# Glyphosate and AMPA concentrations in two types of agroecosystems and in the natural vegetation of Hopelchen, Mexico

Minor thesis report

Diana Pastrana Cervantes
(910226642130)
Plant Sciences - Breeding and Genetic Resources

**Supervisors:**

Dr. CWB (Christian) Bachem
Dr. E (Esperanza) Huerta Lwanga
Dr. X (Xiaomei) Yang

## Course information

| | |
|---|---|
| Name: | MSc Thesis Plant Breeding |
| Code: | PBR-80424 |
| Credits: | 24 credits |
| Submission date: | |


## Author

| | |
|---|---|
| Name: | Diana del Rosario Pastrana Cervantes |
| Registration number: | 910226642130 |
| Study program: | MSc Plant Sciences - Breeding and Genetic Resources |
| E-mail: | diana.pastranacervantes@wur.nl / diana.pastrana91@gmail.com |

## Supervisors

| | |
|---|---|
| Name: | Dr. CWB (Christian) Bachem |
| E-mail: | christian.bachem@wur.nl |
| Name: | Dr. E (Esperanza) Huerta Lwanga |
| E-mail: | esperanza.huertalwanga@wur.nl/ehuerta@ecosur.mx |
| Name: | Dr. X (Xiaomei) Yang |
| E-mail: | xiaomei.yang@wur.nl |

# Index

# 1. Introduction

The global population growth has stimulated the intensification of crop production and the use of pesticides. For most countries detailed information about pesticides is not available. In Mexico the use of pesticides during 2014 was estimated in 98 814 tons of insecticides (33%), herbicides (27%) and fungicides (40%) (FAOSTAT, 2017). The use of pesticides in Mexico lacks of the adequate monitoring strategies and regulatory schemes (Arellano and Rendon, 2016). The Official Catalogue of Pesticides has not been updated since 2004; it includes at least 30 pesticides that are banned in other countries due to their potential toxicity including glyphosate (COFEPRIS, 2017). Glyphosate (N-(phosphonomethyl) glycine) is the active component of the worldwide most heavily used herbicide (Benbrook, 2016). Glyphosate has been prohibited in Belgium, Denmark, France, Sri Lanka, Salvador and The Netherlands while other countries had chosen to restrict its use.

As a non-selective herbicide, glyphosate has the potential to affect a broad spectrum of plants (Krieger, 2001). Glyphosate is delivered on the plant leaves by spraying applications. The solution can reach the soil surface via spray-drift or after being washed off from the leaves (Kremer et al., 2009). Once absorbed in the foliar tissues, glyphosate will move through the phloem and translocate in the meristems (Franz and Sikorski, 1997). Additionally, the roots of treated plants may release glyphosate in the soil spreading the phytotoxic effect to non-target plants (Neumann et al., 2006). The mode of action is by disruption of the shikimate pathway (Steinrücken and Amrhein, 1980), a metabolic route for the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) which are precursors of vital plant products involved in growth, development, reproduction, defence, and environmental responses (Maeda and Dudareva, 2012).

Several of the chemicals present in pesticides may become soil contaminants particularly when the recommended doses are exceeded. The fate pathways of glyphosate in soil are mineralization, immobilization or leaching (Mamy et al. 2005). Degradation of glyphosate into its major metabolite aminomethylphosphonic acid (AMPA), is mainly carried out by microbial mechanisms (Kryuchkova et al., 2014). The half-life of glyphosate in soils ranges from few days up to two or three months (Andrea et al. 2003), after 90 days, it is expected that approximately 40-50% of the glyphosate will be degraded into AMPA (Gimsing et al., 2004). Extensive use of glyphosate may lead to AMPA accumulation in soils, as it is more persistent than glyphosate (Mamy et al. 2005).

The growing trend in glyphosate use has contributed to increase the release of pollutants to the environment and the exposure to glyphosate, AMPA and other additives present in commercial formulas (Benbrook, 2016). The potential impact of glyphosate on the soil biota include changes in microbial activities and populations (Dunfield and Germinda, 2004). Due to the moderate rate of degradation of glyphosate and its strong sorption with soil particles, the risk of leaching is often assumed to be low (Mamy et al., 2005). In the soil surface, these residues are transported to surrounding water bodies via run-off (Borggaard and Gimsing, 2008) or through macropore flow when present in the root zone (Laitinen et al., 2007). Glyphosate and AMPA are water soluble suggesting a persistence of several weeks in aquatic environments (Giesy et al., 2000).

The Yucatan Peninsula (YP) is located in south-eastern Mexico comprising the states of Yucatan, Campeche, and Quintana Roo. People in the rural communities still depending directly or indirectly on agriculture for their livelihood. The traditional agriculture has been facing a transition towards the expansion of the agro-industrial model which is characterized by the use of improved seeds, machinery and agrochemicals to maximize crop yields. In the municipality of Hopelchen the agro-industrial model has progressed more than in any other agricultural zone of Campeche (Ellis et al., 2015). Pesticides are used intensively in crops of maize, sorghum and tomato among others (Dominguez and Sabatino, 2005). Glyphosate proliferation has been emphasized by the implementation of transgenic crop species such as soybeans developed with specific resistance to this herbicide (Arellano and Rendon, 2016).

The soils of this region (karstic) are highly permeable, with a thin organic layer, allowing the infiltration of water into groundwater which is the main supply of freshwater in the YP. Current knowledge about glyphosate is insufficient and the lack of information is particularly notorious for the main source of glyphosate contamination in Campeche: the agricultural fields. In this context, it is relevant to investigate the concentration of glyphosate in soils and plants samples from agroecosystems with intensive management, those in transition and, in the adjacent natural vegetation. The aim of this research was to investigate the glyphosate and AMPA concentrations on soil samples and plants collected in the rain fed corn farms, technified soybeans systems and the natural vegetation in Hopelchen, Mexico.

## 2. Background information

### 2.1. Glyphosate

N-(phosphonomethyl) glycine is a derivate of the amino acid glycine. It is an odourless white crystalline solid that consist of one basic amino function and three ionisable acid sites. The herbicidal properties of glyphosate were discovered by Monsanto chemist John E. Franz in 1970 and four years later it was released in the marked under the name Roundup (Dill et al., 2010). Fast symptom liquid formulations increase the contact of the herbicide with the plant cells. These formulations may contain water-soluble salts or other derivatives of glyphosate and, surfactants as POEA (Polyoxyethyleneamine or polyethoxylated tallow amine) to facilitate the penetration of the leaves' waxy layers (Sihtmäe et al., 2003). In the developing countries, the application of more concentrated glyphosate-based products is a common practice. Continuous applications trigger the emergence of weed phenotypes with high resistance or low sensitivity to glyphosate. In order to deal with the persistent weeds, farmers increase the rate of glyphosate applications or they mix it with different herbicides (Mortensen at al., 2012; Heap, 2014).

The herbicidal activity is based on disruption of the shikimate pathway, this is achieved by inhibiting the enzyme 5-enolpyruvylshikimate 3-phosphate synthase (EPSP) (Steinrücken and Amrhein, 1980). In plants the shikimate pathway is responsible for the synthesis of the aromatic amino acids L-Tryptophan, L-phenylalanine, and L-tyrosine which are essential constituents of proteins leading to produce phytochemicals like pigments, alkaloids, hormones, and cell wall components (Maeda and Dudareva, 2012). Plants, fungi, algae and several microorganisms use this metabolic route but absent in animals so the essential aminoacids are obtained from the diet. After entering the plant cell, glyphosate is translocated in the phloem from the leaves to the apical meristems and root meristems (Shaner, 2009). At this point, glyphosate can be released through the roots and when it reaches non-target plants, inhibitory effects on the shikimate pathway, on uptake of micronutrients, and plant growth are expected (Neumann et al., 2006).

