Leaching of S-metolachlor, terbuthylazine, desethyl-terbuthylazine, mesotrione, flufenacet, isoxaflutole, and diketonitrile in field lysimeters as affected by the time elapsed between spraying and first leaching event

(Article begins on next page)

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Leaching of S-metolachlor, terbuthylazine, desethyl-terbuthylazine, mesotrione, flufenacet, isoxaflutole, and diketonitrile in field lysimeters as affected by the time elapsed between spraying and first leaching event

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Abstract

The effect of elapsed time between spraying and first leaching event on the leaching behavior of five herbicides (terbuthylazine, S-metolachlor, mesotrione, flufenacet, and isoxaflutole) and two metabolites (desethyl-terbuthylazine and diketonitrile) was evaluated during a 2011 and 2012 study in northwest Italy. A battery of 12 lysimeters ($8.4m^2$ large with a depth of 1.8m) were used in the study, each filled with silty-loam soil and treated during pre-emergence with the selected herbicides by applying a mixture of commercial products Lumax (4 L/ha) and Merlin Gold (1 L/ha). At treatment times, no gravity water was in the lysimeters. Irrigation events capable of producing leaching (40mm) were conducted on independent groups of three lysimeters at 1 day after treatment (1DAT), 7 DAT, 14 DAT, and 28 DAT. The series was then repeated fourteen days later. Leachate samples were collected a few days after irrigation; compounds were extracted by SPE and analyzed by HPLC and GC-MS. Under study conditions, terbuthylazine and S-metolachlor showed the highest leaching potentials. Specifically, S-metolachlor concentrations were always found above 0.25 µg/L. Desethylterbuthylazine was often detected in the leached waters, in most cases at concentrations above 0.1 µg/L. Flufenacet leached only when irrigation occurred close to the time of herbicide spraying. Isoxaflutole and mesotrione were not measured (<0.1 µg/L), while diketonitrile was detected in concentrations above 0.1 µg/L at 1DAT in 2011 only.

Keywords: herbicides, metabolite, lysimeters, leaching, water, contamination, time

Introduction

Ground water pollution due to pesticides applied in agriculture has been confirmed by several studies conducted in various settings around the world. [1-3] [4-7] Public concern about pesticide presence in ground waters comes from the possible impact of these chemicals on human health and

42 ecological systems. [8]For Europe as a whole, the 2000 Water Framework Directive (WFD) set

43 protection standards for water resources from any form of pollution. [9] In most European countries

44 where ground water is the main source of drinking water, an offshoot directive of the WFD called

45 the Ground Water Directive (GWD) [10] established specific groundwater pollution prevention and

46 control measures, and Drinking Water Directive 98/83/EC set maximum concentrations for

47 individual pesticides and their metabolites (0.1 μg/L) and total pesticides (0.5 μg/L) for water

48 intended for human consumption. The GWD dictated these same quality standards.

49 Herbicides (47.5%) dominate worldwide pesticide consumption; insecticides (29.5%), fungicides

50 (17.5%), and other products (5%) represent smaller portions. [11] Considering these proportions, it is

51 not surprising that herbicides or their metabolites are the most frequently found pesticides in surface

52 and ground waters. [12-16] Furthermore, the very nature of herbicide application *versus* other

53 pesticide categories exacerbates potential pollution risk as they are generally applied toward the soil

54 since weeds are their target organisms.

55 The risk of leaching by herbicides applied in agriculture is generally highest for those applied in

56 pre-emergence. They are typically applied to crop/weed-free seed beds, where some require soil

57 incorporation, some need good soil moisture or rainfall for activation, and many are characterized

58 by residual activity. [17] All of these factors may enhance chemical transport through the soil profile

59 with a consequent risk of pollution to ground waters.

60 Aquatic environments are more vulnerable to contamination by pre-emergence pesticides. [18] A

61 review by Flury [19] found post-emergence pesticide application reduced leaching relative to pre-

62 emergence application. The reduction was attributed to conditions of lower rainfall amounts and

63 higher temperatures that generally occur during post-emergence applications and likely enhance

64 chemical dissipation. This phenomenon is confirmed by the fact that the majority of the herbicides

65 detected in surface and ground waters are pre-emergence herbicides. [6, 14, 15]

66 Among herbicides, triazines and chloroacetanilides are some of the substances most frequently

67 found in surface and ground waters. [2, 14, 15, 20, 21] Terbuthylazine and S-metolachlor are widely used

68   to control weeds in maize cultivation. In some European countries since 1991, atrazine was replaced

69   by terbuthylazine after the former was banned for its water pollution risk. [22] Its good efficacy,

70   particularly against broad-leaved weeds, resulted in wide use of the herbicide, often in combination

71   with S-metolachlor. A great debate in the last years about triazine- and triazine metabolite-caused

72   environmental problems has led to routine screening of triazine metabolites in most environmental

73   monitoring campaigns today. [23] In recent years, different actions have been carried out to reduce

74   water contamination risk from triazines, such as reduced application rates, excluded use in

75   combination with other herbicides, restricted application in areas classified as vulnerable to

76   pesticide leaching, and interdicted use proximal to water bodies. S-metolachlor was introduced in

77   Italy in 2003 as a selective substitute herbicide for metolachlor to control grasses. It is a selective

78   herbicide, absorbed by the shoots and hypocotyls that inhibit weed germination. S-metolachlor is

79   generally applied during pre-planting, pre-emergence, or early-post-emergence. [24]

80   Mesotrione is another important herbicide applied pre- or post-emergence to control several broad-

81   leaved weeds in maize. It is a member of the triketone family of herbicides that is applied mainly in

82   combination with other herbicides. It equips maize growers with an herbicide that works by

83   extending weed suppression activity and controlling some weeds resistant to triazine and ALS-

84   inhibiting herbicides. [25] Mesotrione is a week acid with a *pKa* of 3.1 [26] that dissociates from the

85   molecular to anionic form as pH rises. Its environmental behavior is strictly related to soil pH and

86   organic matter content; these two parameters make the herbicide more or less available to

87   dissipation and transfer.

