13 wt.% Mn, Stoller, Brazil), Mn-phosphite (MnHPO_3) (8 wt.% Mn, Agrivalle, Brazil), and Mn-glycine (22 wt.% Mn, Stoller, Brazil). The employed Mn-EDTA, Mn-phosphite and Mn-glycine are commercial products, while the MnSO_4 and MnCO_3 are lab reagents. The glyphosate employed in this study was also a commercial product ($\text{C}_3\text{H}_8\text{NO}_5\text{P}$) (48 wt.% active ingredient, 36 wt.% acid equivalent, and 68.5 wt.% of inert ingredients), obtained from Ameribrás (Brazil). It worth mentioning that the real composition regarding the inert ingredients from the glyphosate source is not known. Generally, it is composed by surfactants, diluents or adjuvants which stabilize the formulation and improve the product penetration within the plants, but the real composition is considered confidential business information [14].

The experiments were carried out in triplicate using the five Mn sources, with and without glyphosate.

2.2 Solution and dispersion preparation

The solutions and dispersions containing Mn, with and without glyphosate, were prepared based on the recommended field doses of 350 g ha^{-1} of Mn [15] and 1.5 L ha^{-1} of glyphosate. Considering the application of 50 L ha^{-1} of the spray mix, the employed concentrations were 7 g L^{-1} of Mn and 30 mL L^{-1} of glyphosate (10.8 g L^{-1} acid equivalent). It corresponds to a Mn:glyphosate molar ratio of nearly 2:1. All measurements (pH, electric conductivity, Mn fractionation, thermal analysis, pH assay, and XAS) were carried out in three repetitions.

2.3 pH and electric conductivity measurements

The Mn solutions/dispersion (25 mL) were transferred to 50 mL plastic vials, as the pH and electric conductivity (EC) were measured using InLab[®] Expert Pro-ISM and InLab[®] 731-ISM sensors, respectively, coupled to the SG-23 SevenGo DuoTM equipment (Mettler-Toledo, Switzerland). Subsequently, glyphosate (750 μL) was added in the solution/dispersion and fully homogenized by manual stirring. Immediately after, the pH and EC were measured. From this time on, the plastic vials were closed and left undisturbed, under room temperature and dark. pH and EC measurements were carried out afterwards every 4 h in the first 24 h, with an extra measurement made around 72 h after the glyphosate addition (Figure S1). This time range covers field conditions, the first 24 h being the most critical period, as factually spraying solutions hardly exceeds this time limit.

2.4 Mn fractionation

The Mn fractionation was determined based on Almeida et al. [16], with modifications. The fertilizers solutions and dispersions, with and without glyphosate, were prepared following the sample preparation described above (Sect. 2.2). Aliquots (7 μL) of the solutions/dispersions were sampled right after the mixtures, then the samples were centrifuged for 2 h at 1464 g, in order to separate any precipitate from supernatants, being the supernatants also sampled (7 μL).

The 7 μL aliquots were transferred into 1 mL plastic vials, the surfactant Triton X-100 solution at 5% (v/v) (5 μL), purified water (938 μL), and 1003 mg Ga L⁻¹ internal standard (50 μL) were subsequently added.

The insoluble phase of the precipitated treatments was collected and dried in a laboratory oven (60 °C) until reach constant mass. The precipitates were weighed, ground manually using an agate mortar, and microwave-assisted acid digested. For the latter procedure, the precipitates (20 mg) were transferred into pre-cleaned Teflon TFM tubes, mixed with HNO₃ 20% (v/v) (5 mL) and H₂O₂ 30% (v/v) (2 mL). So, the samples placed in the microwave oven (DGT 100 Plus, Provecto Analítica, Brazil) followed a heating treatment of 7 min at 400 W, 15 min at 850 W and 7 min at 320 W. The final solution was transferred to 50 mL plastic vials and diluted up to 40 mL with purified water. Finally, an aliquot of the solutions (330 μL) was transferred to 1 mL plastic vial, and the surfactant Triton X-100 solution at 5% (v/v) (5 μL), purified water (615 μL), and 1003 mg Ga L⁻¹ internal standard (50 μL) were subsequently added.

The concentration of Mn, P and S in the pristine solutions and dispersions containing Mn, with and without glyphosate, supernatants and in the precipitate (after microwave-assisted digestion) were determined by energy dispersive X-ray fluorescence spectrometer (EDXRF, Shimadzu, EDX-720, Japan). Therefore, 10 μL of each prepared sample were pipetted on the external side of the 31 mm diameter poly(ethylene) XRF cuvette (cat. no. 1530, Chemplex, USA) covered with 6 μm thick polypropylene film (VHG Labs, USA). Then, the samples were dried in laboratory oven (60 °C).

The EDXRF was operated using a Rh X-ray tube at 50 kV and applying an auto-tunable current to a 30% maximum dead time. The analysis was carried out under vacuum and using a 10-mm diameter collimator (Shimadzu, Japan) for 200 s. The X-ray spectrum was acquired by a Si(Li) detector. The quantification was carried out using external calibration (Figure S2).

Limits of detection (LOD) and quantification (LOQ) were determined as stated by Almeida et al. [16] and Kadachi and Al-Eshaikh [17], respectively, following the Eqs. (1) and (2):

$$LOD = \frac{3}{I_{Ga} * S} \sqrt{\frac{BG}{t}} \quad (1)$$

$$LOQ = LOD \times 3.33 \quad (2)$$

where LOD, LOQ, S, I_{Ga}, BG and t are the limit of detection (mg L⁻¹), limit of quantification (mg L⁻¹) relative elemental sensitivity (mg⁻¹ L), Ga intensity (cps), background intensity (cps) of the analyte, and the acquisition time (s).