Soil bacteria and fungi play an important role on the degradation of glyphosate by means of two pathways, one synthetizing intermediates of sarcosine and glycine and the other leading to the formation of its major metabolite: aminomethylphosphonic acid (AMPA) (Kryuchkova et al., 2014). In further steps, AMPA is degraded into inorganic phosphate and methylamine ultimately forming simple carbon and nitrogen metabolites (Gimsing et al., 2004). The rate of this process increases with temperature and it varies in response to the soil type and the topographical features influencing water availability (Stenrod et al., 2006). In comparison to other pesticides, glyphosate present strong sorption characteristics, the molecules are sorbed by anion exchange processes in the soil minerals and trough hydrogen bonds in the organic matter (Veiga et al., 2001).

The assessment of herbicide eco-toxicity under field conditions is usually challenging, effects of glyphosate on the composition, diversity and biomass of the edaphic fauna seems to be dose-dependent but the response in the long term it is not well understood yet (Nguyen et al., 2016). The presence of glyphosate and AMPA in water sources have been frequently reported, the residues are transported from terrestrial to aquatic environments by leaching through the soil and by overland flow (Borggaard and Gimsing, 2008; Bai and Ogbourne, 2016). A relationship between the presence of the POEA in glyphosate-based formulas and the toxicity in the aquatic organisms has been suggested (Edginton et al., 2004; Moore et al., 2012). However, the toxicity levels are not exclusively mediated trough the presence of this surfactant, the effect of other additives in the composition of the particular formulation should also be considered (Sihtmäe et al., 2013).

In the classification of organophosphate pesticides published in 2015 by the IARC (International Agency for Research on Cancer), glyphosate was classified in the Group 2A (Guyton et al., 2004). This means that there is sufficient evidence of glyphosate carcinogenicity in animals to consider it "probably carcinogenic to humans". Oppositely, the European Food Safety Authority (EFSA) (2015) emitted a report indicating that glyphosate is unlikely to be carcinogenic and recommended to the IARC to perform a more extensive study of the relevant information. Clearly, there is no consensus about the effects of glyphosate on human health.

## 2.2. Problem description

In the state of Campeche, the progress of mechanized agriculture, livestock and urbanization have resulted in an annual deforestation rate of -0.74% from 1976 to 2005, higher than the national average of -0.43% for the period 2000-2010 (Esparza and Martinez, 2011). Furthermore, a wide variety of synthetic substances is used for pest control in the agricultural fields. Pesticides are defined as 'any substance, or mixture of substances of chemical or biological ingredients intended for repelling, destroying or controlling any pest, or regulating plant growth' (FAO and WHO, 2004). In 2010 Campeche had one of the highest rates of poisoning due to pesticide exposure in Mexico (Gutiérrez, 2013).

The introduction of transgenic soybeans in Campeche has promoted the expansion and intensification of glyphosate use nevertheless, it is important to mention that glyphosate is not exclusively applied in the soybean fields. Given the importance of apiculture in the regional economy, local beekeepers have hold their position against the indiscriminate use of pesticides and particularly against GMO crops. Since 2010 strong declines of the hives and acute intoxication were detected allegedly due to the contamination with pesticides (Gomez, 2016). Furthermore, pollen of the GMO plants was traced in the honey, situation that represent a disadvantaged position in the market of exportations. The local producers made a legitimate requested to prohibit the cultivation of soybean in Campeche and Yucatan, in November of 2015 Yucatan and The Supreme Court of Justice of the Nation annulled the concession granted to the Monsanto.

Arellano and Rendon (2016) collected samples of water from rivers, sea and lagoons of the YP that were analysed to determine the presence of pesticides. Glyphosate was detected in all samples, the highest concentration corresponds to samples collected in Yucatan followed by Champoton river and Terminos lagoon (both located in Campleche). Glyphosate was also found in the Caribbean Sea, these loads might have their origin in the golf courses from Quintana Roo. There is relevant evidence about the intensive use of pesticides in the YP and the situation of glyphosate in Campeche is emphasized given the fact that 30 years ago it was not reported in the list of the most widely used herbicides (Arellano and Rendon, 2016).

# 3. Materials and methods

## 3.1. Study area

This study was conducted in the Mennonite community 'Las flores' and in the 'Ejido Chenco', both located in Hopelchen. Hopelchen is one of the 11 municipalities that comprises the Mexican state of Campeche (18 ° 57 'and 20 ° 10' north latitude; 89 ° 24 'and 90 ° 06' west longitude) with altitude of 100-200 m.a.s.l. The averages of annual temperatures range from 26° to 28°C and rainfall fluctuate between 800 and 1000 mm. Most precipitation falls during September while March is the driest month. The dominant soil type in Hopelchen is leptosol (50.2%) and to a lesser amount vertisol and luvisol (INEGI, 2009). The climate of Hopelchen has been categorized as Aw (Tropical savannah climate or tropical wet and dry climate) by the Köppen-Geiger system (INEGI, 2014). The main type of vegetation is semi-deciduous tropical forest associated with shrub and tree secondary vegetation.

The local economy is based on agriculture, livestock and forestry (INEGI, 2016). According to data from INEGI (2014) the cultivated area in Campeche during 2013 accounted for 275 968 ha, with the most important crops being maize, sorghum and soybeans with 182 067, 17 817 and 15 704 ha respectively. From the total land dedicated to maize cultivation in Campeche, approximately the 34% correspond to Hopelchen (61 593 ha) with a production volume of 225 711 tons. In the case of soybeans, the municipality contributed with 6 851 ha representing the 43% of the total cultivated land in the Campeche for this crop and a production volume of 12 123 tonnes. All the maize and soy production in Hopelchen rely on rainfall. Beekeeping is another important component of the local economy. This activity is integrated to other local traditional agroecosystems that make use of the forest resources in the peripheries of the communities (Gomez, 2016). In 2002 a total of 2 296 tons of honey were produced in Hopelchen, in the subsequent years the production fell off and by 2011 beekeepers achieved only 480 tons (INEGI, 2016).

Until 2010, the municipality had a total of 37 777 inhabitants. Perez et al. (2015) characterized the rain fed corn farmers in the state of Campeche and concluded that most of them are of Mayan descent, their level of education is five years and often low income. Most farms are in transition with yields ranging from two to five tons per ha and an average cultivated area of 9 ha. The population in Hopelchen was predominantly Mayan until 1990 but by 2010 the 12% of the population was constituted by the Mennonite groups (INEGI 2016), they own or rent most of the mechanized land used for the production of soybeans (Gomez, 2016).