88   Flufenacet is an oxiacetamide herbicide effective in pre- and post-emergence against many grasses

89   in corn, wheat, rice, tomato, and other crops. [27, 28] It is generally associated with a broad-leaved

90   herbicide to control the complete weed spectrum. Isoxaflutole is a broad spectrum pro-herbicide of

91   the isoxazole family used during maize pre-emergence or pre-plant against grass and broad-leaved

92   weed species mostly. [24] Pallet et al., [29] reports the complete mode of action of isoxaflutole.

93   The aim of the study was to evaluate the leaching behavior of five herbicides (terbuthylazine, S-

94   metolachlor, mesotrione, flufenacet, and isoxaflutole) and two metabolites (desethyl-terbuthylazine

95   and diketonitrile) as affected by time elapsed between spraying and the first leaching event.

96

97   **Materials and methods**

98

99   The study was carried out in 2011 and 2012 at the experimental station of the Dipartimento di

100   Scienze Agrarie, Forestali e Alimentari of the University of Torino, Italy. The experimental station

101   is located in the municipality of Carmagnola (NW Italy, 44° 53' 08.99'' N, 7° 41' 11.33'' E;

102   WGS84) in an area of the Po Valley historically cultivated with maize.

103

104   *Lysimeters- Structural feature descrition*

105   The lysimeter set-up consists of 12 lysimeters placed in two adjacent rows built in 1991. Each

106   lysimeter has a surface area of 8.4 m^2 (2.8x3 m) and a depth of 1.8 m with a rectangular high-

107   density polyethylene frame that opens at the top. Each was filled with agriculturally-disturbed soil

108   from the natural layers of the experimental station soil. The soil was *Typic Udifluvent* (34.8% sand,

109   59.9% silt, 5.4% clay), with low organic matter content (0.44% on average in the 0-2.2 m depth).

110   To ensure field-like conditions, the lysimeters were buried and subjected to the same agronomic

111   practices of the neighboring area. To facilitate percolating water discharge, a series of polyethylene

112   tubes arranged horizontally was laid at the base of the each lysimeter. Soil was separated from the

113   tubes by three layers: one of gravel (30 cm), one of sand (30 cm), and one made of non-woven

114   polypropylene fiber. These layers constituted the drain component for each lysimeter.

115

116   *Lysimeters-Physical and hydrological soil characteristic description*

117   The groundwater level was about 6 m deep with minimal seasonal variation, the soil bulk density

118   was 1.30 Mg m^3 in the 0-0.5 m soil depth, and the water content at saturation averaged

119   0.56 mm^3 mm^{-3}. [30, 31] Although the soil profile of the lysimeters was not identical to the original

120   soil, the main soil hydrological parameters and the soil bulk density were very similar, made

121   evident by a physical and hydrological characterization of the lysimeter soil performed eight years

122   after their installation by Zavattaro and Grignani [32]. Lysimeter and undisturbed soil differences

123   were evaluated at two depths (0-20cm and 20-50cm) while the bulk density and water tension were

124   considered at 0 kPa, 33 kPa, and 1500 kPa. Bulk density differences were encountered only in the

125   plowed layer as the lysimeter soil was spade-tilled, so that no soil compaction from machinery

126   transit occurred. In the deeper layer, minimal differences were found between lysimeter soil and the

127   undisturbed one. The abilities of the soils to store water were evaluated at field capacity and at the

128   permanent wilting point; a few differences were observed only for volumetric water content at field

129   capacity. [30] The soil infiltration rate (70 mm h^{-1}) was indicated in a more recent previous study. [33]

130

Lysimeters—Current study set-up description

132   Each lysimeter drained by gravity into a 200L collection tank placed 2.5m deep into an inspection

133   chamber. Each inspection chamber hosted the collection tanks of four lysimeters. The flow from the

134   lysimeter to the tank can be manually regulated by means of a valve situated on the bottom of the

135   lysimeter. After each percolation event, the water was removed from the tank by electric pump, and

136   the percolated volume was measured with an in-line flow meter (K24 Turbine meter, Piusi

137   Instruments, Suzzara, Italy).

138

Lysimeters-Agronomic practices

140   The lysimeters were cultivated with maize following the local agronomic practices used for this

141   crop. Maize was also grown in the soil surrounding the lysimeters to mimic actual crop-like

142   conditions. Two weeks before crop sowing, the lysimeter soil was hand-tilled with a spade to a

143   depth of 30 cm, and then fertilized with 65 kg ha^{-1} of KCl and 45 kg ha^{-1} of triple superphosphate.

144   Crop sowing was done on April 20 in 2011 and May 14 in 2012. In 2012, the crop was sown later in

the season due to unfavorable weather. Total rainfall was 637.4 mm, monitored by experimental station meteorological devices and expressed as a yearly average over the past ten years, with rainfall occurring mainly during the spring and autumn seasons. Herbicides were applied a few days after sowing during pre-emergence.