2.5 X-ray absorption near edge structure (XANES) characterization

XANES measurements were carried out in liquid and solid phases formed before and after the addition of glyphosate into the Mn solutions/dispersion. Glyphosate was added to the liquid samples ca. 2.5 h prior to the analysis, fitting to field conditions. Solid samples preparation was done according to Gomes et al. [18] with modifications. From solid samples (precipitates and MnCO₃) previously dried in a laboratory oven at 60 °C and diluted in cellulose at 1.5 Mn wt.%, were then prepared 100 mg pellets using a 13 mm stainless steel die set pressed at 500 kg for 1 min (Carver 3912, USA).

The liquid samples were placed in acrylic 1 mm thick sample holders covered with Kapton tape (Figure S3A). Solid ones were fixed in sample holders using Kapton tape (Figure S3B).

The measurements were performed in transmission mode at the XRF beamline of the Brazilian Synchrotron Light Laboratory (LNLS). The XRF beamline is equipped with a bending-magnet, Si (111) double crystal monochromator, KB mirror system resulting in a 20 μm diameter spot size. The detection was made using ionization chambers before and after the sample. The 20 μm beam was positioned at the center of the samples and the XANES spectra recorded between - 100 and 200 eV across the Mn-K edge. Three scans per sample were collected and merged to improve the signal-to-noise ratio, each measurement being replicated three times.

The spectra were merged, energy-calibrated using a Mn-foil, and then normalized using Athena software within the IFEFFIT package [19].

2.6 pH assay: chemical speciation of MnSO₄ + glyphosate solutions at different pH

MnSO₄ solutions (7 g L⁻¹ of Mn) were mixed with glyphosate (10.8 g L⁻¹ of acid equivalent) and their pH adjusted to 1, 3, 5 and 7, adding H₂SO₄ or NaOH. The pH was measured using the pH meter Tec-2 (Tecnal, Brazil). Subsequently, these solutions were centrifuged (1464 g) for 2 h, and the

supernatants were separated from the precipitates using Pasteur pipettes.

Chemical speciation analysis of the supernatants was conducted in transmission mode also at the previously cited XRF beamline of the Brazilian Synchrotron Light Laboratory (LNLS).

2.7 Thermal analysis (gravimetric—TGA and differential—DrTGA)

Thermal analysis was made on the precipitates formed after increasing the pH of MnSO_4 solutions with and without glyphosate. To prepare the samples, MnSO_4 was dissolved in purified water at the concentration of 7 g L^{-1} of Mn. The pH, initially approximately 4.4, was adjusted to 7 using NaOH solution. The titration was made under stirring by a magnetic mixer. A precipitate of a brownish compound was noticed due to this pH increase.

For MnSO_4 + glyphosate samples, glyphosate (10.8 g L^{-1} of acid equivalent) was added to the MnSO_4 solution at pH 7. The glyphosate addition causing a pH decrease, and NaOH was employed to readjust the pH to 7. In this case, a lighter brownish compound precipitate was observed.

The supernatants were properly discarded after centrifuging the samples at 1464 g for 1 h. The precipitates were placed into the laboratory oven ($50 \text{ }^\circ\text{C}$) until reach constant mass. After drying, the precipitates were ground manually using an agate mortar, weighted, before a thermal analysis. Each treatment was prepared through three independent experiments.

The thermal analysis was performed by a Shimadzu DTG-60H equipment-simultaneous TG-DrTGA, from room temperature till $1100 \text{ }^\circ\text{C}$ under N_2 atmosphere and heating rate of $10 \text{ }^\circ\text{C min}^{-1}$.

2.8 Dynamic light scattering analysis (DLS) and microelectrophoresis (zeta potential) analysis

The DLS and zeta potential analysis were performed in MnSO_4 + glyphosate solutions under several pH values. A MnSO_4 stock solution was prepared at the concentration of 7 g L^{-1} of Mn, which presented an initial pH of *ca.* 4.4, then 10 mL aliquots of the stock solutions were separated for the pH adjustments to 5 or 7 using NaOH solution. Later, 300 μL of glyphosate were added to the aliquots, aiming a final glyphosate concentration of 10.8 g L^{-1} (acid equivalent). Then, the pH was readjusted to 5 or 7. A treatment with no pH adjustment was also completed, which presented a pH of 2.5 after the glyphosate addition. The Mn and glyphosate concentrations used here fit to field applications [15].

The DLS and zeta potential analysis were performed using a ZetaSizer Nano ZS90 particle analyzer (Malvern Instruments). Samples at pH 2.5 and 5 had to be diluted 10 and 20-fold, respectively, due to their light scattering properties, while samples at pH 7 did not require any dilution. Three repetitions of each treatment were evaluated.

3 Results and discussion

3.1 Monitoring the pH and electric conductivity

The pH and electric conductivity (EC) of the tank mixtures were monitored for *ca.* 72 h. Glyphosate addition promoted changes in pH and EC of solution/dispersion for all Mn sources, especially during the first 5 h after its addition (Fig. 1). Past 5 h, the EC remained constant for all mixtures; however, for MnSO_4 and Mn–glycine, the pH continued to decrease until the last measurement, while it increased from 24 to 72 h after the glyphosate addition for MnCO_3 (Fig. 1a, b). As a general trend, the electrical conductivity and pH were inversely proportional. It is worth mentioning that both MnSO_4 and Mn–glycine presented a white precipitate after mixing with glyphosate, while MnCO_3 precipitated regardless glyphosate addition, since it is a low solubility source.

