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Corresponding Author: Dr. LUIS RODRIGUEZ, Chemical Engineering PhD

Corresponding Author's Institution: University of Castilla-La Mancha

First Author: VIRTUDES SANCHEZ, MEng

Order of Authors: VIRTUDES SANCHEZ, MEng; FRANCISCO JAVIER LOPEZ-BELLIDO, PhD; PABLO CAÑIZARES, PhD; LUIS RODRIGUEZ, Chemical Engineering PhD

ASSESSING THE PHYTOREMEDIATION POTENTIAL OF CROP AND GRASS PLANTS FOR ATRAZINE-SPIKED SOILS

Virtudes Sánchez[1], Francisco Javier López Bellido[2], Pablo Cañizares[3] and Luis Rodríguez[1,*]

[1] Department of Chemical Engineering, School of Civil Engineering, University of Castilla-La Mancha, Avenida Camilo José Cela, s/n, 13071 Ciudad Real (SPAIN)

[2] Department of Plant Production and Agricultural Technology, School of Agricultural Engineering, University of Castilla-La Mancha, Ronda de Calatrava, s./n, 13003 Ciudad Real (SPAIN)

[3] Department of Chemical Engineering, Faculty of Chemical Sciences and Technologies, University of Castilla-La Mancha, Avenida Camilo José Cela, 10, 13071 Ciudad Real (SPAIN)

* To whom correspondence should be addressed. e-mail: Luis.Rromero@uclm.es
Tel.: 34 926 295300 (ext. 6403); Fax: 34 926 295391

ABSTRACT

Pollution of soil and groundwater by atrazine has become an increasing environmental concern in the last decade. A phytoremediation test using plastic pots was conducted in order to assess the ability of several crops and grasses to remove atrazine from a soil of low permeability spiked with this herbicide. Four plant species were assessed for their ability to degrade or accumulate atrazine from soils: two grasses, i.e., ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*), and two crops, i.e., barley (*Hordeum vulgare*) and maize (*Zea mays*). Three different doses of atrazine were used for the contamination of the pots: 2, 5 and 10 mg kg^{-1}. 16 days after spiking, the initial amount of atrazine was reduced by 88.6-99.6% in planted pots, while a decrease of only 63.1-78.2% was found for the unplanted pots, thus showing the contribution of plants to soil decontamination. All the plant species were capable of accumulating atrazine and its N-dealkylated metabolites, i.e., deethylatrazine and deisopropylatrazine, in their tissues. Some toxic responses, such as biomass decreases and/or chlorosis, were observed in plants to a greater or lesser extent for initial soil doses of atrazine above 2 mg kg^{-1}. Maize was the plant species with the highest ability to accumulate atrazine derivatives, reaching up to 38.4% of the initial atrazine added to the soil. Rhizosphere degradation/mineralization by microorganisms or plant enzymes, together with degradation inside the plants, have been proposed as the mechanisms that contributed to a higher extent than plant accumulation to explain the removal of atrazine from soils.

Keywords: atrazine, crops, grasses, phytoremediation, rhizodegradation, polluted soil

1. INTRODUCTION

Atrazine (6-chloro-*N*-ethyl-*N*-isopropyl-1,3,5-triazine-2,4-diamine) is a selective systemic herbicide used for the pre- and-post-emergence control of annual grasses and broadleaf weeds being usually applied, among other crops, to corn, sorghum and sugar cane. Following its first introduction into the market approximately 50 years ago, it has become one of the most heavily used agricultural herbicides in the world, especially in the United States and developing countries such as China (Jablonowski et al., 2011; Mudhoo and Garg, 2011). Atrazine has also been widely used in Europe until 2006, when its commercial use was banned in the European Union.

Atrazine has a moderate aqueous solubility; nevertheless, it has the potential to act as both non-point and point source for the contamination of surface and groundwater, due to its low adsorption and moderate half-life in soils (Ribeiro et al., 2005; Fan and Song, 2014). In fact, high levels of atrazine have been detected in drinking waters in Europe and the USA (Mahía et al., 2007). Bioremediation technologies (including phytoremediation) have been shown to be effective in the removal of atrazine from soils; nevertheless, a recent review by Fan and Song (2014) has concluded that the existence of contradictory results suggests further research is needed in this field.

Phytoremediation is a well-known technology that uses plants to remediate inorganic and organic contaminants in the environment. The role of the plants includes the degradation, adsorption, volatilization and accumulation of pollutants and/or the promotion of the soil rhizosphere activity (Newman and Reynolds, 2004). Phytoremediation has several advantages, such as its low cost and relative simplicity and, moreover, it is environmental

friendly and does not produce secondary pollution. So, there is a bright future for the phytoremediation of contaminated soils (Ibrahim et al., 2013).

The uptake and translocation of organic compounds by plants are dependent on the physical-chemical characteristics of the pollutants, i.e. hydrophobicity (lipophilicity), solubility, polarity and molecular weight, the plant species and environmental factors (Turgut, 2005). Atrazine is moderately hydrophilic (log K_{ow} 2.5) and is likely to partially be adsorbed into roots or to be taken up by roots and move across cell membranes to reach the above-ground portion of plants. The fate of pesticides in the soil is strongly related to vegetation; in fact, organic pollutants are usually removed more quickly in planted soils than in soils without plants (Singh et al., 2004). Rhizosphere, the narrow layer of soil together to plant roots, is a zone of high microbiological activity, which is caused by root exudates containing carbohydrates, amino acids and organics acids (Curl and Truelove, 1986). Therefore, the use of vegetation at the polluted sites can overcome some of the inherent limitations to the biological clean-up approach, such as low microbial population or inadequate microbial activity (Singh et al., 2004).