Chemicals applied and studied

Lysimeters were sprayed immediately after maize seeding with Lumax ® (Syngenta Crop Protection Italia, Milano, Italy), an herbicide containing terbuthylazine (187.5 g L^{-1}), S-metolachlor (312.5 g L^{-1}), and mesotrione (37.5 g L^{-1}), as well as with the herbicide Merlin Gold ® (Bayer CropScience Italia SpA, Milano, Italy) containing flufenacet (428.4 g L^{-1}) and isoxaflutole (51.5 g L^{-1}). Application of these two herbicides occurred at rates of 4.0 L ha^{-1} (Lumax ®) and 1 L ha^{-1} (Merlin Gold ®). The main degradation product of terbuthylazine (desethyl-terbuthylazine) and the first metabolite of isoxaflutole (diketonitrile) were also evaluated in this study. Treatment was applied using a conventional tractor with rear-mounted boom sprayer adjusted to deliver 400 L/ha of herbicide mixture. The sprayer was inspected before herbicide application to ensure proper functioning of the nozzles. Table 1 lists the physical-chemical properties of the studied substances.

Lysimeter preparation

Prior to the start of each growing season trial, the lysimeters were irrigated with a volume of water previously calculated to exceed the field capacity of the upper 0.5 m soil layer by 25%. After irrigation, the lysimeters were left to fully discharge the percolating water to ensure the absence of gravitational water flows. Herbicide application took place only after percolation was complete. In 2011, herbicide application occurred one week after percolation ended due to unfavorable weather conditions for spraying (rainy and windy conditions), whereas the herbicide was sprayed the day after percolation ended in 2012. To avoid interference from forecasted rain during lysimeter

170   preparation and after herbicide application, temporary covers were applied to the lysimeters and

171   removed immediately after rainfall. Weather conditions after herbicide application were generally

172   good in 2011, making it necessary to cover the lysimeters only a few times. By contrast, several

173   events occurred in 2012 after herbicide spraying that caused the lysimeters to be covered for a total

174   of about ten days, distributed across seven events.

175

176   ***Simulation of percolation after herbicide application***

177   Lysimeter percolation was induced with irrigations using water pumped from a 30m-deep well

178   placed about 150 m from the lysimeters. The irrigations were performed on independent groups of

179   three lysimeters each, randomly selected at either 1 DAT (days after treatment), 7 DAT, 14 DAT, or

180   28 DAT. The process was repeated 14 days later, resulting in a second set of irrigations either at 15

181   DAT (1+14), 21 DAT (7+14), 28 DAT (14+14), or 42 DAT (28+14). Each lysimeter received 336

182   L of water during a single irrigation, which corresponded to a 40 mm rainfall event calculated on a

183   combination of the meteorological pattern of the zone over the past 10 years and the high likelihood

184   of rainfall events during herbicide spraying. It was also an amount considered capable of producing

185   leaching during the trial. The water was hand-delivered by hose with a dispersion device attached to

186   its end. Each lysimeter was irrigated individually, and required about 30 minutes to deliver the

187   specified water quantity.

188

189   ***Water sampling***

190   Starting from the day after irrigation, each lysimeter was monitored for the presence of percolated

191   water. When present, water was collected and the full volume was measured. Three samples per

192   lysimeter and for each pesticide formulation (Lumax ® and Merlin ®) were collected from the

193   entire volume of leached water with a submersible drainage pump (Calpeda, Montorso Vicentino,

194   Italy). The water samples were then put into 0.5-1L graduated square polyethylene bottles (Kartell,

Noviglio, Italy) and immediately stored in a -25°C cold room until analysis. Each water sample collected for Merlin ® pesticide extraction was immediately split in two (0.5 L each) sub-samples and stored at the same conditions indicated above. In the 2012 spring, three samples of leached water were collected from the lysimeters to evaluate the prior year background residues of all the chemicals.

Herbicide extraction and analysis

Terbuthylazine, desethyl-terbuthylazine, S-metolachlor, and mesotrione

Herbicides were extracted from the water samples using solid phase extraction (SPE) cartridges. The cartridges (SupelcoSil LC-18, 6 mL, 0.5 g C18 sorbent material) (Supelco, Bellefonte, PA, USA) were activated with 6 mL of acetonitrile (Sigma Aldrich, Steinheim, Germany) and then washed with 6 mL of distilled water. The entire sample volume (1 L) flowed through the cartridges under vacuum at 500 mL h^{-1}. The cartridges were let to dry, and then adsorbed herbicides were eluted with acetonitrile until a final volume of 5 mL was attained.

HPLC was performed using an Agilent 1200 instrument (Agilent Tecnologies Italia, Cernusco sul Naviglio, Italy) equipped with a C18 Supelco ABZ, a UV detector at 215 nm, a mobile phase H$_2$O pH3/CH$_3$CN 50/50, and the flow rate set to 1 mL min^{-1}. Analytical-grade S-metolachlor, terbuthylazine, desethyl-terbuthylazine, and mesotrione supplied by Sigma Aldrich, were used as analytical standards. Resulting retention times were 11.1 min, 9.31 min, 5.63, and 6.00 min, for S-metolachlor, terbuthylazine, desethyl-terbuthylazine, and mesotrione, respectively.

Terbuthylazine, desethyl-terbuthylazine, S-metolachlor and mesotrione mean water recoveries were 101%, 94%, 95%, and 98% respectively, and the limit of quantification (LOQ) achieved in the water samples was 0.1 µg L^{-}1 for all chemicals.