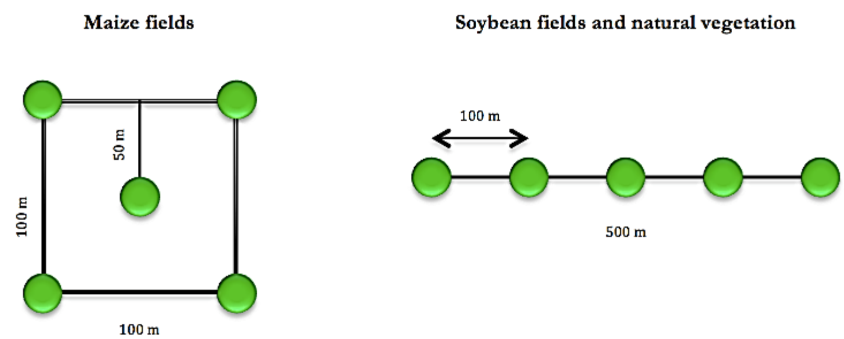
This study is part of a larger research project investigating the interaction between pesticides and the functional diversity of soil macroinvertebrates in agricultural sites of Campeche. In order to obtain samples from the two types of agroecosystems and the natural area, a total of 12 parcels were selected. The selection consisted of four representative parcels of a) the corn farms in Chenco, b) the soybean fields in the ejido Las Flores and c) the natural vegetation (consisting of the bordering vegetation from two parcels in both Chenco and Las Flores). The selection was based on the information obtained from prospective interviews in which seven owners per crop type have participated.

According to the respondents, all the parcels have been under mechanization for intervals ranging from 10 to 20 years. Dose and frequency of glyphosate applications is the same in both agroecosystems (3 times per crop cycle at dose of 3 l/ha). In the maize fields the product used is 'Diablosato' (Isopropylamine salt of N-(phosphonomethyl)-glycine) which has been provided by the government to support farmers. The owners of the soybean fields indicated that they use 'Faena' (potassium salt of N-(phosphonomethyl)-glycine), the product is acquired in agricultural input stores. The differences among crop types are related to the management practices, the intensity of pesticide use and the parcel size. For a constant parcel size, it has been chosen an extension of 2 Ha for maize and 30 Ha for soybean. The parcels included within each agroecosystem are similar with regard to the land use history, extension, soil type and surrounding natural vegetation.

## 3.2. Sampling methods

In each parcel, five monoliths of surface area 25 x 25 cm and 25 cm depth were obtained in accordance with the international method TSBF (Anderson and Ingram, 1993). Due to the characteristics of the fields, two spatial arrangements for the establishment of monoliths where chosen. In the soybeans fields and in the natural vegetation, samples were collected in 500 m transects at each 100m (Figure 1). The maize fields are relatively smaller and of irregular shape hence, monoliths were obtained in the corners and center of a frame with an area 100 x 100 m. In order to avoid the border effect, the sampling sites were carefully established near to the central part of the parcel and in the natural vegetation at a distance of 5 meters from the fields. Annex 1 contains the description and coordinates of the monoliths.

**Figure 1. Spatial arrangements for the establishment of monoliths.**




## 3.3. Sample collection

### a. Soil samples

The soil of each monolith was inspected for the presence of soil macroinvertebrates and samples were collected for further identification and quantification. Nevertheless, this data is not included in this report. A total of 60 samples were collected (five samples per parcel) for the determination of glyphosate and AMPA concentrations. Each sample consisted of 500 g of the inspected soils collected in a plastic bag and subsequently stored in a portable fridge.

### b. Plant samples

Leave samples were obtained from the closest plant to the monolith. As shown in figure 1, four plant samples were collected in each parcel (the samples corresponding to the last monolith were not included) hence, a total of 48 plant samples were obtained for glyphosate and AMPA determination. Young leaves were preferably selected and deposited in paper bags. In the parcels of Chenco and Las Flores all the samples correspond successively to maize and soybeans. In the natural vegetation the samples correspond to representative species.

## 3.4. Sample preparation

Sample preparation took place in the soil laboratory of El Colegio de la Frontera Sur Unidad Campeche (located in the city of Campeche, Mex). The containers and tools were cleaned after processing a sample to prevent contamination. The processed samples were air-transported to the Netherlands.

### a. Soil samples

The samples were dried at room temperature for one week. Subsequently, the soil samples were grinded and sieved. From each sample, four grams of the processed soil were packaged inside a plastic bag.

### b. Plant samples

The leaves were dried in an oven at a temperature of 35°C for 48 hours. The dried leaves were crushed into powder with a mortar, the powder was placed in plastic back.

## 3.5. Glyphosate and AMPA determination

Glyphosate and AMPA concentrations were determined by LC–MS/MS using an XBridge™ Shield RP C18 column. The methods and procedures used in this study were carried out as described by Yang et al. (2015). The limits of detection (LOD) (the lowest concentration reliably detected) for glyphosate and AMPA were obtained from Bento et al. (2016). The LOD for glyphosate was 0.024 mg $kg^{-1}$ and 0.036 mg $kg^{-1}$ for AMPA. Annex 2 contains all the results obtained for glyphosate and AMPA per monolith.

## 3.6. Statistical analysis

### a. Soil samples

Mean values and standard error of the means were estimated for the soil samples that shown glyphosate and AMPA concentrations within the limits of detection. Comparisons between agroecosystems (soybeans and maize) were only possible for the variable AMPA. According to the results of the Shapiro-Wilk test (Annex 3), the available samples do not follow a normal distribution ($P$= <0.001) therefore, the means were compared with the Mann-Whitney U test, the significance level was set at 5% (Annex 4). The analyses were carried out in Genstat 18$^{th}$ edition.

### b. Plant samples

The results of the plant samples were not included in the statistical analysis due to lack of reliability, the majority of the plant samples presented concentrations of AMPA and glyphosate below the limits of detection. The residues were only detected in few samples from soybean parcels, mean glyphosate concentration was 2.$^{77}$ ± 1.67 mg $kg^{-1}$ (n=4). AMPA was detected only in two samples with concentrations of 0.44 and 0.69 mg $kg^{-1}$.

# 4. Results

## 4.1. Glyphosate in soil samples

Glyphosate was detected (>LOD) in nine samples collected across parcels 1 (n=3), 3 (n=5) and 4 (n=1) with soybean cultivation, maximum and minimum values of glyphosate concentration for each parcel was 0.05 and 0.39 mg $kg^{-1}$. The average concentration of glyphosate for soil samples in the soybean crop was 0.21 ± 0.10 mg $kg^{-1}$. In maize cultures, glyphosate values were under the limit of detection (see annex 2).

**Table 1. Average glyphosate concertation in soil samples per soybean parcel and total (n = number of samples)**

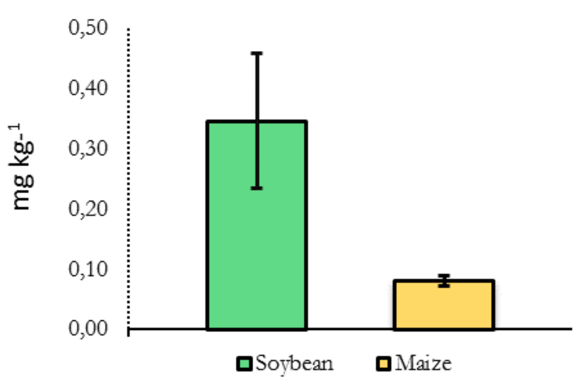
| Crop | Parcel # | n | Glyphosate |
|---|---|---|---|
| Soybean | 1 | 3 | 0.39 |
| | 2 | - | <LOD |
| | 3 | 5 | 0.13 |
| | 4 | 1 | 0.05 |
| **Total** | | **9** | **0.21** |

## 4.2. AMPA in soil samples

The presence of AMPA in soil was detected for all the parcels from both soybean and maize crops. Figure 2 shows the average concentration of AMPA in soil per crop type. In the soybean fields 17 soil samples were found to contain AMPA corresponding to two samples from parcel 2 and five samples from each of the three remaining parcels in Las Flores. The average concentration was 0.35 ± 0.11 mg $kg^{-1}$ with values per parcel ranging from 0.05 to 0.66 mg $kg^{-1}$.