Following dissolution in water, $\text{MnSO}_{4(\text{aq})}$ forms an outer-sphere complex $[\text{Mn}(\text{H}_2\text{O})_6]^{2+}\text{SO}_4^{2-}$ [12], while the solubility product constant (K_{sp}) for Mn–glyphosate solution buffered at pH 7 is 0.955×10^{-6} [20]. The reduction in pH followed by the EC increasing is likely related to the correlation between conductivity and availability of H^+ ions in solution. The pH decreased more intensively for MnSO_4 (from 4.6 to 2.6) and Mn–glycine (from 5.5 to 3.0). This was probably due to the deprotonation of glyphosate molecules, i.e. providing H^+ ions to the solution, which also released adsorption sites for Mn^{2+} complexation.

Chahal et al. [21] also investigated the influence of glyphosate addition to a Mn solution using a commercial source based on MnSO_4 (Nutrisol 8% Mn, Coastal Agro-Business). The concentrations were *ca.* 0.8 g L^{-1} of Mn and 6.7 g L^{-1} of glyphosate. Differently from our data, they found an initial pH of 3 for the Mn solution, which is more acid than ours, and glyphosate raised the pH to 4.2 right after its addition, then remained steady until the 72 h elapsed. Conversely to our observations, these authors did not observe any precipitate formation.

The pH of the tank mixtures is considering an important parameter to assure the efficiency of the involved compounds. Machado et al. [22] reported severe epidermal cell shrinking due to the application of very acid Mn solutions (pH 1.4) on soybeans leaves, but further studies are still necessary to unravel the effects of the solution pH on foliar

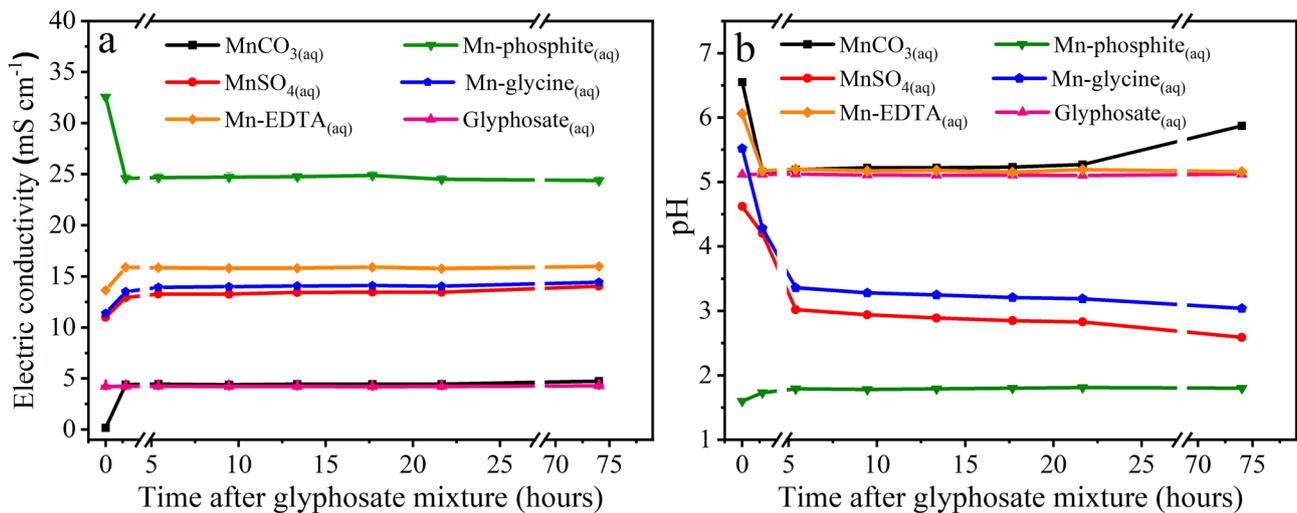


Fig. 1 Electric conductivity (a) and pH (b) of Mn aqueous solutions/dispersion as function of time after glyphosate mixture. The data shows that glyphosate increased the electric conductivity (EC) of

the solutions/dispersion, except for Mn-phosphite. On the other hand, the dispersion/solutions' pH decreased with the glyphosate addition, except for Mn-phosphite

fertilization efficiency. On the other hand, the influence of the pH on glyphosate's efficiency was better investigated. Previous studies had demonstrated that glyphosate's activity is higher in acidic pH than that in alkaline [23–25]. Shea and Tupy [23] observed that the increase of the glyphosate solution pH from 4 to 5.5 was sufficient to reduce its efficiency on weed control, but no significant difference was resultant due to the adjustment of the pH from 5.5 to 6 or 10.

3.2 Mn fractionation

Values above the LOQ (4.6×10^{-7} , 5.8×10^{-7} , and 4.2×10^{-7} mol for Mn, P and S, respectively) were considered to determine the Mn, P and S content in the obtained fractions (solutions, dispersions, supernatants, and precipitates). For $\text{MnSO}_{4(aq)}$ (Fig. 2a) and $\text{Mn-glycine}_{(aq)}$ (Fig. 2c) without glyphosate, no significant changes were observed for pH, EC, Mn and S content before and after the centrifugation process.

Most of the Mn and P provided by the mixture of $\text{MnSO}_{4(aq)}$ and glyphosate remained in solution (Table S1), however, nearly 29% of the Mn precipitated along with ca. 28% of the P. A ca. 2:1 Mn:P molar ratio was found in the MnSO_4 precipitate (Table S2). Regarding the Mn-glycine , one should expect that the Mn complexation by glycine could ward off some interactions with glyphosate; however, the addition of glyphosate to the Mn-glycine solution caused the precipitation of ca. 43% of the employed Mn, forming a precipitate with a Mn:P molar ratio of ca. 3:1 (Table S2), indicating that the stability constant of glyphosate is higher than that of glycine.