Flufenacet and isoxaflutole

220   Herbicide extraction from the water samples employed solid phase extraction (SPE) cartridges. The

221   cartridges (SupelcoSil LC-18, 6 ml, 0.5 g C18 sorbent material) (Supelco) were previously activated

222   with 6 ml of acetonitrile (Sigma Aldrich), and then washed with 20 ml of distilled water. The

223   extraction was carried out on half (0.5 L) of the water samples. Water flowed through the cartridges

224   under vacuum at 500 mL h^{-1}. The cartridges were left to dry and eluted with acetonitrile until a final

225   volume of 5 mL was reached. The eluted volume of 10 mL was then filtered through a 0.20 μm

226   nylon filter (Whatman, Maidstone, UK) to eliminate impurities. An Agilent 6890N GC and Agilent

227   5975 MS single-quadrupole (Agilent) analyzer, equipped with MS detector, autosampler (Agilent),

228   and split-splitless injector connected to an Agilent Chemstation was used. The column was a

229   Supelco Equity5 TM (30 m x 0.25 mm i.d.) that contained 5% diphenyl and 95% dimethyl siloxane.

230   The MS source temperature was 270°C and the gas carrier was helium. Analytical-grade flufenacet

231   (Sigma Aldrich) served as the analytical standard. Retention times were 23.60 min for flufenacet

232   and 26.90 min for isoxaflutole.

233   Flufenacet and isoxaflutole mean recoveries in water were 98% and 87%, respectively. The LOQs

234   achieved in the water samples were 0.1 μg L-1 for flufenacet and 0.13 μg L-1 isoxaflutole.

235

236   *Diketonitrile*

237   Diketonitrile extraction from water samples was carried out using solid phase extraction cartridges.

238   The cartridges (Bakerbond SDB-1, 6 ml, 0.2 g Styrene Divinyl Benzene) (J.T. Baker, Avantor

239   Group, Deventer, The Netherlands) were activated with 6 ml of acetonitrile (Sigma Aldrich) and

240   then washed with 20 ml of distilled brought water (pH 2). The entire water sample volume (0.5 L)

241   had been brought to pH 2 with H_3PO_4. The volume then flowed through the cartridges under

242   vacuum at 500 mL h^{-1}. The cartridges were let to dry and then eluted with acetonitrile/water not

243   brought (50/50 v/v) until a final volume of 5 mL was reached. The eluted volume was then filtered

244   through a 0.20 μm nylon filter (Whatman, Maidstone, UK) to eliminate impurities.

245 Analysis was performed by HPLC using an Agilent 1200 equipped with a C18 Zorbax (Agilent)

246 SB-C18 column (25 cm x 4.6 mm, 5 μm particle sizes), a UV detector at 300 nm, a mobile phase

247 H_2O pH2/CH_3CN 50/50 and the flow rate set to 1 mL min^{-1}. Analytical-grade diketonitrile (Bayer,

248 Germany) was used as the analytical standard. The retention time was 9.15 min.

249 The mean recovery of diketonitrile in water was 100%, and the LOQ achieved in the water samples.

250 ***Results***

251 The percolated volumes measured in the lysimeters during the two-year study are reported in Table

252 2. Volumes observed in 2011 resulted lower than those in 2012. No actual measures of water

253 content in the lysimeter soil profile were made with devices, so preferential soil flow channel

254 development was assumed unlikely. Our result might have arisen from the longer time interval

255 between lysimeter preparation and herbicide application in 2011 compared to 2012. The good

256 weather conditions in 2011 that allowed the lysimeters to remain uncovered from herbicide

257 application forward may also have had an effect, whereas the frequent rain in 2012 after herbicide

258 application forced the lysimeters to be covered. These facts led us to assume the soil humidity level

259 in 2012 was greater than in 2011, which may explain the observed leached volume differences.

260 During 2011, the water volumes measured on 1, 7, and 14 DAT (irrigation) were low. Indeed, on 28

261 DAT no percolated water was present. Then, just two weeks later and after the first watering, the

262 repeated irrigation produced leached volumes not dissimilar to those observed in 2012. In effect, the

263 water supplied by the second irrigation restored the level of soil moisture, bringing it close to field

264 capacity. The irrigation fourteen days later (28+14) produced important percolation volumes (Table

265 2), which confirmed this explanation. The effects of initial water content on leaching intensity is

266 well understood and relates strictly to soil texture. Sandy soil leaches less under dry conditions, as

267 opposed to loamy and clayey soils that leach more when rainfall occurs shortly after pesticide

268 application (Flury, 1996).

269 *S-metolachlor*

270 Tables 3 and 4 report the S-metolachlor concentrations detected in 2011 and 2012. During both

271 years, S-metolachlor was found in percolated waters on every sampling date, save for no recorded

272 percolation in any lysimeter at 28 DAT. In 2011, the highest concentrations were detected in

273 percolated samples from the irrigation at 1 DAT (2.88 μg/L) and 1+14 DAT (0.95 μg/L). On

274 succeeding dates, observed concentrations were much lower, yet still above 0.1 μg/L. The second

275 highest concentration was detected in percolated water samples collected after the 1+14 DAT

276 irrigation. In 2012, S-metolachlor residues were detected at all sampling dates at concentrations

277 above 0.2 μg/L, with the peak concentration observed after the irrigation at 7 DAT (1.2 μg/L).

278

279 *Terbuthylazine and desethyl-terbuthylazine*

280 Tables 3 and 4 report the S-metolachlor concentrations detected in 2011 and 2012. In 2011, as

281 observed for S-metolachlor, the highest concentrations of terbuthylazine were found in percolated

282 water collected at 1 DAT and at 1+14 DAT, which were 0.96 μg/L and 0.52 μg/L, respectively

283 (Table 3). Among sampling dates, terbuthylazine was present in concentrations above the maximum

284 allowable limit only at 7+14 DAT (0.15 μg/L). The reduced 2011 percolation flows observed after

285 the first irrigation likely transferred less terbuthylazine in its percolating water, which can explain

286 the less than LOQ concentrations recorded at 7 DAT, 14+14 DAT, and 28+14 DAT.