Eight samples from the maize fields contained AMPA, four were collected in parcel 3, two in parcel 1 and one in both parcel 2 and 4. The average concentration in the maize fields was 0.08 ± 0.01 mg $kg^{-1}$, concentrations per individual parcel were between 0.06 and 0.11 mg $kg^{-1}$. According to the results of the Mann-Whitney U test, differences between the average concentration of AMPA in soil of maize and soybean parcels are significant (*P* = 0.011).

**Figure 2. Average concentration of AMPA in soil samples across two crop types (Bars represent standard error of the means).**



## 5. Discussion

The occurrence of AMPA in the soybean and maize parcels indicate that glyphosate is being applied in both agroecosystems. Under the warm and rainy conditions present in the study area, the dissipation of glyphosate and AMPA is expected to be fast (Bento et al., 2016). Glyphosate was only detected in the soybean fields, the incidence of glyphosate in soils that have been collected approximately two months after the crop establishment may indicate the presence of glyphosate-resistant plants. The transgenic variety enables the possibility to apply glyphosate along the crop cycle (interval of six months) suggesting intensive or continue applications of the herbicide. Furthermore, the persistence of glyphosate is expected to be higher in soil when treated plants are left on the surface because the trapped residues are not readily available to soil microorganisms (Mamy et al., 2016). After soybean cultivation, farmers grow sorghum in the parcels from Las Flores. This could also contribute to the results obtained in this study, if glyphosate is being applied at least before crop establishment.

The difference in AMPA concentration of soils from the maize and soybean seems to be related to the management practices. However, the behaviour and fate of glyphosate and AMPA is complex and multifactorial (Todorovic et al., 2013). The concentration of AMPA in soils of the maize fields was four times smaller than the one found in the soybean fields. In the maize fields, glyphosate applications take place before starting the crop cycle (this was approximately two months before sample collection) and most farmers only grow maize once per year due to the rain dependence. Glyphosate sprayed in these fields have already been degraded into AMPA, the high persistence of AMPA compared with glyphosate have been reported in other studies (Roy et al., 1989; Grunewald et al., 2001; Mamy et al., 2016). Furthermore, the parcels with soybean cultivation show more variation with regard to AMPA concentrations. These differences suggest less homogeneity in the amount and concentrations of the glyphosate-based products used in the soybean fields. According to the farmers, different glyphosate-based herbicides are used in the studied agroecosystems, comparison are challenging since the chemical identity of these formulations is confidential.

Most of the available studies dealing with the occurrence of glyphpsate and AMPA have been conducted for environmental water samples (Guo et al., 2016; Mörtl, et al., 2013; Sanchís et al., 2012; Hao et al., 2011; Qian et al; 2009; Battaglin et al., 2009; Struger et al., 2008; Skark et al., 1998). The results of Arellano and Rendon (2016) for the water samples collected in Campeche indicate that glyphosate concentrations are between 1.5 and 2.75 mg $L^{-1}$. Peruzzo et al. (2008) determined the concentration of glyphosate in soils near to transgenic soybean fields in north Argentina, the doses 1.5 kg $ha^{-1}$ and 1.0 kg $ha^{-1}$ were respectively applied one and two months after the sowing date, glyphosate concentrations were between 0.5 and 5.0 mg $kg^{-1}$. In forest soils from north-west Spain treated once after planting with glyphosate-based herbicide at dose of 5-8 l $ha^{-1}$, concentrations of 0.14 and 0.11 mg $kg^{-1}$ were detected respectively for glyphosate and AMPA (Viega et al., 2001). The first study corresponds to the area with highest agricultural production in Argentina and the second to a silvicultural system, differences between the concentrations of AMPA might be related to the intensity and frequency of herbicide applications. The concentration of glyphosate obtained in this study for the soybean fields is higher than in the forest soil but below the range found for Argentinian soils. The concentration of AMPA in the forest soil is similar to the results of the maize fields but lower when compared with the soybean fields.

As previously mentioned, the risk of glyphosate residues leaching is supposed to be low but vertical transportation of glyphosate and AMPA in soils have been demonstrated (Busse et al., 2001; Landry et al., 2005 Mörtl, et al., 2013 Sanchís et al., 2012). The adsorption of glyphosate is influenced by physical soil conditions such as structure, organic matter content and water infiltration rate (Veiga et al., 2001; Kjaer et al. 2005; Candela et al. 2007). In karstic environments, such as in the YP, the aquifers and coastal ecosystem are vulnerable to pollution due to the high infiltration rate and rapid flow of these soils (Aranda-Cirerol et al., 2011). Further studies are required for evaluating the concentration of Glyphosate and AMPA in groundwater.

With respect to the natural vegetation Glyphosate and AMPA were not detected in the soil and plant samples. This might mean that the applications of glyphosate do not reach these areas but other conjectures are possible. In the natural vegetation, the higher microbial activity is expected to increase the dissipation of glyphosate and AMPA. It will also be difficult to detect residues of the herbicide if the content in the samples is below the limits of detection.

## 6. Conclusions

Glyphosate-based herbicides are being used for weed management in maize and soybean fields. The differences between these agroecosystem seems to be related to the management practices. In the technified agroecosystem (soybean), glyphosate and AMPA concentrations in soils corresponds to herbicide use intensification along the crop cycle or the year. In the semi-technified maize farms, glyphosate was not detected and AMPA concentrations was four times smaller than the one found in soybean fields suggesting less intensive herbicide use. The determination of glyphosate and AMPA concentrations in the local agroecosystems and surrounding natural vegetation is an essential step to recognize the impact of different agricultural practices. The intensification of crop production should be pursued in a sustainable manner in order to prevent and reduce environmental pollution.

## 7. References


Anderson, J., & Ingram, J. (1993). Tropical soil biology and fertility (TSBF). Handbook of Methods. CAB. Internacional. 171p.

Andréa, M. M., Peres, T. B., Luchini, L. C., Bazarin, S., Papini, S., Matallo, M. B., & Savoy, V. L. T. (2003). Influence of repeated applications of glyphosate on its persistence and soil bioactivity. *Pesquisa Agropecuaria Brasileira*, *38*(11), 1329-1335.

ArandaCirerol, N., Herrera-Silveira, J. and Comín, F. (2006). Nutrient water quality in a tropical coastal zone with groundwater discharge, northwest Yucatan, Mexico. *Estuarine, Coastal and Shelf Science*, 68, 445–454.

Arellano, O. and Rendon, J. 2016. *La huella de los plaguicidas en México*. Greenpeace. Retrieved from: http://www.greenpeace.org/mexico/Global/mexico/Graficos/2016/comida-sana/Plaguicidas_en_agua_ok_EM.pdf

Bai, S. H., and Ogbourne, S. M. (2016). Glyphosate: environmental contamination, toxicity and potential risks to human health via food contamination. *Environmental Science and Pollution Research*, *23*(19), 18988-19001.