The addition of glyphosate in both sulfur-containing sources (MnSO_4 and Mn-glycine) did not change the S content in the solution, revealing that the SO_4^{2-} does not precipitate along with glyphosate molecules (Fig. 2b, d). It is valuable to emphasize that glycine ($\text{C}_2\text{H}_5\text{NO}_2$) does not contain sulfur, even though this element was detected in the mixture. It suggests that the Mn-glycine fertilizer was prepared through an attempt to complex Mn^{2+} with glycine displacing the SO_4^{2-} , not completely purified.

The precipitation suggests that both MnSO_4 and Mn-glycine can lose efficiency on foliar fertilization. Thereon, minimal research is available about Mn-glycine as fertilizer. However, the MnSO_4 performance appeared negatively affected by tank mixtures with glyphosate [22]. Likewise, the herbicidal action of glyphosate may also be affected due to precipitation. Bernards et al. [12], by tracing the glyphosate movement within *Abutilon theophrasti* leaves (velvetleaf) using ^{14}C radiolabeled glyphosate, verified that the absorption of glyphosate by the plant was reduced in the presence of Mn-ethylaminoacetate (Mn:glyphosate ca. 5:1), Mn-lignin sulfonate (Mn:glyphosate ca. 6:1), and MnSO_4 (Mn:glyphosate ca. 25:1), while Mn-EDTA (Mn:glyphosate ca. 7:1) was the least likely to diminish glyphosate absorption, translocation and efficacy.

$\text{Mn-EDTA}_{(aq)}$ presented stable Mn and P contents before and after the centrifugation, regardless of the glyphosate mixture. Although the glyphosate changed the pH and EC of the Mn-EDTA solution, no precipitate was formed. The Mn^{2+} stability constant of EDTA, $\log K = 13.81$, being much

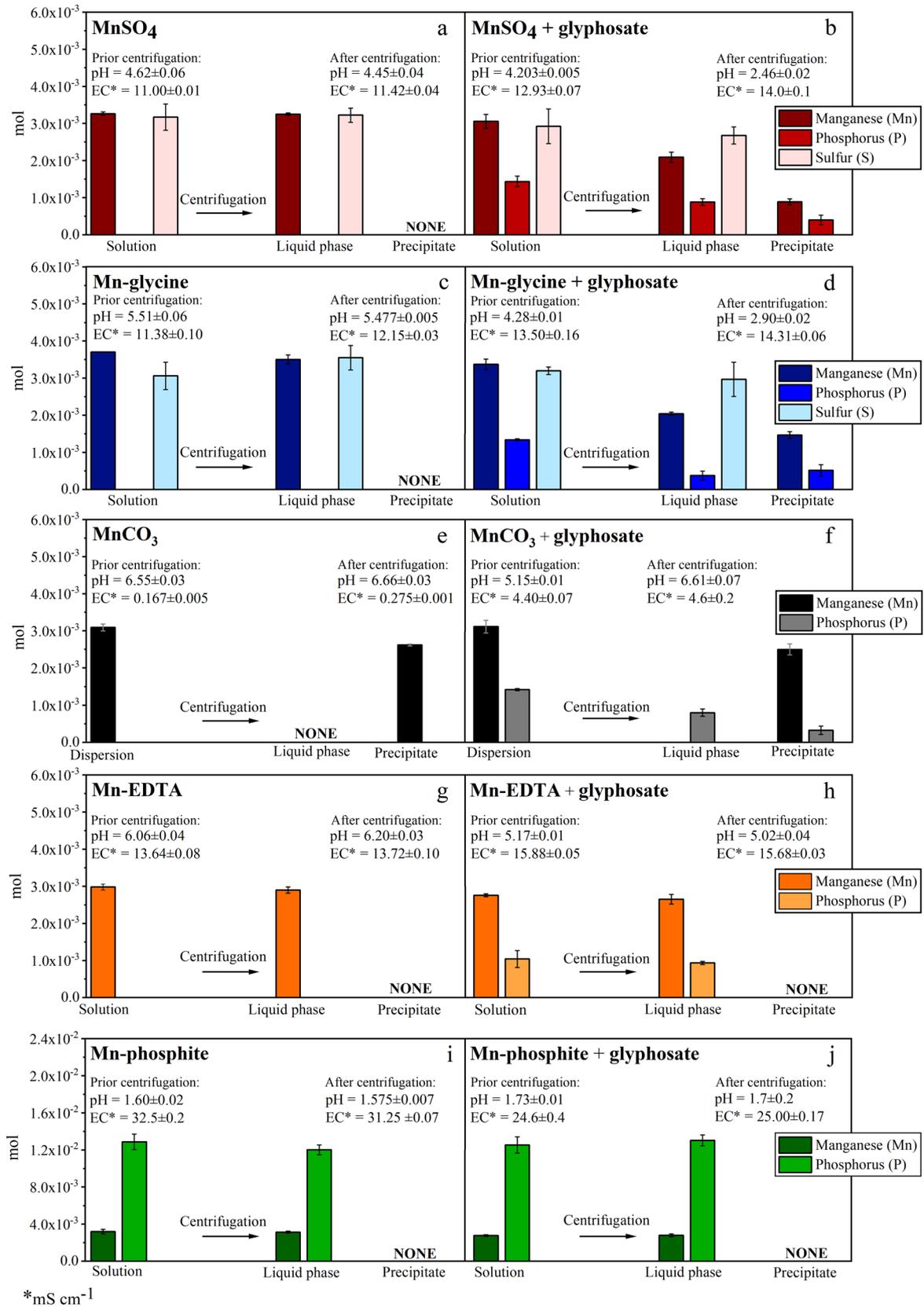


Fig. 2 Fractions formed by Mn-containing fertilizers before and after mixing with glyphosate, with their respective Mn, P and S content (when present). MnSO_4 (a); MnSO_4 +glyphosate (b); Mn-glycine (c); Mn-glycine+glyphosate (d); MnCO_3 (e); MnCO_3 +glyphosate (f); Mn-EDTA (g); Mn-EDTA+glyphosate (h); Mn-phosphite (i); Mn-phosphite+glyphosate (j). Only values above LOQ (4.6×10^{-7} , 5.8×10^{-7} , and 4.2×10^{-7} mol for Mn, P and S, respectively) were considered