287 In 2012, all collected samples were found to contain terbuthylazine ranging from 0.2 μg/L to 1.1

288 μg/L, with the highest concentrations recorded in samples from 7 DAT. In 2011, desethyl-

289 terbuthylazine was found in waters sampled at 1 DAT, 7 DAT, 14 DAT, and 1+14 DAT, with

290 concentrations ranging between 0.13 μg/L and 0.34 μg/L. No samples exceeded 0.34 μg/L (Figure

291 4), and the concentrations detected in all other samples were below quantification limits. In 2012,

292 desethyl-terbuthylazine was found in percolated waters at concentrations slightly higher than in the

293 previous season.

294

Mesotrione, isoxaflutole, and diketonitrile

Isoxaflutole was never found in percolated waters at concentrations above the quantification limit (0.1 µg/L) at any point in the two-year study (Table 3 and Table 4). As expected, its low application rate (40 g ha^{-1}) and rapid degradation to diketonitrile prevented isoxaflutole from leaching easily through the soil profile. Typically, diketonitrile is more prone to leaching, but residues of this compound were generally quite low, and above 0.1 µg/L only in 2011 on water samples collected at 1 DAT (0.12 µg/L) and 1+14 DAT (0.11 µg/L). In 2012, diketonitrile residues in leached waters always fell below the quantification limit as did those of mesotrione, an herbicide characterized by a low rate of application and rapid soil dissipation. Per our results, this chemical did not move along the soil profile and might not be a groundwater threat.

Flufenacet

The 2011 and 2012 detected concentrations of flufenacet are reported in Tables 3 and 4. Only at two sampling times in 2011 was flufenacet found at concentrations above the quantification limit (0.1 µg/L)— at 1 DAT (1.30 µg/L) and at 1+14 DAT (0.11 µg/L). In 2012, product residues were also detected twice—at 1 DAT (0.11 µg/L) and at 7 DAT (1.12 µg/L). As observed for S-metolachlor and terbuthylazine, the highest concentrations came at the first sampling dates when some of the flufenacet transported to deeper layers by the first irrigation was dissolved and leached by the second irrigation. At all other sampling dates, concentrations were below the limit of quantification.

Background concentration

Leached water was sampled from each lysimeter before every season to establish background concentrations. In both years, residues of terbuthylazine, S-metolachlor, flufenacet, mesotrione, and diketonitrile all measured as less than the quantification limit. Desethyl-terbuthylazine residues were found only in 2012 at an average of 0.17 (± 0.13) µg/L across all lysimeters.

320 **Discussion**

321 The study focused on the leaching behavior of several important herbicides used in maize

322 cultivation as affected by the time elapsed between herbicide application and the first leaching

323 event. Water pollution risk via pesticide runoff and leaching is generally higher in the early days

324 following pesticide application; that is, larger losses are encountered when runoff or leaching events

325 occur close to spraying times. [19, 34-37] However, the tendency of any individual pesticide to be

326 leached depends on its own characteristics, local climate, and agronomic factors. [19] The data in

327 Tables 3 and 4 show herbicide concentration differences reflecting these unique behaviors

328 throughout the experiment.

329

330 *S-metolachlor and Terbuthylazine*

331 Among the studied herbicides, S-metolachlor and terbuthylazine were shown to carry the highest

332 leaching potentials, which mirrors literature reports that each is frequently detected in ground

333 waters. [2, 3, 5, 6, 15, 38] Metolachlor and S-metolachlor are frequently the subjects of study in the

334 literature as they behave similarly in soil. [39, 40] However, S-metolachlor is considerably more

335 active than metolachlor against weeds; in fact, the manufacturer suggests a 35% lower application

336 rate compared to metolachlor. While O'Connel et al., [39] have put forth that the reduced application

337 rate might lower the risk of ground water contamination, its high water solubility, moderate

338 persistence, and low Koc (Table 1) suggest that this herbicide is at high risk of leaching, [41]

339 particularly in permeable soil and when rain falls close to herbicide application. [42, 43] Indeed, in a

340 lysimeter study conducted by Jebellie et al., [44], metolachlor showed itself to be highly mobile as it

341 leached easily through the soil profile. Its high mobility in different soils was also found by

342 Procopio et al. [45]

343 Here, the highest concentrations of S-metolachlor and terbuthylazine were found in water samples

344 collected after leaching events occurred close to herbicide spraying time. In particular, the S-

345 metolachlor concentrations found were always above current EU legal limits (>0.1μg/L). The

346 tendency of S-metolachlor to be transported from the upper soil layer was also reported in a

347 previous study on runoff conducted on the same soil. [37] Runoff losses of S-metolachlor were

348 detected only when rain fell close to herbicide application. In addition, the study indicated that

349 rainfall events in the weeks after herbicide spraying—even those not causing runoff—might likely

350 transport the most soluble chemicals to deeper soil layers. [37]

351 Overall, the S-metolachlor concentrations observed during the two years were not dissimilar,

352 whereas terbuthylazine residues were different, resulting higher in 2012 than in 2011. One possible

353 explanation for this behavior may come from the different leaching volumes recorded in the two

354 years. In the Results section, we reasoned that the different volumes observed were for high water

355 soluble chemicals (such as, S-metolachlor) that are easily transported even in reduced water

356 volumes. S-metolachlor and terbuthylazine have similar Koc values, but very different water

357 solubility values (Table 1). These chemical property differences clearly determined the different

358 leaching behaviors observed across the two years. Therefore, despite their similar high leaching

359 potentials, S-metolachlor leached more easily and more rapidly than did terbuthylazine.