Battaglin, W. A., Rice, K. C., Focazio, M. J., Salmons, S., and& Barry, R. X. (2009). The occurrence of glyphosate, atrazine, and other pesticides in vernal pools and adjacent streams in Washington, DC, Maryland, Iowa, and Wyoming, 2005–2006. Environmental Monitoring and Assessment, 155(1-4), 281-307.

Benbrook, C. M. (2016). Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe*, *28*(3).

Bento, C. P., Yang, X., Gort, G., Xue, S., van Dam, R., Zomer, P., Molf, H., Ritsemaa, C.J. and Geissen, V. (2016). Persistence of glyphosate and aminomethylphosphonic acid in loess soil under different combinations of temperature, soil moisture and light/darkness. *Science of The Total Environment*, *572*, 301-311.

Borggaard, O. K. and Gimsing, A. L. (2008). Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest management science*, *64*(4), 441-456.

Busse, M.D., Ratcliff, A. W., Shestak, C.J. and Powers, R.F. (2001) Glyphosate toxicity and effects of long-term vegetation control on soil microbial communities. *Soil Biology & Biochemistry*, 33, 1777–1789.

Candela, L., Alvarez-Benedi, J., De Melo, M. C., & Rao, P. S. C. (2007). Laboratory studies on glyphosate transport in soils of the Maresme area near Barcelona, Spain: Transport model parameter estimation. *Geoderma, 140*(1), 8-16.

COFEPRIS (2017). Official Catalogue of Pesticides. Retrieved from: http://www.cofepris.gob.mx/AZ/Paginas/Plaguicidas%20y%20Fertilizantes/CatalogoPlaguicidas.aspx

Díaz, G., Botello, A. and Ponce, G. (2005). *Plaguicidas organoclorados en pastos y peces de los sistemas Candelaria-Panlau y Palizada del Este, laguna de Términos, Campeche, México*, 207-224. In: A. Botello, V., Rendon, J., Gold, G. and Agraz, C. (Eds.). Golfo de México Contaminacion e Impacto Ambiental: Diagnostico y Tendencias, 2da Edicion. Universidad Autonoma de de Campeche, Univiversidad Autonoma de México, Instituto Nacional de Ecología.

Dill, G. M., Sammons, R. D., Feng, P. C. C., Kohn, F., Kretzmer, K., Mehrsheikh, A., Bleeke, M., Honegger, J. L., Farmer, D., Wright, D. and Haupfear, E. A. (2010) *Glyphosate: Discovery, Development, Applications, and Properties*, 1-33. In Glyphosate Resistance in Crops and Weeds: History, Development, and Management (ed V. K. Nandula), John Wiley and Sons, Inc. Hoboken, NJ, USA.

Domínguez, D. and Sabatino, P. (2005). La muerte que viene en el viento: los problemática de la contaminacion por efecto de la agricultura transgénica en Argentina y Paraguay. CLACSO. Retrieved from: http://bibliotecavirtual.clacso.org.ar/ar/libros/becas/2005/soja/domsa.pdf.

Dunfield, K.E. and Germida, J.J. (2004). Impact of genetically modified crops on soil- and plant-associated microbial communities. *Journal of environmental quality*, 33, 806–815.

Edginton, A.N., Sheridan, P.M., Boermans, H.J., Thompson, D.G., Holt, J.D. and Stephenson, G.R., (2004). A comparison of two factorial designs, a complete 3 × 3 factorial and a central composite rotatable design, for use in binomial response experiments in aquatic toxicology. *Environmental Contamination and Toxicology*, 46, 216–223.

EFSA (European Food Safety Authority) (2015) Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate 1. EFSA, *13*(11), 4302.

Ellis, E., Romero, A. and Hernández, I. 2015. *Evaluacion y mapeo de los determinantes de deforestacion en la Península Yucatán*. Agencia de los Estados Unidos para el Desarrollo Internacional (USAID), The Nature Conservancy (TNC), Alianza México REDD+. México, Distrito Federal. 104 .

Esparza, L. and Martinez, E. 2011. Deforestacion en Campeche: Causas y Efectos. *Revista Fomix Campeche 3*(10): 6-11.

FAO and WHO. 2004. *The International Code of Conduct on Pesticide Management*. Retrieved from: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Code/CODE_2014Sep_ENG.pdf

FAOSTAT. (2017). Pesticide inputs use by country. Retrieved from: http://www.fao.org/faostat/es/#data/RP

Franz, J.E., Mao, J.A. and Sikorski, J.A. (1997). Glyphosate: A Unique Global Herbicide. ACS Monograph No. 189. *American Chemical Society*. Washington, DC. 653.

Giesy, J., Dobson, S., Solomon, K. (2000). Ecotoxicological risk assessment for Roundup® herbicide. *Environmental Contamination and Toxicology*,167, 35–120.

Gimsing, A.L., Borggaard, O.K., Jacobsen, O.S., Aamand, J. and Sorensen, J. (2004) Chemical and microbiological soil characteristics controlling glyphosate mineralization in Danish surface soils. *Applied Soil Ecology*, 2, 233–242.

Gomez, I. 2016. A honey-sealed alliance: Mayan beekeepers in the Yucatan Peninsula versus transgenic soybeans in Mexico's last tropical forest. *Journal of Agrarian Change*, 1-9.

Grunewald, K., Schmidt, W., Unger, C. and Hanschmann, G. (2001), Behavior of glyphosate and aminomethylphosphonic acid (AMPA) in soils and water of reservoir Radeburg II catchment (Saxony/Germany). J Plant Nutr Soil Sci 164: 65–70. (2001).

Guo, H., Riter, L. S., Wujcik, C. E., & and Armstrong, D. W. (2016). Direct and sensitive determination of glyphosate and aminomethylphosphonic acid in environmental water samples by high performance liquid chromatography coupled to electrospray tandem mass spectrometry. Journal of Chromatography A. A, 1443, 93-100.

Gutiérrez, J. 2013. Panorama historico de morbilidad y mortalidad por Intoxicacion por plaguicidas en México 1995-2012. *Boletín Epidemiologico*, 30(34):1-5.

Guyton, K. Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N. Scoccianti, C., Mattock, H. and Straif, K. (2015). Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *Lancet Oncology*, 16, 490–491.

Hao, C., Morse, D., Morra, F., Zhao, X., Yang, P., and& Nunn, B. (2011). Direct aqueous determination of glyphosate and related compounds by liquid chromatography/tandem mass spectrometry using reversed-phase and weak anion-exchange mixed-mode column. Journal of Chromatography A, 1218(33), 5638-5643.

Heap, I. M. (2014) Global perspective of herbicide-resistant weeds. *Pest Management Science*, 70, 1306–1315.

INEGI. (2009). *Prontuario de informacion geográfica municipal de los Estados Unidos Mexicanos Hopelchén, Campeche, Clave geoestadística 04006*. Retrieved from: http://www3.inegi.org.mx/sistemas/mexicocifras/datos-geograficos/04/04006.pdf

INEGI. (2014). *Anuario estadístico y geográfico de Campeche 2014*. Retrieved from: http://internet.contenidos.inegi.org.mx/contenidos/productos/prod_serv/contenidos/espanol/bvinegi/productos/anuario_14/702825066246.pdf.