This work forms part of a wider research project focused on the combination of phytoremediation and electrokinetic remediation to decontaminate soils polluted by atrazine. As a prior step, it is advisable to carry out preliminary studies in order to select plant species capable of degrading and/or accumulating atrazine. The main goals of this work were to assess the tolerance of several crops and grass plants to increasing atrazine soil concentrations and to study the role of plants in the removal of atrazine by phytoremediation. This paper shows the results from a pot experiment conducted with a low-permeability soil spiked with atrazine using four different plant species: the grass species *Festuca arundinacea* (tall fescue)

and *Lolium perenne* (ryegrass), and the crop species *Hordeum vulgare* (barley) and *Zea mays* (maize). These plant species were selected based on previous studies about biomass production and survival capability in soils contaminated by atrazine and/or other organic pollutants. Moreover, all of them have been reported to be effective in the removal of organics by phytoremediation (Singh et al., 2004; Huang et al., 2007; Li et al., 2012; Ibrahim et al., 2013).

2. MATERIALS AND METHODS

2.1. Soil

TABLE 1

The soil used in the present study was a clay loam collected from a region of high agrarian activity in central Spain (Mora de Toledo, Toledo), which could be vulnerable to pollution by pesticides. The most important physical-chemical characteristics of the soil are shown in Table 1. Soil pH and electrical conductivity (EC) were measured in a 1:5 soil/water mixture; organic carbon was determined using a total organic carbon analyser (Shimadzu TOC-VCSH, Columbia, USA); particle size distribution (clay, silt and sand content) was determined using laser diffractometry (Beckman Coulter LS, Fullerton, USA); cation exchange capacity (CEC) was measured by the ammonium acetate saturation method (MAPA, 1994). Specific gravity and water holding capacity were determined according to the ASTM Standards D854 and D2980, respectively. The soil was air dried and passed through a 2-mm sieve to remove stones and any plant residues prior to the experiment.

2.2. Plants and experimental design

Four plant species were used in the phytoremediation experiment: tall fescue (*Festuca arundinacea*), ryegrass (*Lolium perenne*), barley (*Hordeum vulgare*) and maize (*Zea mays*). Barley (spring two rows cultivar) and maize (dent corn hybrid cultivar) commercial varieties were used. Ryegrass and tall fescue seeds were purchased as certified seed at the Oregon Seed Certification Service (Corvallis, USA). All seeds were pre-germinated using a 0.5 mM calcium sulphate solution; pre-germination was carried out in a germination chamber for 3 days at 28 °C. After the germination period, only healthy seedlings with uniform size were selected. Plants were then gently removed from the growing medium and then transplanted to the soil of the plastic pots used in the experiment.

The phytoremediation experiment was carried out in a growth chamber with a 16-hour day length and mean day and night temperatures of about 27 ˚C and 16 ˚C, respectively. Relativity humidity was maintained between 60% and 70%. The light source consisted of Sylvania Gro-lux Cool White Fluorescent Light (36 w) and Philips Master HPI-T Plus Metal Halide Lamps (400 w) in a 14:1 ratio, supplying a light intensity of 64,000 lux and photosynthetically active radiation (PAR) of 1,010 μmol m^{-2} s^{-1} at the top of the canopy. 350 g of air-dried soil were put in plastic pots of 10 cm diameter and 7 cm depth. Twenty seedlings of tall fescue and ryegrass, twelve seedlings of barley and five seedlings of maize were planted per pot. After planting, soil was maintained at 70% water holding capacity. 49 days after planting, the soils were spiked with aqueous solutions of atrazine (prepared from a 250 mg L^{-1} solution of atrazine in methanol; solid atrazine was supplied by Sigma-Aldrich, USA) in order to achieve

concentrations of 2, 5 and 10 mg of atrazine per kg of soil (corresponding to 3.25, 8.12 and 16.24 µmol per pot, respectively). The solution of atrazine was carefully added to the pots, avoiding direct contact with the plants' shoots. The doses used here were based on typical application rates of atrazine in agricultural soils, i.e., 1.12-2.24 kg ha^{-1} equivalent to soil concentrations of 3-6 mg kg^{-1} (Lesan and Bhandari, 2003). The plants were harvested after 65 days, that is, sixteen days after atrazine application. Shoots were cut at the soil surface, while roots were carefully separated and washed with deionized water to completely remove the soil particles. Roots and shoots were air-dried for seven days until they reached a constant weight. Bulk soil was sampled at the end of the experiment; it was air-dried and disaggregated prior to being analysed.

The experimental design of the phytoremediation test was a completely randomized design. It consisted of nineteen different treatments, each one consisting of three replicates, using the four plant species and three different values for the initial soil concentration of atrazine, i.e., 2, 5 and 10 mg kg^{-1}; controls consisting of unplanted pots spiked with the three doses of atrazine and planted pots without atrazine addition were also used.

2.3. Soil and plants extraction and analysis

Soil and plant samples were analysed in order to quantify the concentration of atrazine and its main metabolites. Atrazine derivatives were extracted from soil and plant samples, using an extraction procedure previously described by Amadori et al. (2013). A mass of 2.0 g of soil or plant tissues (previously chopped, crushed and blended) was weighed in 25 mL glass flasks, suspended in 3 mL of acetonitrile (Acetonitrile Chromasolv for HPLC, gradient grade, Sigma-Aldrich, USA) and shaken for 30 minutes in a rotary shaker. Then, the suspensions were

centrifuged for 15 minutes at 3500 rpm and the supernatant phases were reserved. The extraction step was carried out three times, and the respective supernatant phases were combined. The extracts were transferred to 10.0 mL volumetric flasks and filtered using 0.45 µm nylon membranes before HPLC determination.

The concentration of atrazine residues, i.e., atrazine and the dealkylated metabolites deethylatrazine (DEA) and deisopropylatrazine (DIA) in soil and plant extracts, was analysed using high-performance liquid chromatography (HPLC). 20 µL aliquots samples were injected into a HPLC system (Shimadzu Prominence UFLC XR, Japan) and analysed using a C18 reversed-phase column (240 mm x 4 mm) kept at 30 ºC. The mobile phase consisted of an acetonitrile and 0.01 M phosphate buffer solution (pH = 7.1) at a flow rate of 0.5 mL min^{-1} for 25 minutes. The operating conditions were: linear gradient from a 30:70 (v/v) acetonitrile:phosphate buffer to 40:60 from 0 to 3 min; linear gradient from 40:60 to 50:50 from 3 to 7 min; linear gradient from 50:50 to 30:70 from 7 to 10 min; and, finally, isocratic gradient of a 30:70 acetonitrile:phosphate buffer for 10-25 min. The UV detection was made at 222 nm for atrazine and its metabolites. Under these conditions, the retention times (RT) of atrazine, deethylatrazine and deisopropylatrazine were 16.1, 7.9 and 4.5 minutes, respectively.