360 As seen for S-metolachlor, the presence of terbuthylazine in ground water is commonly reported

361 across the world. [4, 46] In our study, despite the low water solubility, terbuthylazine was found

362 frequently in water leached in both 2011 and 2012. Bowman [47] in a field lysimeter study, indicated

363 that terbuthylazine moves deeper into the soil than does metolachlor because of its longer soil

364 persistence, rather than from mobility. According to Donati and Funari [48] and other authors,

365 terbuthylazine is poorly retained by the soil and can be easily transported by leached waters. [49, 50]

366 The soil may actually act as a reservoir from which terbuthylazine leaches after application. These

367 characteristics might explain why terbuthylazine is often found in groundwaters at levels above

368 allowable limits. [49]

369 Between the two years, terbuthylazine was found in leached water to a larger extent during 2012

370 than in 2011. As previously indicated, during initial 2011 irrigations we measured lower leached

371 water volumes than we did in the year following and at 28 DAT, no leaching was recorded. S-

372   metolachlor soil profile movement was little affected by the different leaching amounts observed,

373   whereas terbuthylazine leaching was greatly influenced. In 2012, residues of terbuthylazine were

374   found in samples from all the sampling dates. The lower mobility observed for terbuthylazine in

375   2011 likely related to the smaller leached volumes, and in particular to the lower solubility of the

376   herbicide as the same water volumes were applied to the lysimeters in both years.

377   Flury [19] may provide some insights regarding terbuthylazine behavior as be showed that smaller

378   solute amounts are transported out of wet soil relative to dry. The reduced water solubility and high

379   koc of terbuthylazine account for its 2011 behavior and correspond to its observed reduced leached

380   volumes (Table 2). Overall, our results made clear that significant losses of terbuthylazine and S-

381   metolachlor can occur even when leaching events take place at times far from herbicide application,

382   which is keenly important in Europe, where terbuthylazine is one of the most applied herbicides.

383   Different water solubility levels certainly influence leaching predisposition, but as seen for

384   terbuthylazine, and to a lesser extent for flufenacet, reduced water solubility does not imply a minor

385   leaching risk.

386

387   *Desethyl-terbuthylazine*

388   Desethyl-terbuthylazine is formed in the soil as a degradation product of terbuthylazine. Desethyl-

389   terbuthylazine is more water-soluble, and therefore more vulnerable to leaching than its parent

390   compound (Table 1), as evidenced by the GUS Index (*Ground Water Ubiquity Score*) values of the

391   two compounds (desethyl-terbuthylazine, 3.5 and terbuthylazine, 3.1) shown in Table 1. Desethyl-

392   terbuthylazine was not only more frequently found, but also at higher concentrations in 2012 *versus*

393   2011. As mentioned earlier, the different behaviors may be related to the greater volumes of water

394   percolated in 2012. Nonetheless, the effect of background concentrations of desethyl-terbuthylazine

395   must be taken into account, as this metabolite was present in waters sampled during lysimeter

396   discharge operations in 2012 before trial start. At that time, background concentrations in the

397   lysimeters averaged 0.17 µg/L (± 0.13).

398   Another consideration is that desethyl-terbuthylazine is the first metabolite of terbuthylazine; hence,

399   its presence in leached waters is a function of the degradation of its parent compound, which can

400   result in its delayed release and extended presence in the soil system (Table 2). Several

401   environmental authorities [5, 15], have published on the presence of desethyl-terbuthylazine and this

402   metabolite and consider them high risk pollutants for groundwaters. [4, 51] In Northern Italy,

403   widespread groundwater contamination by these compounds is mentioned by the environmental

404   agencies of two important regions of the Po Valley, Piemonte and Lombardia, areas of widespread

405   terbuthylazine use. [15, 23, 52]

406

407   *Desethyl-terbuthylazine/terbuthylazine ratio*

408   The behavior of desethyl-terbuthylazine is strongly dependent on the degradation of its parent

409   compound, which makes their water environment fates inseparable. The desethyl-terbuthylazine

410   terbuthylazine to ratio was calculated for all sampling dates. When either compound was measured

411   to be below the quantification limit, the calculation was completed using the quantification limit

412   value of the respective compound (0.1µg/L). The DTA/TBA ratios calculated for the two years are

413   presented in Table 5. According to recent research, groundwater DTA/TBA ratios can be used as

414   indirect measures of herbicide and soil interaction. [23, 51] DTA dissipates more slowly in soil than

415   does TBA, so DTA/TBA ratios of <1 indicate point source contamination has occurred.

416   DTA/TBA ratios in 2011 grew from 0.28 to 2.83 (DAT 14) in water samples collected after the first

417   irrigation (DAT 1). Irrigations repeated 14 days later showed more leaching as evidenced by ratios

418   of <1 in earlier samples (1+14 and 7+14 DAT) *versus* ratios in later samples (14+14 and 28+14

419   DAT) of 1 because DTA presence was below the quantification limit. The second irrigation event

420   carried out at 1+14 and 7+14 DAT caused further movement of terbuthylazine through the soil. At

421   14+14 and 28+14 DAT, residues of terbuthylazine and desethyl-terbuthylazine were already close

422   to their quantification limits, thus explaining the DTA/TBA ratio values.