higher than that of glyphosate, $\log K=5.53$, [26] prevents Mn complexing with glyphosate (Fig. 2h).

Several studies highlighted that Mn-EDTA did not antagonize glyphosate efficacy [11, 12, 27], on the contrary, it can even increase its herbicidal action [12, 27]. The EDTA can chelate the cations present in tap water, normally used to prepare spray mixture, hence it prevents the formation of metal-glyphosate complexes. From the plant nutrition standing point, the use of in vivo X-ray spectrometry revealed that Mn foliar absorption and transport kinetics were not affected by the mixture of Mn-EDTA with glyphosate as well [22].

The MnCO_3 (80–100 nm) precipitated regardless of glyphosate addition (Fig. 2e, f). The Mn content remaining in the supernatant was lower than the established limit of quantification. When glyphosate was added ca. 23% of the total P precipitated with 81% of the total Mn applied (Table S2). EC and pH increased after the centrifugation of the MnCO_3 dispersion (Fig. 2e). Then, when glyphosate was added up, the pH decreased while the EC increased. After centrifugation, the pH raised back to a value near to the one observed in the absence of glyphosate, while the EC increased only 0.2 mS cm^{-1} (Fig. 2f).

Based on the behavior of the MnCO_3 dispersion, one can notice that the MnCO_3 source employed in this study showed itself as an inadequate source for foliar fertilization, regardless of glyphosate mixture. Since it precipitated in its entirety, no Mn remained available in solution for foliar uptake. Accordingly to this statement, Migliavacca [28] found that foliar application of MnCO_3 did not circumvent Mn deficiency in soybean plants. Likewise, Machado et al. [22] found that MnCO_3 was not able to increase the Mn content in soybean petioles 48 h after leaf spraying, regardless of glyphosate mixture.

Regarding to the Mn-phosphite_(aq) solution, the addition of glyphosate slightly increased the pH and decreased the EC. This is related to the acidic behavior of phosphite compounds. Besides, Mn-phosphite also presents high P content (P concentration ca. fourfold higher than Mn). Therefore, P content was not significantly changed by the glyphosate mixture. It was not observed any formation of precipitates after the addition of glyphosate to the Mn-phosphite_(aq) solution, since at such low pH (ca. 1.7), the glyphosate molecules remain protonated that ultimately provide few Mn^{2+} adsorption sites.

Considering the absence of visual interactions, such as precipitate formation, one should expect none negative consequences on the mixture between glyphosate and Mn-phosphite_(aq). However, attention must be paid regarding the pH of the solution. Actually, on the one hand, such acid pH should not disturb glyphosate efficacy on weed control, once its activity increases as pH decreases [23]. On the other hand, it can be harmful to the main crop. Machado et al. [22] showed that Mn-phosphite solutions, with and without glyphosate (pH 1.8 and 1.6, respectively), caused severe epidermal cell shrinking on soybean leaves. Additionally, it was also shown that glyphosate reduces the Mn leaf uptake from Mn-phosphite.

Among other factors, impairment of micronutrient root to shoot transport was also reported due to glyphosate, though the mechanisms behind were not elucidated. Thus, it has been demonstrated that foliar applied glyphosate can be translocated and further exudate by roots [29], potentially precipitating Mn in soil or nutrient solution. Moreover, Duke et al. [30] listed several studies showing no restriction of micronutrients availability for GR crops treated with glyphosate.

3.3 Chemical speciation

Figure 3a presents the spectra for pristine Mn fertilizers and nanopowder without glyphosate, while (b) shows the spectra for the supernatants obtained after glyphosate addition and centrifugation, and (c) the spectra for recovered precipitates. The pristine solutions of Mn-glycine, MnSO_4 and Mn-phosphite present similar spectra, while Mn-EDTA and MnCO_3 demonstrate features that clearly differentiate them from the others.

Although the stoichiometry let appearing that part of the glyphosate remained in liquid phase, the spectra for the supernatants did not present remarkable spectral changes to support a glyphosate reaction with Mn^{2+} that would yield an inner-sphere Mn-glyphosate complex (Figure S4). This would be consistent with electronic paramagnetic resonance (EPR) measurements of Mn^{2+} +glyphosate performed by Bernards et al. [12] in a similar pH condition. On the other hand, likewise in Fig. 3b, these overlapped spectra clearly show similarities between the chemical environment of the suspended Mn-glycine, MnSO_4 and Mn-phosphite.