TABLE 2

Throughout the text, concentrations of atrazine, DEA and DIA in soils and plants have been expressed as µmol (per g or per pot) instead of µg, due to the different molecular weight of the atrazine and its metabolites (Table 2).

2.4. Statistical analysis

Data were evaluated statistically by one-way ANOVA, and comparison of mean values (mean±SD) was done by using Duncan's honestly significant difference test at $p < 0.05$. The data normality was checked by using the Kolmogorov-Smirnov test. The SPSS 22 Statistical Software was used for the analysis.

3. RESULTS AND DISCUSSION

3.1. Effect of atrazine on plant biomass

FIGURE 1

Figure 1 shows the total root and shoot plant biomass at the end of the experiment. As can be seen, the exposure to atrazine affected plant growth in a different way, depending on both plant species and the amount of atrazine initially applied to the soil. The addition of atrazine in doses of 5 and 10 mg kg^{-1} caused a significant decrease of shoot, root and total biomass for all plant species, as compared to those of control pots (with the exception of maize roots for the 5 mg kg^{-1} dose) (Figure 1). Roots were affected to a higher extent than shoots; thus, root biomass reduction was in the range of 50-82% (excluding maize for the 5 mg kg^{-1} dose) (Figure 1B), while shoot biomass decreased between 22 and 60% (Figure 1C). Visual toxicity symptoms such as chlorosis, stunting and, in some cases, leaf death, were observed for the 10 mg kg^{-1} dose from approximately seven days after applying atrazine; visual effects were less

pronounced for the 5 mg kg^{-1} dose, with the exception of barley, which showed a similar condition for both 5 and 10 mg kg^{-1} doses. Addition of an atrazine dose of 2 mg kg^{-1} significantly affected the growth of the grass species but not the crops (Figure 1); nevertheless, that decrease was less pronounced than that found for higher doses of atrazine. On the whole, it can be said that the growth of the four plant species was markedly affected by the 10 mg kg^{-1} dose of atrazine, maize being the most resistant plant for the lower doses.

The toxic response of some plants to different levels of atrazine in soils has been reported elsewhere. Fang et al. (2001) found that *Lolium perenne* and *Festuca arundinacea* were not able to grow in a soil-perlite mixture spiked with 5 mg kg^{-1} of atrazine; however, Sudan grass (*Sorghum vulgare*) and switchgrass (*Panicum virgatum*) grew in the same soil. In another study, Merini et al. (2009) reported germination and growth of *Lolium multiflorum* in soil spiked with 1 mg kg^{-1} of atrazine, but only some plantlets survived in atrazine concentrations of 5 and 10 mg kg^{-1}. Ryegrass, tall fescue and spring onion have also been shown to develop symptoms of toxicity and death in long-term polluted soil with high concentrations of atrazine (Singh et al., 2004). Ibrahim et al. (2013) carried out a study to assess the performance of eight plant species for the phytoremediation of atrazine. They found that the descendent order of tolerance of plants to atrazine was: maize, bitter lupine, sweet sorghum, radish, sweet lupine, oat, soya bean and wheat. Those authors also found that atrazine affected plant roots more than shoots. Huang et al. (2007) also reported that maize growth was not affected by atrazine soil concentrations up to 5 mg kg^{-1}. Therefore, those studies strongly agree with our results, showing the difficulties of grasses in growing in soils with atrazine concentrations above 5 mg kg^{-1} and the higher tolerance of maize to that level of pollution. The toxic response of plants to atrazine is closely related to its uptake and accumulation (Li et al., 2012; Ibrahim et al., 2013); this issue will be further discussed in the next section.

3.2. Accumulation of atrazine in plant tissues

TABLE 3

Table 3 shows the concentration of atrazine (ATR) residues, i.e., atrazine, deethylatrazine (DEA) and deisopropylatrazine (DIA), in roots, shoots and the whole plant at the end of the experiment for the three doses of ATR initially applied to the soil. Plant uptake of atrazine and/or its metabolites was observed for all tested plants and for all initial doses of atrazine in soils. ATR residues concentrations in shoots greatly exceeded those corresponding to roots for tall fescue, barley and ryegrass; the values of the translocation factor (calculated as the ratio between shoot and root concentrations) were in the range 4.0-17.7, with the highest values corresponding to ryegrass. Conversely, root concentrations of maize were higher than those of shoots for all initial ATR doses, with translocation factors in the range 0.24-0.79. Shoot concentration of ATR residues was higher for grasses than crops, while the highest values of root concentration were found for maize (Table 3). In general, ATR residues plant concentrations increased as the pollution level of the soil was increased; nevertheless, some variations were not statistically significant ($p < 0.05$, Table 3) due to large differences between replicates and/or the high impact of atrazine toxicity on plant growth (e.g., maize for the 10 mg kg^{-1} dose). More specifically, the differences in ATR residues plant concentrations between the 2 mg kg^{-1} and 10 mg kg^{-1} doses were statistically significant in all the cases, while data obtained for the 5 mg kg^{-1} dose were not significantly different to those of the 2 and 10 mg kg^{-1} doses, depending on the plant species and/or the tissue considered (shoots or roots).