INEGI. (2016). *Mexico en cifras, informacion nacional por entidad federativa y municipio*. Retrieved from: http://gaia.inegi.org.mx/mdm6/?v=bGF0OjE5LjU0MjUwLGxvbjotODkuNjEwNzQsejo1LGw6Y3VzdjU=

Kjær, J., Olsen, P., Ullum, M. and Grant, R. (2005). Leaching of glyphosate and amino-methylphosphonic acid from Danish agricultural field sites. *Journal of Environmental Quality*, *34*(2), 608-620.

Kremer, R.J. and Means, N.E. (2009) Glyphosate and glyphosate-resistant crop interaction with rhizosphere microorganisms. *European Journal of Agronomy*, 31, 153–61.

Krieger, R. (Ed.). (2001). *Book of Pesticide Toxicology, Two-Volume Set: Principles and Agents* (Vol. 1). Academic Press, 1667.

Kryuchkova, Y. V., Burygin, G. L., Gogoleva, N. E., Gogolev, Y. V., Chernyshova, M. P., Makarov, O. E., Fedorov, E. E. and Turkovskaya, O. V. (2014). Isolation and characterization of a glyphosate-degrading rhizosphere strain, Enterobacter cloacae K7. *Microbiological research*, *169*(1), 99-105.

Laitinen, P., Rämö, S. and Siimes, K. (2007). Glyphosate translocation from plants to soil — does this constitute a significant proportion of residues in soil?. *Plant Soil*, 300, 51–60.

Landry D, Dousset S, Fournier J-C and Andreux F. (2005). Leaching of glyphosate and AMPA under two soil management practices in Burgundy vineyards. *Environmental pollution*, 138, 191–200.

Maeda, H., and Dudareva, N. (2012). The shikimate pathway and aromatic amino acid biosynthesis in plants. *Annual review of plant biology*, 63, 73-105.

Mamy, L. and Barriuso, E. (2005). Glyphosate adsorption in soils compared to herbicides replaced with the introduction of glyphosate resistant crops. *Chemosphere*, 61, 844–855.

Moore, L.J., Fuentes, L., Rodgers Jr., J.H., Bowerman, W.W., Yarrow, G.K., Chao, W.Y. and Bridges, W.C., (2012). Relative toxicity of the components of the original for- mulation of Roundup to five North American anurans. *Ecotoxicology and Environmental Safety*, 78, 128–133.

Mortensen, D.A., Egan, J.F., Maxwell, B.D., Ryan, M.R. (2012). Navigating a critical juncture for sustainable weed management. *Bioscience*, 62, 75–84.

Mörtl, M., Németh, G., Juracsek, J., Darvas, B., Kamp, L., Rubio, F., and& Székács, A. (2013). Determination of glyphosate residues in Hungarian water samples by immunoassay. Microchemical Journal, 107, 143-151.

Neumann, G., Kohls, S., Landsberg, E., Stock-Oliveira Souza, K., Yamada, T. and Römheld, V. (2006) Relevance of glyphosate transfer to non-target plants via the rhizosphere. *The Journal of Plant Diseases and Protection*, 20, 963–969.

Nguyen, D. B., Rose, M. T., Rose, T. J., Morris, S. G., and Van Zwieten, L. (2016). Impact of glyphosate on soil microbial biomass and respiration: A meta-analysis. *Soil Biology and Biochemistry*, 92, 50-57.

Perez, A. (2012). Identificacion de residuos toxicos en miel de diferentes procedencias en la zona centro del Estado de Veracruz. *Revista Iberoamericana de las Ciencias Biologicas y Agropecuarias*, *1*(2):1-42.

Pérez, N., González, A., Méndez, J. and Arellano, J. (2015). Caracterizacion de los productores de maíz de temporal en el Estado de Campeche, México. Revista Mexicana de Agronegocios, *19*(36): 1295-1305.

Qian, K., Tang, T., Shi, T., Wang, F., Li, J., and& Cao, Y. (2009). Residue determination of glyphosate in environmental water samples with high-performance liquid chromatography and UV detection after derivatization with 4-chloro-3, 5-dinitrobenzotrifluoride. Analytica chimica acta, 635(2), 222-226.

Rendon, J., Canepa, M. and Ek, N. (2005). *Plaguicidas orgánicos persistentes (POPs) en sedimentos de la costa sur de Campeche, México*, 249-260. In: A. Botello, V., Rendon, J., Gold, G. and Agraz, C. (Eds.). Golfo de México Contaminacion e Impacto Ambiental: Diagnostico y Tendencias, 2da Edicion. Universidad Autonoma de de Campeche, Univiversidad Autonoma de México, Instituto Nacional de Ecología.

Roy, N.D., Konar, S.K., Banerjee, S., , Charles, D.A., Thompson, D.G. and Prasad, R,. (1989). Persistence, movement and degradation of glyphosate in selected Canadian boreal forest soils. J Agric Food Chem 37: 437–440 (1989).

Sanchís, J., Kantiani, L., Llorca, M., Rubio, F., Ginebreda, A., Fraile, J. and, ... & Farré, M. (2012). Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. Analytical and bioanalytical chemistry, 402(7), 2335-2345.

Shaner, D. (2009). Role of Translocation as a Mechanism of Resistance to Glyphosate. *Weed Science*, *57*(1), 118-123. Retrieved from http://www.jstor.org.ezproxy.library.wur.nl/stable/40586983

Sihtmäe, M., Blinova, I., Künnis-Beres, K., Kanarbik, L., Heinlaan, M. and Kahru, A., (2013). Ecotoxicological effects of different glyphosate formulations. *Applied Soil Ecology*, 72, 215 –224.

Skark, C., Zullei-Seibert, N., Schöttler, U. , and& Schlett, C. (1998). The occurrence of glyphosate in surface water. International Journal of Environmental Analytical Chemistry, 70(1-4), 93-104.

Steinrücken, H. C., and Amrhein, N. (1980). The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. *Biochemical and biophysical research communications*, *94*(4), 1207-1212.

Stenrod, M., Charnay M.P., Benoit, P. and Eklo, O.M., (2006). Spatial variability of glyphosate mineralization and soil microbial characteristics in two Norwegian sandy loam soils as affected by surface topographical features. *Soil Biology and Biochemistry*, 38, 962–971.

Struger, J., Thompson, D., Staznik, B., Martin, P., McDaniel, T. and, & Marvin, C. (2008). Occurrence of glyphosate in surface waters of southern Ontario. Bulletin of Environmental Contamination and Toxicology, 80(4), 378-384.

Todorovic, G., Mentler, A., Popp, M., Hann, S., Köllensperger, G., Rampazzo, N and Blum, W. (2013). Determination of Glyphosate and AMPA in Three Representative Agricultural Austrian Soils with a HPLC-MS/MS Method. *Soil and Sediment Contamination: An International Journal*, 22(*3*), 332-350.

Veiga, F., Zapata, J. M., Marcos, M. L. F. and Alvarez, E. (2001). Dynamics of glyphosate and aminomethylphosphonic acid in a forest soil in Galicia, north-west Spain. *Science of the Total Environment*, 271, 135-144, 2001.

Yang, X., Wang, F., Bento, C.P.M., Xue, S., Gai, L., van Dam, R., Mol, H., Ritsema, C.J., Geissen, V. (2015). Short-term transport of glyphosate with erosion in Chinese loess soil - A flume experiment. *Science of the Total Environment*. 512-513, 406-414.