Machado et al. [22] obtained the Mn chemical speciation in the petioles of soybean plants which were foliar fertilized with MnSO_4 , Mn-EDTA and Mn-phosphite, mixed or not with glyphosate. The acquired Mn XANES spectra found no Mn-glyphosate complexes inside the plants regardless of the applied Mn source. While MnSO_4 and Mn-phosphite provided Mn for plants in a similar chemical

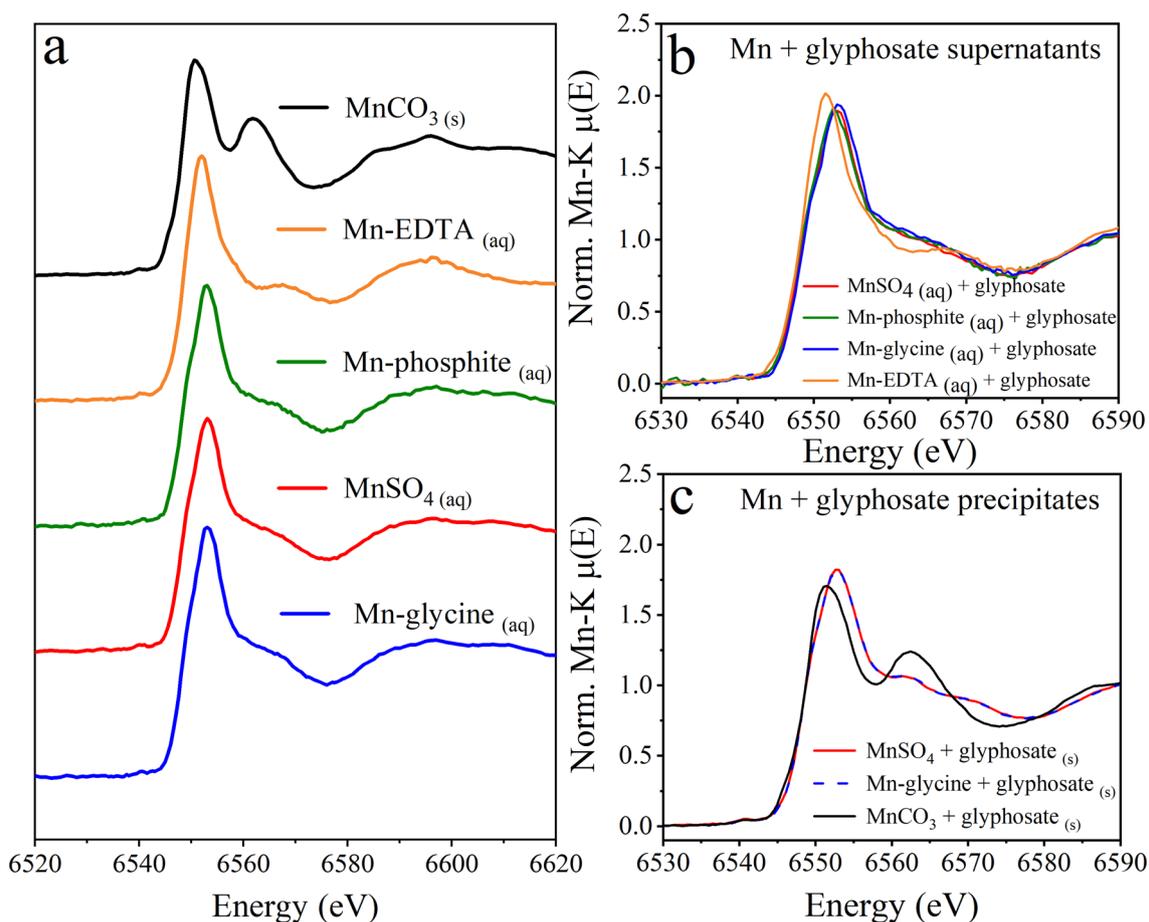


Fig. 3 XANES spectra for the Mn sources in aqueous solution and solid MnCO₃ (a), liquid phase (supernatant) (b) and solid phase (precipitated) (c) obtained after mixing glyphosate to the Mn

sources. Data shows that the Mn chemical environment of the liquid phase after glyphosate mixture are different, however, it is the same for the precipitates coming from Mn-glycine and MnSO₄

environment, the Mn-EDTA was still found in its pristine form, showing that the bonding force between Mn and EDTA is powerful enough to keep the Mn chelated all over the way from the leaf to the petiole.

Despite the reactions between aqueous Mn²⁺ and glyphosate within tank mixtures, the above-mentioned results [22] show that Mn remaining in solution is being absorbed and transported in a non-complexed form. These data, together with Bernards et al. [12], also support the rejection of the soluble complexes formation hypothesis, since they indicate that glyphosate does not interfere with the Mn chemical environment inside the plants, and that both Mn and glyphosate remaining in solution can accomplish their physiological impacts. It seems that the major cause for the loss of efficiency observed when Mn²⁺ and glyphosate are mixed, is due to the relative amount of both species available in solution.

The MnSO₄ and Mn-glycine formed similar precipitated products with glyphosate (Fig. 3c). Their spectra presented a slightly reduction of whiteline intensity compared to

the pristine and supernatant forms (see also Figure S4). The reduction of whiteline intensity is associated to the increase of electron density on p unoccupied states of Mn²⁺ ions, while edge shifts towards lower energies can be caused by increase of Mn-ligand bond length [31]. This corroborates the chemical affinity between Mn-glyphosate which yields a more stable molecule than MnSO₄ and Mn-glycine.

As previously reported, glyphosate can form low soluble complexes with Ca²⁺ which cannot be absorbed by the plants leaves [32]. The MnSO₄ + glyphosate precipitate presenting clear spectral differences with soluble Mn (Figure S4) support that such changes in the Mn chemical environment may also make the Mn-glyphosate complexes unavailable for plant uptake. This precipitates formation is a serious practical problem, since the formed particles can clog the sprayer nozzles during field application, which compromises the operation efficiency.