The uptake of atrazine and its accumulation and/or degradation by several plant species has been previously reported (Fan and Song, 2014). Maize has been shown as capable of accumulating atrazine in its tissues, reaching higher ATR concentrations in roots than shoots; moreover, in agreement with our results, ATR plant concentrations rose with increasing concentrations of atrazine in the growth media (Huang et al., 2007; Li et al., 2012). Several studies have also reported the respective ability of different grass species in atrazine accumulation and phytodegradation (Fang et al., 2001; Henderson et al., 2007; Lin et al., 2008; Merini et al., 2009; Murphy and Coats, 2011; Albright III et al., 2013). Henderson et al. (2007) found higher concentrations of ATR residues in the leaves than in the roots of a mixture of three native prairie grasses grown over 21 days (big bluestem, yellow indiangrass and switchgrass); however, Murphy and Coats (2011) reported higher concentrations of atrazine residues in the roots than in the shoots of switchgrass, although this study only lasted seven days. Therefore, the accumulation pattern between roots and shoots could be dependent on the specific plant species and/or experimental conditions (initial soil concentration, duration of tests, etc.).

FIGURE 2

Figure 2 shows the total plant accumulation of ATR residues, expressed as μmol of ATR, DEA and DIA per pot. The overall performance of the studied plant species in the accumulation of atrazine and its metabolites was barley < tall fescue ≈ ryegrass < maize. More specifically, the accumulation of ATR residues in tall fescue, barley and ryegrass was between 4.2 and 36.2% of the total amount accumulated by maize. The superior ability of maize to take up and accumulate atrazine could be attributed on one hand to its greater

biomass and on the other hand to its higher leaf surface, and therefore its higher transpiration rate, which has been reported as one of the key factors related to the rate of absorption and translocation of triazine herbicides (Burken and Schnoor, 1997; Lin et al., 2008). Nevertheless, it must be pointed out that high soil doses of atrazine (i.e., 10 mg kg^{-1}, Figure 2) led to a lower accumulation yield in maize due to the significant damage caused to plant growth (Figure 1). A significant negative correlation between the total plant accumulation of atrazine and the total plant biomass was found for tall fescue, barley and ryegrass (Pearson's correlation coefficient; $r = -0.599$, $p < 0.001$). In the case of maize, the low amount of atrazine and metabolites accumulated for the highest dose of atrazine (Figure 2) did not indicate a significant correlation with plant biomass. Therefore, it can be concluded that the increase in plant toxicity observed for increasing doses of atrazine applied to the soil (Section 3.1) can be attributed to a higher uptake and accumulation of atrazine in the plant tissues.

Taking into account previous studies about atrazine uptake and degradation in plants (Murphy and Coats, 2011; Albright III et al., 2013; Fan and Song, 2014), the present study was initially focused on atrazine and its main dealkylated metabolites, i.e., DEA and DIA; according to this, the methods of extraction and analysis of soil samples were only optimized for atrazine, DEA and DIA (Section 2.4). However, other hydroxylated atrazine metabolites such as hydroxyatrazine (HA), deethylhydroxyatrazine (DEHA) or deisopropylatrazine (DIHA) have also been detected in plant tissues for experiments using different grasses (Henderson et al., 2007; Lin et al., 2008). The presence of those ATR metabolites cannot be discarded in our study, although no significant chromatographic peaks other than ATR, DEA or DIA were found in this experiment.

DEA and DIA metabolites were detected in measurable concentrations in practically all plant species and for all doses of atrazine added to soils (Figure 2). DEA was the major derivative of atrazine present in shoots, reaching even higher concentrations than those of atrazine in all plant species for the 2 and 5 mg kg^{-1} soil ATR doses. However, ATR shoot concentrations were similar or higher than those of DEA for the highest soil ATR dose used (Figure 2). The results from roots were somewhat different: tall fescue, barley and ryegrass showed root ATR concentrations similar or higher than those of DEA (with the only exception being ryegrass, when an initial dose of 2 mg kg^{-1} of ATR was used); however, maize accumulated DEA to a higher extent than atrazine for the 2 and 5 mg kg^{-1} soil ATR doses, while atrazine was prevalent for the highest soil dose. Plant concentrations of DIA were much lower than those of DEA, with some values under the detection limit and, in general, reached higher concentrations in roots than shoots; the high values of DIA concentrations found for maize were also remarkable (Figure 2).

It must be pointed out that the experimental methodology used in this study does not let us distinguish between degradation of atrazine occurring in the soil and degradation within the plant after uptake. This problem has also been explicitly indicated in a previous work by Lin et al. (2008); nevertheless, it seems to be considered as generally agreed that the metabolites found in plant tissues come mainly from the degradation inside the plant (Lin et al. 2008; Murphy and Coats, 2011; Fan and Song, 2014). The observed increase of the ratio parent atrazine:DEA in plant tissues found for the highest ATR soil dose (Figure 2) could be evidence that most ATR metabolites come mainly from the degradation inside the plants. Moreover, it means that the ability of plants to degrade atrazine is limited and/or the atrazine uptake is a process faster than its degradation inside the plant.

In any case, the results obtained here showed that atrazine degradation proceeded preferentially by the deethylation, leading to DEA formation. The other N-dealkylated metabolite, DIA, was also produced but to a much lesser extent than DEA; only maize showed significant DIA concentrations (Figure 2). It has been reported that the deethylation rate of atrazine is approximately 2–3 times higher than the rate of deisopropylation (Fan and Song, 2014), being usually the main path found for the atrazine metabolism in grasses (Lin et al., 2008; Murphy and Coats, 2011) and hybrid poplars (Burken and Schnoor 1997). The degradation of atrazine to DEA and DIA in maize roots has been also reported elsewhere (Huang et al., 2007). According to the literature, atrazine phytodegradation could have been produced in both roots and shoots, although the much greater concentrations of DEA found in shoots of tall fescue, barley and ryegrass would indicate that DEA had been quickly translocated from roots. Together with the preferential DEA formation, the higher degradation rate of DIA could also contribute to the low DIA concentrations found for those plant species (Burken and Schnoor, 1997; Murphy and Coats, 2011). However, the higher concentrations of DEA and DIA found in maize roots would be due to a slow transfer rate of atrazine residues from roots to shoots; Li et al. (2012) have suggested that maize could reduce its transpiration rate and, therefore, the transportation of atrazine from roots to shoots, as a mechanism to avoid atrazine toxicity. It should be taken into account that deethylation and deisopropylation of atrazine are mechanisms of plant detoxification, because those metabolites are less toxic than the parent atrazine (Fan and Song, 2014). The significant higher proportion of parent atrazine as compared to metabolites in the plants corresponding to the 10 mg kg^{-1} soil dose highlights the difficulties of plants in attaining atrazine detoxification at such high soil concentration.