# 8. Annex

## Annex 1. Description and coordinates of the monoliths.

(*NV = natural vegetation, M=maize and S= soybean)

| Site | Type of vegetation* | Parcel | Monolith | N Coordinate | O Coordinate | Soil sample ID | Plant sample ID |
|---|---|---|---|---|---|---|---|
| Chenco | NV | 3 | 1 | 19°24'51.67" | 89°47'46.38" | 1 | 61 |
| Chenco | NV | 3 | 2 | 19°24'52.28" | 89°47'44.67" | 2 | 62 |
| Chenco | NV | 3 | 3 | 19°24'50.26" | 89°47'39.69" | 3 | 63 |
| Chenco | NV | 3 | 4 | 19°24'49.25" | 89°47'37.13" | 4 | 64 |
| Chenco | NV | 3 | 5 | 19°24'48.84" | 89°47'29.85" | 5 | |
| Chenco | NV | 4 | 1 | 19°26'10.26" | 89°48'41.33" | 6 | 65 |
| Chenco | NV | 4 | 2 | 19°26'7.57" | 89°48'45.47" | 7 | 66 |
| Chenco | NV | 4 | 3 | 19°26'6.26" | 89°48'47.44" | 8 | 67 |
| Chenco | NV | 4 | 4 | 19°26'5.16" | 89°48'49.78" | 9 | 68 |
| Chenco | NV | 4 | 5 | 19°26'10.32" | 89°48'49.81" | 10 | |
| Flores | NV | 1 | 1 | 19° 8'41.19" | 89°54'48.73" | 11 | 69 |
| Flores | NV | 1 | 2 | 19° 8'43.56" | 89°54'45.72" | 12 | 70 |
| Flores | NV | 1 | 3 | 19° 8'45.29" | 89°54'42.75" | 13 | 71 |
| Flores | NV | 1 | 4 | 19° 8'46.64" | 89°54'39.63" | 14 | 72 |
| Flores | NV | 1 | 5 | 19° 8'48.86" | 89°54'36.68" | 15 | |
| Flores | NV | 2 | 1 | 19°12'13.35" | 89°53'47.71" | 16 | 73 |
| Flores | NV | 2 | 2 | 19°12'16.17" | 89°53'45.04" | 17 | 74 |
| Flores | NV | 2 | 3 | 19°12'18.39" | 89°53'42.52" | 18 | 75 |
| Flores | NV | 2 | 4 | 19°12'20.63" | 89°53'39.82" | 19 | 76 |
| Flores | NV | 2 | 5 | 19°12'22.62" | 89°53'37.09" | 20 | |
| Chenco | M | 1 | 1 | 19°25'24.17" | 89°48'51.75" | 21 | 77 |
| Chenco | M | 1 | 2 | 19°25'23.24" | 89°48'48.46" | 22 | 78 |
| Chenco | M | 1 | 3 | 19°25'27.03" | 89°48'50.29" | 23 | 79 |
| Chenco | M | 1 | 4 | 19°25'26.29" | 89°48'47.57" | 24 | 80 |
| Chenco | M | 1 | 5 | 19°25'25.52" | 89°48'49.26" | 25 | |
| Chenco | M | 2 | 1 | 19°24'41.15" | 89°47'48.43" | 26 | 81 |
| Chenco | M | 2 | 2 | 19°24'42.89" | 89°47'51.38" | 27 | 82 |
| Chenco | M | 2 | 3 | 19°24'40.45" | 89°47'52.64" | 28 | 83 |
| Chenco | M | 2 | 4 | 19°24'39.34" | 89°47'49.95" | 29 | 84 |
| Chenco | M | 2 | 5 | 19°24'40.77" | 89°47'50.82" | 30 | |
| Chenco | M | 3 | 1 | 19°24'39.85" | 89°47'46.09" | 31 | 85 |
| Chenco | M | 3 | 2 | 19°24'39.02" | 89°47'42.69" | 32 | 86 |
| Chenco | M | 3 | 3 | 19°24'36.95" | 89°47'43.54" | 33 | 87 |
| Chenco | M | 3 | 4 | 19°24'37.39" | 89°47'46.25" | 34 | 88 |
| Chenco | M | 3 | 5 | 19°24'38.24" | 89°47'44.90" | 35 | |
| Chenco | M | 4 | 1 | 19°26'17.10" | 89°48'43.67" | 36 | 89 |
| Chenco | M | 4 | 2 | 19°26'16.41" | 89°48'40.25" | 37 | 90 |
| Chenco | M | 4 | 3 | 19°26'14.06" | 89°48'39.57" | 38 | 91 |
| Chenco | M | 4 | 4 | 19°26'14.42" | 89°48'42.63" | 39 | 92 |
| Chenco | M | 4 | 5 | 19°26'15.46" | 89°48'41.00" | 40 | |
| Flores | S | 1 | 1 | 19° 8'44.62" | 89°55'2.16" | 41 | 93 |
| Flores | S | 1 | 2 | 19° 8'46.14" | 89°55'5.13" | 42 | 94 |
| Flores | S | 1 | 3 | 19° 8'47.70" | 89°55'8.01" | 43 | 95 |
| Flores | S | 1 | 4 | 19° 8'49.36" | 89°55'11.07" | 44 | 96 |
| Flores | S | 1 | 5 | 19° 8'49.91" | 89°55'14.44" | 45 | |
| Flores | S | 2 | 1 | 19° 8'43.54" | 89°54'56.54" | 46 | 97 |
| Flores | S | 2 | 2 | 19° 8'46.90" | 89°54'55.89" | 47 | 98 |
| Flores | S | 2 | 3 | 19° 8'49.99" | 89°54'55.80" | 48 | 99 |
| Flores | S | 2 | 4 | 19° 8'53.24" | 89°54'55.19" | 49 | 100 |
| Flores | S | 2 | 5 | 19° 8'56.31" | 89°54'54.37" | 50 | |
| Flores | S | 3 | 1 | 19°10'23.63" | 89°54'21.15" | 51 | 101 |
| Flores | S | 3 | 2 | 19°10'24.46" | 89°54'24.48" | 52 | 102 |
| Flores | S | 3 | 3 | 19°10'25.20" | 89°54'27.85" | 53 | 103 |
| Flores | S | 3 | 4 | 19°10'26.00" | 89°54'31.19" | 54 | 104 |
| Flores | S | 3 | 5 | 19°10'26.79" | 89°54'34.50" | 55 | |
| Flores | S | 4 | 1 | 19°11'49.96" | 89°53'50.72" | 56 | 105 |
| Flores | S | 4 | 2 | 19°11'49.99" | 89°53'54.14" | 57 | 106 |
| Flores | S | 4 | 3 | 19°11'49.90" | 89°53'57.64" | 58 | 107 |
| Flores | S | 4 | 4 | 19°11'49.91" | 89°54'4.44" | 59 | 108 |
| Flores | S | 4 | 5 | 19°11'49.85" | 89°54'7.82" | 60 | |