423 In 2012, desethyl-terbuthylazine residues (0.17 ± 0.13 µg/L) were found in the blank samples

424 collected during lysimeter discharge performed before herbicide application. Even without the

425 dissipation rate of desethyl-terbuthylazine in lysimeter soil at different depths, these data show that

426 desethyl-terbuthylazine persists longer in the soil and can be released longer than its parent. In

427 general, the DTA/TBA ratio was above 1, suggesting a greater prevalence of desethyl-

428 terbuthylazine in 2012, as opposed to 2011 leached waters.

429

430 *Flufenacet*

431 The results of this study showed that flufenacet is not very mobile in the experimental conditions

432 considered, but when rainfall events happen close to herbicide application, some herbicide can be

433 transported through the soil. We undertook several experiments to study the leaching behavior of

434 flufenacet in field lysimeters of different soil types.

435 Most of these studies employed small-sized experimental devices, so flufenacet showed little soil

436 profile leaching. [28, 53, 54] A leaching study conducted in an alluvial Indian soil column showed that

437 even after continuous rainfall, flufenacet did not advance more than 35 cm, and most of the

438 herbicide remained in the 0-35 cm soil depth. [53] A biennial greenhouse bioassay study of the

439 percolation effects of different herbicides in two differently-textured soil columns found that

440 flufenacet, even under simulated high rainfall (100mm), moved no more than 11 cm in loamy-

441 clayey soil and 14 cm in sandy soil. [55] These results likely stem from low water solubility and high

442 soil adsorption of flufenacet (Table 1). On less compacted soils, the lower adsorption could actually

443 facilitate movement of the herbicide in the soil. The American Environmental Protection Agency

444 fact sheet on flufenacet highlights this risk of groundwater contamination under certain conditions,

445 particularly in soils high in permeability. [56]

446

447 *Isoxaflutole and Diketonitrile*

448   Isoxaflutole demonstrated little leaching vulnerability under our experimental conditions. The soil

449   half-life of this herbicide is generally less than 1 day [57] as it is rapidly converted to DKN. A

450   previous study [36] conducted on the same soil found the same results: very quick dissipation and

451   short half-life (<1 day). The present study showed that the fast dissipation and low application rates

452   of isoxaflutole make it a negligible groundwater leaching risk.

453   The characteristics of diketonitrile make it more prone to leaching; it is more water-soluble and

454   persists longer in the soil than its parent compound (Table 1). The chance of groundwater

455   contamination by this chemical appears sizeable. [57, 58] Despite these rather unfavorable

456   characteristics, residues of this chemical were measured mostly below the limit of quantification;

457   the low application rate of its parent compound probably limited its residuals. This warns, however,

458   that higher diketonitrile losses may appear at higher application rates of isoxaflutole, or as some

459   authors say, in soils low in organic matter content. [57]

460

461   *Mesotrione*

462   Mesotrione also showed reduced leaching risk, which is a result that seems inconsistent with the

463   chemical properties of the product. Mesotrione is a weak acid and its solubility is strictly related to

464   pH; it ranges from 220 mg/L at pH=4.8 to 2200 mg/L at pH=9. [59] Lysimeter soil was silty-loam in

465   texture with a low organic content (OC) content and a pH=8.2. In these conditions, we expected the

466   herbicide to leach with percolating water along the soil profile. However, its reduced application

467   rate (150 g ha^{-1}) and short half-life (DT50=3-7 days in European soils) [60] reduced its soil

468   environment permanence, and consequently, its leaching potential. Under the soil conditions of this

469   study, mesotrione represented no great groundwater concerns, even when important precipitations

470   occurred soon after its application, which is consistent with discussions in other technical reports.

471   [60] Finally, while its high water solubility in basic pH soils may facilitate its movement through the

472   soil, this condition also speeds degradation of the herbicide. [58]

Conclusions

The leaching behavior of widely-used maize cultivation herbicides (terbuthylazine, S-metolachlor, flufenacet, mesotrione, and isoxaflutole) and some of their metabolites (desethyl-terbuthylazine and diketonitrile) was evaluated in a two-year study conducted on a battery of field lysimeters. The herbicides were applied in pre-emergence using two commercial formulations containing terbuthylazine, S-metolachlor, mesotrione, flufenacet, and isoxaflutole.

The results of this study should be considered of particular interest because the leaching behavior of the above mentioned herbicides and metabolites have been studied in large-scale lysimeters (8.4 m^2) under field-like conditions. With few exceptions [61] most pesticide leaching studies are routinely carried out on small-scale lysimeters, often with a surface area less than 1 m^2. [38, 62-64]

The leaching was induced by artificial irrigation, with the highest potential for groundwater contamination observed with S-metolachlor and terbuthylazine. In fact, S-metolachlor was still present in leached waters collected post irrigations occurring one month after herbicide application. This was also documented in cases of reduced percolation flows. The data suggested that percolated water volumes influenced terbuthylazine leaching and that the soil may become a reservoir of these chemicals. Once they are transferred into the soil profile, they are less affected by degradation phenomena. This is particularly true for quite persistent and less retained herbicides, like terbuthylazine. Desethyl-terbuthylazine showed delayed soil release.

Flufenacet showed little mobility. However, our study demonstrated that relevant rainfall close to flufenacet application might move the chemical through the soil profile.

In our conditions, isoxaflutole and mesotrione posed no groundwater threats. Desethyl-terbuthylazine, the metabolite of terbuthylazine, was frequently detected in percolated waters at concentrations up to 0.34µg/L, which confirmed the risk reported by national and international monitoring on the movement of this metabolite toward groundwater. Diketonitrile appeared a little more problematic compared to its parent compound; however, the maximum allowable limit (0.1 µg/L) in water was overcome only once under study conditions.