3.3. Atrazine residues in soil

FIGURE 3

TABLE 4

The total amount of atrazine residues (atrazine, DEA and DIA) remaining in the soil after the phytoremediation test is shown in Figure 3. Firstly, it can be seen that regardless of the initial dose of atrazine, the amounts of parent atrazine remaining in the soil of the planted pots after the experiment were significantly lower ($p < 0.05$) than those of the unplanted ones (with the exception of tall fescue for the 2 mg kg^{-1} soil dose) (Figure 3). Specifically, the initial ATR of the unplanted pots decreased by 63.1-78.2%, depending on the initial ATR dose, while the reductions observed for the planted pots were in the range 88.6-99.6% (Table 4). So, the presence of plants increased ATR removal with respect to unplanted pots by approximately 10-36%. Moreover, while the parent atrazine accounted for 75-96% of the ATR residues remaining in the soil of the control pots after the experiment, ATR degradation products were the majority in the planted pots, i.e., 57-88% of the total ATR residues (excluding tall fescue and maize for the 10 mg kg^{-1} soil dose) (Figure 3). Therefore, those results clearly demonstrated the decisive role of the plants in the atrazine removal from soils. In our experiment, tall fescue was the plant species with the lowest overall effectiveness on atrazine removal, while maize, barley and ryegrass showed similar results (Table 4). These results are in agreement with previous studies in which an increase in atrazine mineralization and/or degradation of 20-45% was observed by using *Lolium multiflorum* (Merini et al., 2009) and several grass species, including tall fescue (Lin et al., 2008; Murphy and Coats, 2011). Maize has been also shown capable of degrading atrazine to a higher extent than non-planted soils (Ibrahim et al., 2013). However, these studies contrast with others in which there was no

enhancement of atrazine dissipation by using several grasses (Henderson et al., 2007) or even that it was greater in the non-planted soils (Fang et al., 2001).

Atrazine removal from soils would be the result of several processes, such as degradation by plant enzymes both inside or outside the plant (phytodegradation), microbial degradation (bioremediation) and/or the enhancement of the microbial activity by the presence of plant roots (rhizodegradation) (Albright III et al., 2013; Fan and Song, 2014). With the experimental methodology used here it was not possible to totally discriminate the contribution of the different mechanisms from the overall atrazine removal. However, some comments can be made from Table 4. Maize was capable of accumulating up to 38.4% of the atrazine (as parent atrazine or as atrazine metabolites) initially added to the soil (this value corresponded to the initial soil dose of 2 mg kg^{-1}), while the rest of the plant species were only capable of accumulating around 2% of the initial atrazine. Tall fescue, barley and ryegrass are C_3 plant species while maize is a C_4 species; the difference between these two types of plant species in degradation and mineralization of atrazine in soils has been previously reported (Lin et al., 2005, 2008).The accumulation rates showed in Table 4 for tall fescue and ryegrass are in good agreement with those previously reported by Lin et al. (2008) for several grasses (tall fescue, orchardgrass, timothy, smooth bromegrass and switchgrass) and by Henderson et al. (2007) for a mixture of prairie grasses (big bluestem, yellow indiangrass and switchgrass). Therefore, rhizodegradation and/or plant degradation seem to be the main processes responsible for the atrazine removal from soils in our study. However, the high percentage of atrazine removal and the low proportion of ATR degradation products remaining in the soil of the unplanted pots suggests that in addition to chemical and biochemical degradation, other processes such as atrazine volatilization (probably in the first

hours after its application to soils) cannot be discounted (Kookana et al., 2010; Amadori et al., 2016; De Paula et al., 2016).

N-dealkylation has been cited as the preferred degradation pathway when the degradation of atrazine in soils is mainly carried out by microorganisms (Mudhoo and Garg, 2011). Therefore, the significant higher proportion of N-dealkylated metabolites in the soil of the planted pots relative to the unplanted ones after the phytoremedation test would give additional evidence of the improvement of bacterial activity in the rhizosphere. It has been reported that *Pennisetum clandestinum* increased significantly both microbial biomass and dehydrogenase activity, leading to a subsequent enhancement of the atrazine degradation effectiveness (Singh et al., 2004). Similar increases in microbial biomass and atrazine degradation yields have been reported for several grass species (Fang et al., 2001; Lin et al., 2008). The N-dealkylated metabolites DEA and DIA have been shown to be less phytotoxic than atrazine, since although they also exhibit photosynthetic inhibition, it is less than half that of atrazine (Lerch et al., 1995; Burken and Schnoor, 1997). In contrast to what was found for plant tissues (Section 3.2), DIA was the prevalent metabolite found in the soils of the planted pots for tall fescue, barley and ryegrass. In the case of maize, DIA concentration was higher than that of DEA only for the 2 mg kg^{-1} soil dose. Since the biochemical degradation of atrazine proceeds via deethlylation are preferable to deisopropylation (Henderson et al., 2007; Lin et al., 2008; Murphy and Coats, 2011), one possible reason to explain the prevalence of DIA in soils at the end of our experiment could be that DEA is taken up by plant roots preferentially to DIA; this hypothesis is based on the fact that log K_{ow} of DEA is higher than that of DIA (1.52 vs 1.13, Table 2) and therefore, its transport through the membranes of the roots would be easier (Briggs et al., 1982; Burken and Schnoor, 1997). The

different behaviour of maize again points out the specificity of phytodegradation pathways and absorption mechanisms for different plant species (Lin et al., 2008; Li et al., 2012).