## Annex 2. Glyphosate and AMPA concentrations (µg g-[1]) for soil and plant samples.

(Results in bold indicate values above the limits of detection)

| Site | Vegetation* | Parcel | Monolith | Soil samples | | | Plant samples | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Sample ID | GLY | AMPA | Sample ID | GLY | AMPA |
| Chenco | NV | 3 | 1 | 1 | 0.014 | 0.010 | 61 | 0.223 | 0.099 |
| Chenco | NV | 3 | 2 | 2 | 0.018 | 0.021 | 62 | 0.255 | 0.040 |
| Chenco | NV | 3 | 3 | 3 | 0.016 | 0.015 | 63 | 0.086 | 0.046 |
| Chenco | NV | 3 | 4 | 4 | 0.006 | 0.006 | 64 | 0.193 | 0.044 |
| Chenco | NV | 3 | 5 | 5 | 0.007 | 0.011 | | | |
| Chenco | NV | 4 | 1 | 6 | 0.005 | 0.007 | 65 | 0.682 | 0.033 |
| Chenco | NV | 4 | 2 | 7 | 0.011 | 0.005 | 66 | 0.236 | 0.204 |
| Chenco | NV | 4 | 3 | 8 | 0.004 | 0.010 | 67 | 0.034 | 0.056 |
| Chenco | NV | 4 | 4 | 9 | 0.001 | 0.004 | 68 | 0.130 | 0.021 |
| Chenco | NV | 4 | 5 | 10 | 0.000 | 0.003 | | | |
| Flores | NV | 1 | 1 | 11 | 0.008 | 0.014 | 69 | 0.055 | 0.011 |
| Flores | NV | 1 | 2 | 12 | 0.005 | 0.001 | 70 | 0.040 | 0.008 |
| Flores | NV | 1 | 3 | 13 | 0.004 | 0.004 | 71 | 0.012 | 0.021 |
| Flores | NV | 1 | 4 | 14 | 0.002 | 0.009 | 72 | 0.038 | 0.018 |
| Flores | NV | 1 | 5 | 15 | 0.001 | 0.004 | | | |
| Flores | NV | 2 | 1 | 16 | 0.000 | 0.004 | 73 | 0.100 | 0.095 |
| Flores | NV | 2 | 2 | 17 | 0.001 | 0.008 | 74 | 0.367 | 0.127 |
| Flores | NV | 2 | 3 | 18 | 0.001 | 0.000 | 75 | 0.301 | 0.046 |
| Flores | NV | 2 | 4 | 19 | 0.012 | 0.007 | 76 | 0.047 | 0.012 |
| Flores | NV | 2 | 5 | 20 | 0.005 | 0.002 | | | |
| Chenco | M | 1 | 1 | 21 | 0.018 | 0.007 | 77 | 0.055 | 0.011 |
| Chenco | M | 1 | 2 | 22 | 0.005 | 0.011 | 78 | 0.046 | 0.040 |
| Chenco | M | 1 | 3 | 23 | 0.007 | 0.013 | 79 | 0.032 | 0.041 |
| Chenco | M | 1 | 4 | 24 | 0.007 | **0.093** | 80 | 0.077 | 0.054 |
| Chenco | M | 1 | 5 | 25 | 0.008 | **0.058** | | | |
| Chenco | M | 2 | 1 | 26 | 0.006 | 0.011 | 81 | 0.044 | 0.017 |
| Chenco | M | 2 | 2 | 27 | 0.006 | 0.008 | 82 | 0.097 | 0.083 |
| Chenco | M | 2 | 3 | 28 | 0.002 | 0.012 | 83 | 0.091 | 0.032 |
| Chenco | M | 2 | 4 | 29 | 0.007 | 0.020 | 84 | 0.081 | 0.015 |
| Chenco | M | 2 | 5 | 30 | 0.013 | **0.106** | | | |
| Chenco | M | 3 | 1 | 31 | 0.020 | **0.093** | 85 | 0.073 | 0.125 |
| Chenco | M | 3 | 2 | 32 | 0.011 | **0.077** | 86 | 0.126 | 0.104 |
| Chenco | M | 3 | 3 | 33 | 0.009 | **0.109** | 87 | 0.019 | 0.015 |
| Chenco | M | 3 | 4 | 34 | 0.009 | 0.021 | 88 | 0.007 | 0.001 |
| Chenco | M | 3 | 5 | 35 | 0.027 | **0.053** | | | |
| Chenco | M | 4 | 1 | 36 | 0.002 | 0.005 | 89 | 0.078 | 0.037 |
| Chenco | M | 4 | 2 | 37 | 0.003 | 0.016 | 90 | 0.245 | 0.012 |
| Chenco | M | 4 | 3 | 38 | 0.001 | 0.001 | 91 | 0.046 | 0.029 |
| Chenco | M | 4 | 4 | 39 | 0.025 | **0.062** | 92 | **0.719** | 0.018 |
| Chenco | M | 4 | 5 | 40 | 0.001 | 0.012 | | | |
| Flores | S | 1 | 1 | 41 | 0.025 | **0.152** | 93 | **4.459** | **0.442** |
| Flores | S | 1 | 2 | 42 | **0.046** | **0.159** | 94 | **2.777** | 0.056 |
| Flores | S | 1 | 3 | 43 | **0.120** | **0.389** | 95 | **3.399** | **0.689** |
| Flores | S | 1 | 4 | 44 | **0.996** | **1.963** | 96 | 0.294 | 0.317 |
| Flores | S | 1 | 5 | 45 | **0.283** | **0.639** | | | |
| Flores | S | 2 | 1 | 46 | - | - | 97 | 0.354 | 0.154 |
| Flores | S | 2 | 2 | 47 | 0.004 | **0.053** | 98 | 0.037 | 0.146 |
| Flores | S | 2 | 3 | 48 | 0.015 | 0.017 | 99 | **0.460** | 0.040 |
| Flores | S | 2 | 4 | 49 | 0.001 | 0.005 | 100 | 0.108 | 0.056 |
| Flores | S | 2 | 5 | 50 | 0.001 | **0.042** | | | |
| Flores | S | 3 | 1 | 51 | **0.075** | **0.659** | 101 | 0.129 | 0.036 |
| Flores | S | 3 | 2 | 52 | **0.056** | **0.278** | 102 | 0.133 | 0.052 |
| Flores | S | 3 | 3 | 53 | **0.195** | **0.249** | 103 | 0.395 | 0.055 |
| Flores | S | 3 | 4 | 54 | **0.183** | **0.218** | 104 | 0.112 | 0.014 |
| Flores | S | 3 | 5 | 55 | **0.151** | **0.203** | | | |
| Flores | S | 4 | 1 | 56 | 0.010 | **0.119** | 105 | 0.193 | 0.087 |
| Flores | S | 4 | 2 | 57 | 0.007 | **0.044** | 106 | 0.072 | 0.025 |
| Flores | S | 4 | 3 | 58 | 0.023 | **0.108** | 107 | 0.027 | 0.015 |
| Flores | S | 4 | 4 | 59 | **0.048** | **0.503** | 108 | 0.298 | 0.011 |
| Flores | S | 4 | 5 | 60 | 0.019 | **0.118** | | | |

## Annex 3. Results of Shapiro-Wilk test for AMPA concertation in soil samples

**Data variate:** AMPA

**Test statistic W:** 0.5424

**Probability:** <0.001

## Annex 4. Results of Mann-Whitney U test for AMPA concertation in soil samples

| Group | N | Mean rank | Sum of ranks | Value of U | P-value |
|---|---|---|---|---|---|
| Maize | 8 | 8 | 61 | 25.0 | 0.011 |
| Soybean | 17 | 16 | 264 | | |

.