Figure 4 shows the XANES spectra recorded for Mn-glyphosate precipitate, Mn₃(PO₄)_{2(s)}, Mn-malate_(aq)

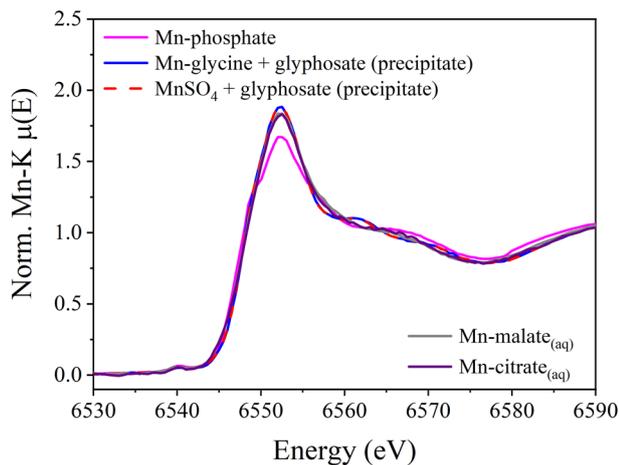


Fig. 4 XANES spectra recorded for Mn-phosphate(s), Mn-malate_(aq), Mn-citrate_(aq) and two Mn-glyphosate precipitates, one obtained from MnSO₄+glyphosate and the other from Mn-glycine+Glyphosate. Both Mn-glyphosate precipitates formed the same compound, which is different from Mn-phosphate, Mn-malate_(aq) and Mn-citrate_(aq). In aqueous solution, Mn-malate and Mn-citrate present a similar spectrum between them, while Mn-phosphate differs from all the overlapped spectra in this image

and Mn-citrate_(aq). The XANES spectra are highly affected by symmetry and oxidation state. Although the precipitate retains Mn²⁺, its structure is much closer to carboxyl complexed Mn²⁺ (Mn-malate and Mn-citrate) than Mn₃(PO₄)_{2(s)}. Subramaniam and Hoggard [33] prepared non-soluble glyphosate complexes with Cu, Ni, and Fe. The crystalline nature of the formed compounds was solved by X-ray diffraction. Infrared Fourier transform evidenced bulk glyphosate and solid complexes changes on several bands which, in principle, would suggest that amine, carboxyl and phosphonate groups participate of Mn²⁺ binding.

Nowack and Stone [34] reported that Mn²⁺ quickly decomposed nitrilotris(methylene)phosphonic acid releasing orthophosphate ions, imino(dimethylene) phosphonic acid, and *N*-formylimino-(dimethylene) phosphonic acid. Conversely, Barrett and McBride [35] reported that Mn³⁺ cleavage on glyphosate produced glycine and orthophosphate, while this did not happen for Mn²⁺ at 1:1 Mn:glyphosate ratio. Otherwise, increasing Mn²⁺:glyphosate proportion to 4:1 lead to a breakdown of glyphosate molecules. The present survey employed a 2:1 Mn:glyphosate molar ratio. Even if the orthophosphate had not been determined in solution, the XANES spectra recorded for the precipitates (Fig. 4) do not suggest formation of Mn₃(PO₄)₂ which would have happened case the glyphosate would have been decomposed by Mn²⁺.

Barrett and McBride [35] reported that less than 1% of Mn²⁺ should be complexed to glyphosate at pH 5 and it should increase to *ca.* 10% at pH 7. They estimate these figures based on Motekaitis and Martel [36]. Incidentally,

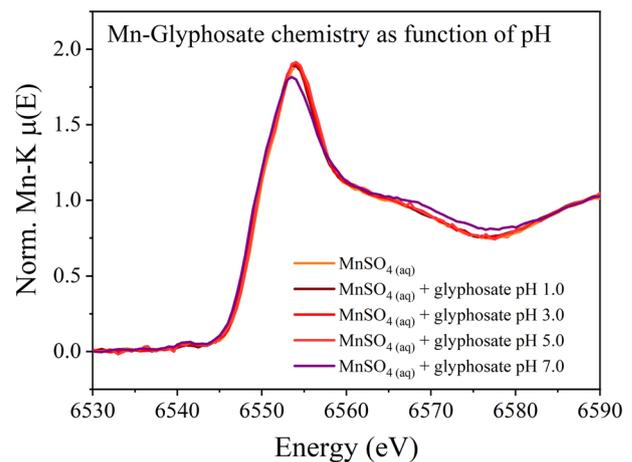


Fig. 5 XANES spectra recorded from MnSO_{4(aq)}+glyphosate supernatants at pH ranging from 1 to 7. Data show different spectral features only at the pH 7, while the other pH conditions offer the same one Mn chemical environment

our experiments showed that *ca.* 29% of Mn²⁺ precipitate at pH 2.5. A possible explanation for the deviation from theory observed in our study might be related to the concentration of ligands and Mn²⁺. Since our study mimicked a field application tank mixture, the concentration of reactive chemical species, i.e. Mn²⁺ and glyphosate, was nearly 60-fold higher than that employed by Motekaitis and Martel [36].

3.4 Mn-glyphosate chemistry as function of pH

Among the fertilizers tested in this study, manganese sulphate is the most common, cheap and glyphosate-reactive source of Mn. Therefore, the chemistry of MnSO₄+glyphosate solutions as function of pH prevailed.

Glyphosate has four pKa values: < 2, 2.6, 5.6, and 10.6 [37]. From pH 3–5 both carboxyl and phosphite groups are ionized. In such condition, an equilibrium between aqueous and precipitate Mn was observed. Precipitation immediately occurred for samples at pH 3 and 5, and to a larger extent at pH 5. At pH 7, glyphosate net charge was 2- and negligible brownish precipitate was found. Zeta potential values for samples at pH 2.5, 5 and 7 were close to neutrality, which means the suspended particles were within the micrometric size range (Table S3). The hydrodynamic diameter and zeta potential decreased as the pH increased.