4. CONCLUSIONS

A 16-day phytoremediation experiment was conducted in order to assess the ability of four plant species, i.e., tall fescue, barley, ryegrass and maize, for atrazine degradation in spiked soils. The data obtained here showed that the four plant species were capable of taking up and detoxifying atrazine to some extent. Nevertheless, toxic responses of plants, such as visual symptoms and biomass decreases, were detected for initial doses of atrazine in soils above 2 mg kg^{-1}; this was related with the accumulation of atrazine in their tissues. Atrazine residues (atrazine, deethylatrazine and deisopropylatrazine) were mainly accumulated in shoots of tall fescue, ryegrass and barley; conversely, root concentrations of ATR residues in maize were higher than those of shoots. Maize accumulated a total amount of atrazine residues, much higher than the rest of the plant species tested here; it was capable of accumulating up to 38.4% of the initial atrazine (as parent atrazine or as atrazine metabolites), which was attributed to its higher biomass and transpiration rate. Between atrazine degradation products, deethylatrazine was the main metabolite detected in the plant tissues, although deisopropylatrazine was also detected in measurable concentrations.

Plants were able to increase by approximately 10-36% the yields of atrazine removal with respect to that of the unplanted pots, demonstrating that phytoremediation of atrazine is more effective than natural attenuation. Since atrazine accumulation in plant tissues was only significant in maize, other mechanisms of atrazine removal have been proposed. Thus, the prevalence of ATR metabolites in the soil of the planted pots suggested that an important part

of atrazine was removed by biochemical degradation (to intermediate metabolites or to complete mineralization), carried out by the microorganisms of the rhizosphere and/or by enzymes coming from plants. Unfortunately, the experimental methodology used here does not allow us to discriminate between the different mechanisms of atrazine degradation in soils, nor if the metabolites found in plant tissues come from the degradation inside the plant or were taken up from the soil.

Acknowledgements

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FIGURE CAPTIONS

Figure 1. Total plant (A), root (B) and shoot (C) biomass (g DW per pot) of the different plant species (Fa: tall fescue; Hv: barley; Lp: ryegrass; Zm: maize) at the end of the experiment for the different initial soil doses of atrazine (Control: not spiked soil). Error bars represent the SD of three replicates. Different letters indicate significant differences ($p<0.05$, Duncan's test) between different initial soil doses of atrazine.

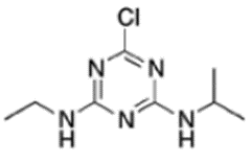
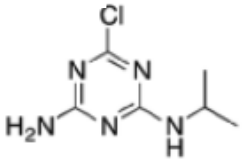
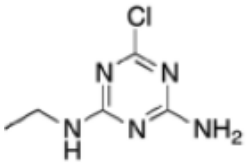
Figure 2. Total atrazine residues uptake (µmol 10^{-3} per pot) and the distribution of the atrazine derivatives (ATR: atrazine, DEA: deethylatrazine; DIA: deisopropylatrazine) in shoots and roots of the plants at the end of the experiment for the different initial soil doses of atrazine (Dose2: 2 mg kg^{-1}; Dose5: 5 mg kg^{-1}; Dose10: 10 mg kg^{-1}). Error bars represent the SD of three replicates. Different letters indicate significant differences ($p<0.05$, Duncan's test) between different initial soil doses of atrazine in each plant species.

Figure 3. Total atrazine residues and the distribution of the atrazine derivatives (ATR: atrazine, DEA: deethylatrazine; DIA: deisopropylatrazine) remaining in the soil at the end of the experiment for the different treatments (C: control/unplanted pots; Fa: tall fescue; Hv: barley; Lp: ryegrass; Zm: maize). Error bars represent the SD of three replicates. Different letters indicate significant differences ($p<0.05$, Duncan's test) between different soil treatments in each dose.

Table 1. Selected properties of soil used in the experiment.

Property	Value
Soil organic carbon content (%)	0.60
Specific gravity (g cm^{-3})	1.54
Water holding capacity	33%
pH	9.42
CEC (cmol kg^{-1})	23.42
Electrical conductivity (mS cm^{-1})	0.15
Clay (<0,002 mm)	39.6%
Silt (0,002-0,05 mm)	22.6%
Sand (0,05-2 mm)	37.8%

Table 2. Physical-chemical characteristics of atrazine and its main metabolites.

Property	Atrazine (ATR)	Deethylatrazine (DEA)	Deisopropylatrazine (DIA)
Molar mass (g mol^{-1})	215.68	187.68	173.68
Water solubility (mg L^{-1}) [a]	33	3200	670
pK_a	1.68-1.71	1.30-1.65	1.30-1.58
log K_{ow} [a]	2.20-2.70	1.52	1.13
Chemical structure			

[a] Taken from Amadori et al. 2016

Table 3. Accumulation of atrazine residues (µmol kg^{-1} DW biomass) in tall fescue (Fa), barley (Hv), ryegrass (Lp) and maize (Zm) plants at the end of the phytoremediation experiment.

Values are given as the mean ± standard desviation (n = 3). The different letters indicate significant differences ($p<0,05$, Duncan's test) between soil treatments.

		ATR+DEA+DIA (µmol kg^{-1})		
Plant	**Treatment**	**Root**	**Shoot**	**Total plant**
Fa	Dose 2	11.1±4.60a	59.6±17.8a	45.0±11.0a
	Dose 5	9.8±3.9a	119.2±37.8b	90.2±28.0b
	Dose 10	12.2±5.0a	180.7±21.9c	150.9±49.1c
Hv	Dose 2	2.1±0.9a	8.4±2.7a	7.0±2.0a
	Dose 5	4.8±0.8b	23.7±2.8b	20.5±1.8b
	Dose 10	6.7±1.7b	37.7±9.4c	31.6±4.0c
Lp	Dose 2	2.1±0.8a	37.1±1.6a	23.4±1.8[a]
	Dose 5	8.0±3.2ab	85.7±17.3a	60.1±11.9[a]
	Dose 10	12.3±5.0b	169.4±56.9b	140.9±54.8b
Zm	Dose 2	102.2±23.4ab	25.0±4.4a	52.9±10.9a
	Dose 5	182.5±87.8b	71.8±12.9b	106.4±19.5b
	Dose 10	108.4±76.9a	85.7±37.0b	70.8±22.6ab

Table 4. Distribution of atrazine and its metabolites at the end of the phytoremediation experiment and atrazine removal from soils.