499 To mitigate the leaching of terbuthylazine and desethyl-terbuthylazine in vulnerable areas, the

500 Italian Ministry of Health, in concert with regional environmental authorities, established

501 terbuthylazine application restrictions: dose reductions (maximum rate 850 g ha^{-1}), buffer strip

502 utilization to limit runoff toward surface waters (5m), and alternate year use along maize rows. In

503 addition, to measures aimed at preventing and reducing water contamination from herbicides, it is

504 advisable to select non-mobile and non-persistent herbicides, both for pre- and post-emergence

505 weed control.

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513

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651 Transport through Field Lysimeters via Preferential Flow Names are necessary to report factually on

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Table 1 Physico-chemical properties of terbuthylazine, desethyl-terbuthylazine, S-metolachlor, mesotrione, flufenacet, isoxaflutole and diketonitrile (PPDB, The Pesticide Properties Database, AERU, University of Hertfordshire, 2009).

Chemical	TBA	DTA	S-MET	FLU	ISO	MES	DKN
Water solubility (mg L^{-1})	6.6	327	480	56	6.2	160	300
Koc (ml g^{-1})	231	121	226.1	401	145	122	92
DT50 in field (days)	22.4	28.6	21	40	1.3	5	9
GUS index	3.1	3.5	1.9	2.4	0.6	3.4	-

Note 1: TBA (terbuthylazine); DTA (desethyl-terbuthylazine); S-MET (S-metolachlor); FLU (flufenacet); ISO (isoxaflutole); MES (mesotrione); DKN (diketonitrile). GUS: Ground water Ubiquity Score

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Table 2. Volumes of waters percolated during the two years. Values are the arithmetic mean of three data.

Days after treatment (I irrigation)	2011	2012
	Percolated water (L)	
1	40 (±4)	213 (±26)
7	45 (±8)	114 (±29)
14	36 (±9)	132 (±29)
28	NL	135 (±31)
Days after I irrigation (II irrigation)	**Percolated water (L)**	
(15) 1+14	153 (±3)	222 (±6)
(21) 7+14	165 (±13)	244 (±6)
(28) 14+14	146 (±11)	158 (±31)
(42) 28+14	125 (±25)	184 (±43)

Note: NL: no leaching

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Table 3. Concentration of chemicals studied in water samples collected after each leaching event in
2011 and 2012. Values are expressed in µg L^{-1}. Arithmetic mean of 9 replications ± SE.

DAT	TBA	DTA	S-MET	MES	FLU	ISO	DKN
2011							
µg/L							
1	0.96±0.34 a*	0.27±0.12 b	2.88±1.10 a	< 0.1	1.30±0.34 a	< 0.1	0.12±0.06
7	< 0.1	0.13±0.06 b	0.24±0.06 b	< 0.1	< 0.1	< 0.1	< 0.1
14	0.10±0.02	0.27±0.07	0.27±0.05	< 0.1	< 0.1	< 0.1	< 0.1
28	NL	NL	NL	NL	NL	NL	NL
1+14	0.52±0.17 a	0.34±0.07	0.95±0.26 a	< 0.1	0.27±0.05	< 0.1	< 0.1
7+14	0.15±0.03	< 0.1	0.33±0.13	< 0.1	< 0.1	< 0.1	< 0.1
14+14	< 0.1	< 0.1	0.59±0.05 b	< 0.1	< 0.1	< 0.1	< 0.1
28+14	< 0.1	< 0.1	0.25±0.05	< 0.1	< 0.1	< 0.1	< 0.1
2012							
µg/L							
1	0.60 ±0.20 b	0.53 ±0.20 a	0.71 ±0.21 b	< 0.1	0.15 ±0.05 b	< 0.1	< 0.1
7	1.11 ±0.07	1.07 ±0.08 a	1.19 ±0.10 a	< 0.1	0.38 ±0.03	< 0.1	< 0.1
14	0.28 ±0.09	0.53 ±0.20	0.25 ±0.07	< 0.1	< 0.1	< 0.1	< 0.1
28	0.63 ±0.05	0.72 ±0.06	0.55 ±0.00	< 0.1	< 0.1	< 0.1	< 0.1
1+14	0.28 ±0.02 b	0.33 ±0.02	0.28 ±0.03 b	< 0.1	< 0.1	< 0.1	< 0.1
7+14	0.60 ±0.08	0.64 ±0.10	0.37 ±0.12	< 0.1	< 0.1	< 0.1	< 0.1
14+14	0.20 ±0.03	0.28 ±0.06	0.61 ±0.02 a	< 0.1	< 0.1	< 0.1	< 0.1
28+14	0.58±0.03	0.65±0.03	0.52±0.02	< 0.1	< 0.1	< 0.1	< 0.1

DAT, Days after treatment.

TBA: terbuthylazine; DTA: desethyl-terbuthylazine; S-MET: S-metolachlor; MES: mesotrione; FLU: flufenacet; ISO:

isoxaflutole; DKN: diketonitrile.

*: Same letter values are not significantly different (t test; α= 0.05).

Table 4. DTA/TBA ratio

DAT	DTA/TBA ratio	
	2011	2012
1	0,28	0,9
7	1,30	1,0
14	2,83	1,9
28	-	1,1
1+14	0,65	1,2
7+14	0,69	1,1
14+14	1,00	1,4
28+14	1,00	1,1

DAT, Days after treatment.

TBA: terbuthylazine; DTA: desethyl-terbuthylazine;