Figure 5 shows the XANES spectra recorded for liquid phase after centrifugation of MnSO₄-glyphosate mixture. The non-normalized XANES spectra for the MnSO₄+glyphosate solutions in ranging pH can be found in the supporting information (Figure S5). Under pH 1–5, the Mn²⁺ remains in the same structure; only a

slight whiteline intensity reduction was observed for pH 7. These outputs are in agreement with Bernardis et al. [12] who outlined identical EPR spectra for $\text{MnSO}_{4(\text{aq})}$ and $\text{MnSO}_{4(\text{aq})}$ + glyphosate in pH range of 2.8–4.5. Under pH 6–7.5 they reported reduction on EPR signal amplitude which would be associated with the displacement of water and coordination by the glyphosate. The values of attenuation coefficient (Figure S5) that reflects the concentration of Mn in solution, are consistent with the observed precipitation yield in Figure S6, i.e. most of the Mn–glyphosate precipitated at pH = 5. The brownish suspended particles were not detected by XANES since they were precipitated during the centrifugation.

No Mn^{2+} redox state change appeared in the XANES spectrum recorded for the supernatant in pH 7. However, a significant weight loss between 350 and 400 °C was noted in the thermogravimetric analysis (Figure S7) which indicates the occurrence of dihydroxylation and the loss of SO_4 between ca. 730 and 1060 °C. This is consistent with Figueira et al. [38] that reported 10 wt.% weight loss between 350 and 400 °C for manganite ($\gamma\text{-MnOOH}$), with an endothermic peak around 370 °C related to the oxidation of $\gamma\text{-MnOOH}$ and $\beta\text{-MnO}_2$ (pyrolusite) formation. Hem [39] also obtained brown or black precipitates when Mn solutions were adjusted to pH higher than 8, due to the formation of oxides, since such pH and oxidation–reduction potential (Eh) conditioned the oxide-stability region. Hem [39] also states that oxidation by air may occur at pH conditions above 8, which decreases the concentration of dissolved Mn in solution. The thermogravimetric curves obtained from the precipitate at pH 7 (Figure S8) do not assert the structure of the Mn–glyphosate as solid complex.

Before ending the discussion on glyphosate and Mn interactions, it is worth mentioning that there are several kinds of glyphosate formulations available. Some of them present distinct active ingredients, like isopropylamine salt of glyphosate (glyphosate–IPA) or potassium salt of glyphosate (glyphosate–K), for example. Others present the same active ingredient, but with distinct formulants employed for the product stabilization. The formulants are often composed by surfactants, diluents or adjuvants, but companies do not need to declare their composition, this information being considered confidential business [14]. The glyphosate formulation employed in this study is a glyphosate–IPA based, with 48 wt.% of active ingredient, 36 wt.% of acid equivalent and 68.5 wt.% of formulants. The formulants are considered inert ingredients according to the products label. Recent studies found that some non-declared formulants of glyphosate based herbicides can be even more toxic than glyphosate itself [14, 40]. Given the unknown chemistry of the formulants, attention must

be paid to the fact that different glyphosate brands may present distinct performances in tank mixtures.

4 Conclusions

This study demonstrates the importance of the Mn source and the solutions pH when preparing spray mixtures with glyphosate. Indeed, glyphosate can form low solubility complexes with free Mn^{2+} ions in a pH ranging from 2.5 to 5. Thus, complexes were found with a Mn:glyphosate molar ratio of nearly 2:1.

A similar chemical environment was found for the precipitates retrieved from the XANES analysis, regardless of the employed Mn source. Further studies using the X-ray diffraction and extended absorption fine structure shall be conducted aiming at unraveling the structure of Mn–glyphosate solids.

Our results did not support the hypothesis of a soluble inner sphere Mn–glyphosate complex under pH 1–7. For sake of clarity, it is important mentioning that due to intrinsic errors introduced during the normalization of the spectra, the XANES spectra unlikely detect a fraction of soluble complex below 5 wt.% of the total manganese. Hence, if present, Mn–glyphosate soluble complexes in tank mixtures would be below ca. 5 wt.% concentration range.

The use of chelated fertilizers, such as Mn–EDTA, or the decreasing of the pH of the tank mixture to values < 2.5 are alternatives to circumvent the formation of precipitates.

Acknowledgements The authors are grateful to the Brazilian Synchrotron Light Laboratory for the beamtimes under the proposals 20180150 and 20190117, and to the support from Dr. Carlos A. Perez (XRF beamline scientist). We are also grateful to the professor F.R.P. Rocha and Prof. C.R. Montes (USP-CENA) for the enriching discussions and TGA analysis, respectively. We thank Prof. L.F. Fraceto (UNESP) for DLS analysis.

Funding The authors are grateful to the São Paulo Research Foundation (FAPESP) in the framework of the Grant Nos. 2018/13401-7 and 2015/05942-0, and to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001, for the financial support. R. Otto is the recipient of a research productivity fellowship from the Brazilian National Council for Scientific and Technological Development (CNPq) (Grant No. 308007/2016-6).

Compliance with ethical standards

Conflict of interest The authors declare the following competing financial interest(s): In addition to resources obtained from Government agencies, this study was partially funded by Agrivalle Brasil Industria e Comercio de Produtos Agricolas LDTA do Brasil and Stoller do Brasil LDTA through the Fundacao de Estudos Agrarios Luiz de Queiroz. The results do not imply endorsement of or preference for any source of manganese listed by the authors.

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