	% of the initial ATR				
	Control	**Tall fescue**	**Barley**	**Ryegrass**	**Maize**
Total soil	29.1-38.5	5.79-30.4	1.70-10.8	1.85-12.8	3.81-11.4
Atrazine	21.8-36.9	3.73-11.4	0.65-3.64	0.39-3.39	1.38-2.85
DEA	0.61-3.73	0-0.32	0-0.64	0.37-1.58	0.68-5.20
DIA	0.66-3.54	1.74-19.0	0.51-7.16	1.08-7.83	1.12-4.85
Plant accumulation		1.23-2.43	0.71-1.96	1.40-2.16	3.66-38.4
Atrazine		0.17-0.59	0.04-0.29	0.10-1.00	0.10-1.45
DEA		0.61-2.00	0.38-1.20	0.35-1.89	1.74-25.0
DIA		0.04-0.18	0.05-0.60	0.05-0.17	0.46-12.8
Atrazine removal[a]	63.1-78.2	88.6-96.7	96.4-99.4	96.6-99.6	97.2-98.6

[a] Calculated using only the amount of the parent atrazine remaining in the soil at the end of the experiment (2nd row of the Table)

Figure
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FIGURE 1

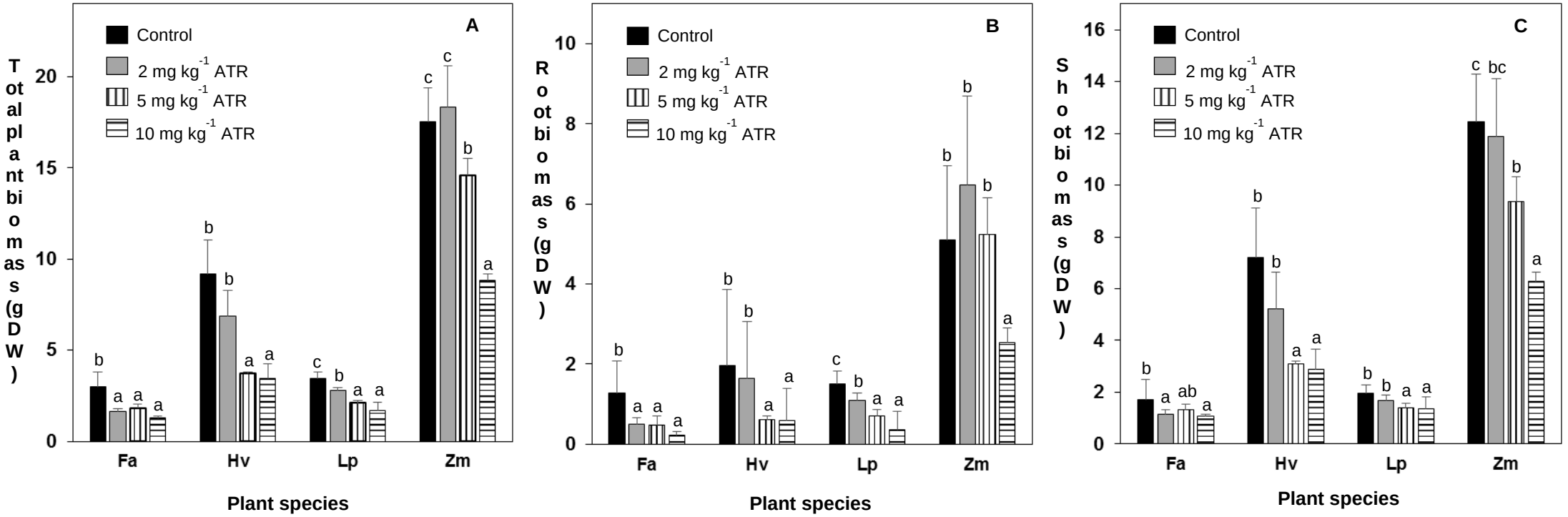


FIGURE 2

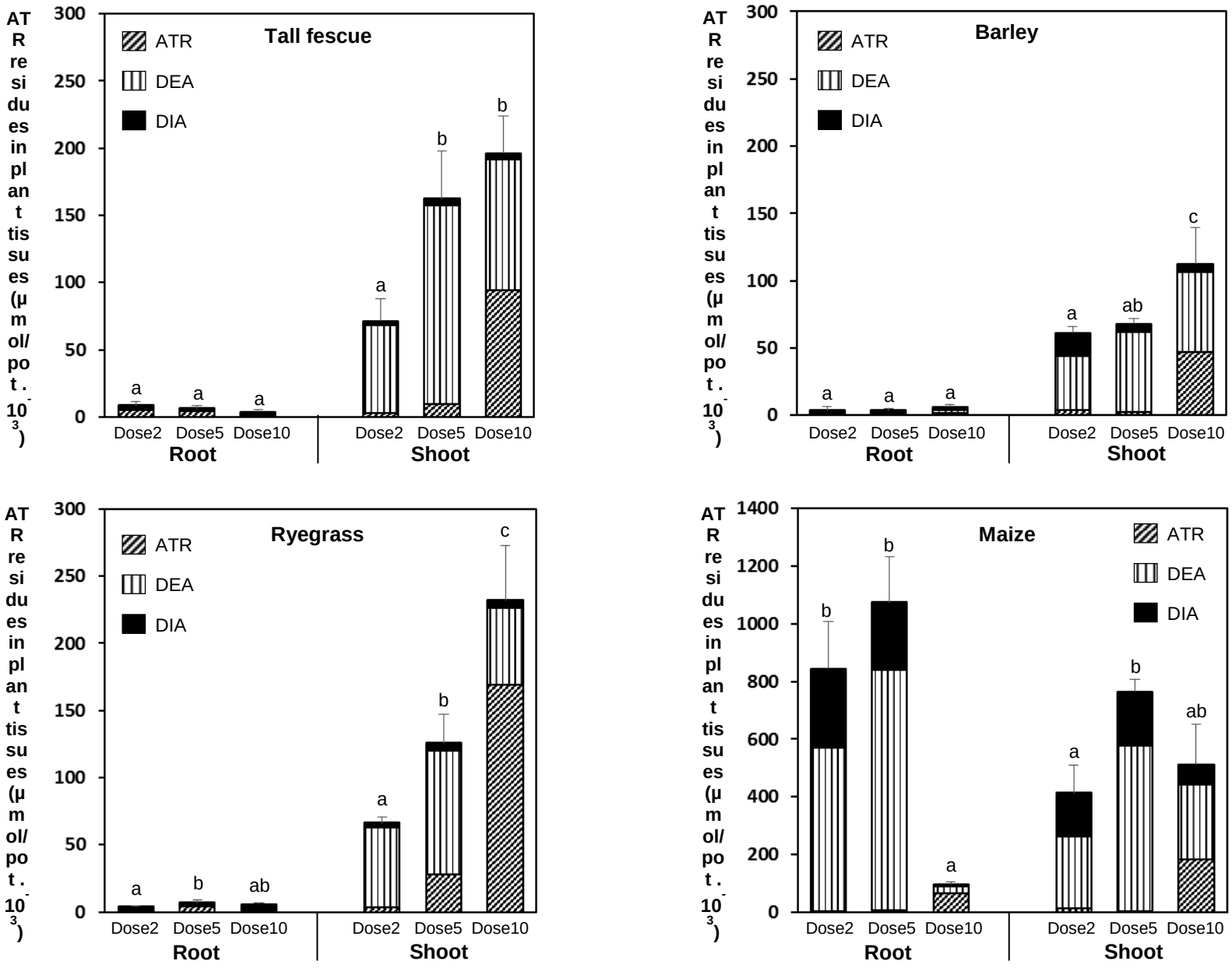
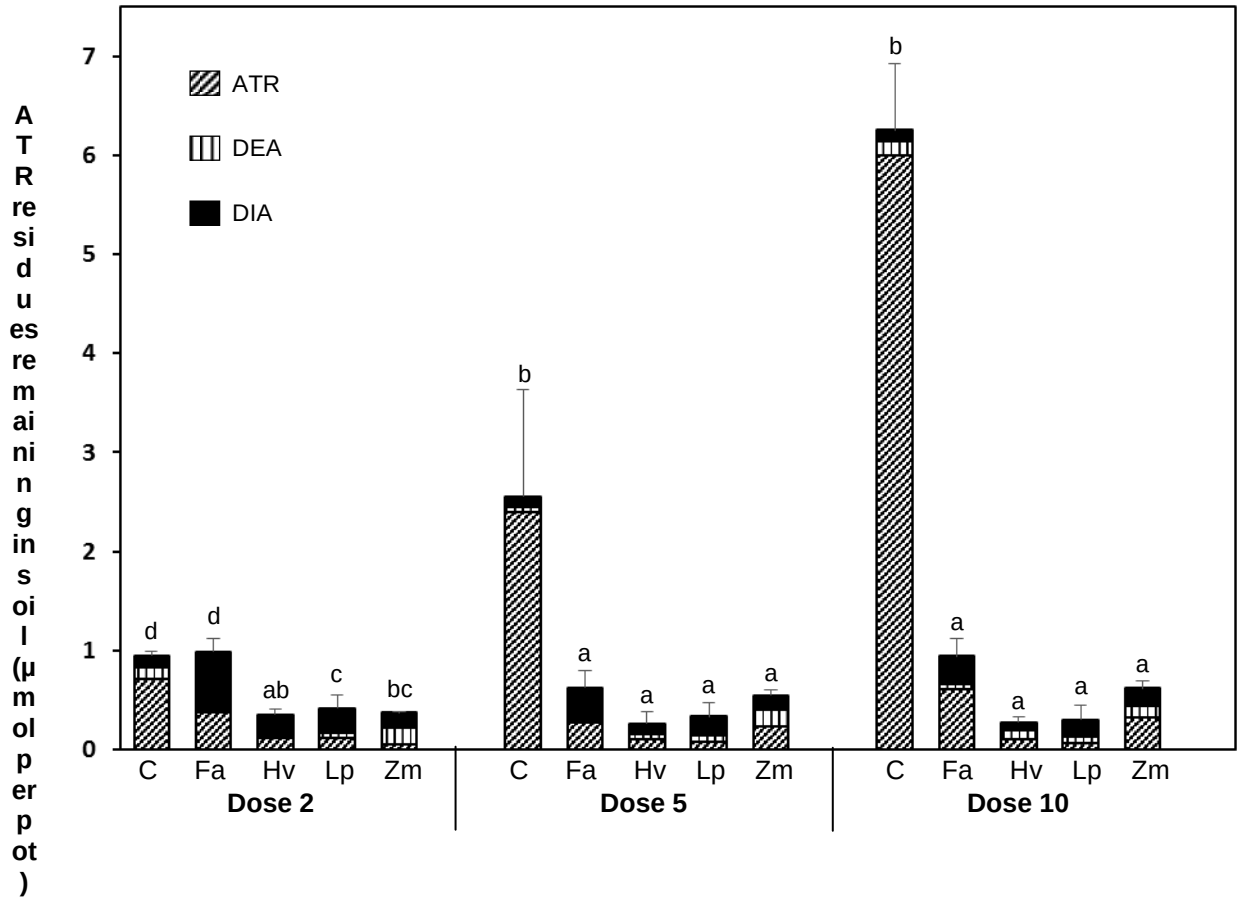

ATR residues in plant tissues (µmol/pot.10-3)
Tall fescue
Barley
Ryegrass
Maize
ATR
DEA
DIA
Dose2
Dose5
Dose10
Root
Shoot

FIGURE 3


ATR residues remaining in soil (µmol per pot)
ATR
DEA
DIA
0
1
2
3
4
5
6
7
d
d
ab
c
bc
b
a
a
a
a
b
a
a
a
C
Fa
Hv
Lp
Zm
Dose 2
Dose 5
Dose 10