REFEREED PAPER

DETERMINATION OF RESIDUAL ATRAZINE IN SOIL AND DRAIN WATERS USING GC-TOF-MS

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Abstract

Residual atrazine in the soil and drainage water at the Zimbabwe Sugar Association Experiment Station was determined using Dispersive Solid Phase Extraction (dSPE) coupled with Gas Chromatography-Mass Spectrometry (GC-MS). An experiment at three levels of atrazine treatment was set up, and residual atrazine in soil and leached solution was determined every week after planting sugarcane setts in pots. The amount of atrazine detected in the soil ranged from 29 to 1 ppm nine weeks after the application for the standard two litres per hectare (2 L/ha). The amount of atrazine in the leachate from the pots ranged from 33.71 to 0.71 pbb and was below the maximum permissible limit. In borehole water, atrazine concentration ranged from 48.76 to 49.00 ppb and in drainage ranged from 21.71 to 109.21 ppb. Soil samples from fields where atrazine had been applied had residual atrazine ranging from 5.66 to 13.21 ppm. Results indicate that the atrazine levels in the borehole and drainage ditch exceed the US Environmental Protection Agency concentration limit of 2 ppb. However, this is below the 1 ppm maximum permissible limit of atrazine in surface and ground water. The results indicate that there is a need to monitor potential contamination of atrazine in surface and ground water. Monitoring of other herbicides used in the sugarcane industry should also be pursued. Breakdown or transformation products of atrazine and other herbicides are not an exception, since the parent compound was detected both in the soil and water.

Keywords: atrazine, dispersive solid phase extraction, gas chromatography-mass spectrometry, herbicides, drainage, borehole

Introduction

Sugarcane is grown in the South Eastern Lowveld of Zimbabwe under irrigation. The major cost drivers in sugarcane production are water, fertilisers, pesticides and labour. Herbicides are used extensively for the control of weeds. Weeds compete with sugarcane for nutrients, light and water. As such, it is imperative to control the weeds so as to attain high sugarcane yields. There are several methods used for weed control, among which are mechanical, manual and chemical control. Chemical control of weeds using herbicides is efficient, effective and less costly. As such the Zimbabwe Sugar Industry uses many herbicides to control weeds. However, the extensive use of these herbicides presents a possible environmental pollution source (Gupta, 2011).

Use of herbicides, although being effective in controlling weeds, is sometimes concomitant with the occurrence of and persistence of herbicide residues in soil and water (Monaco *et* al., 2002). Herbicides reach the soil through direct application in spraying. Herbicides can enter surface and ground water through runoff and leaching. The fate of herbicides in the soil is influenced by the physico-chemical properties of the herbicide, the properties of the soil and temperature and moisture conditions, (Monaco *et al.*, 2002). Herbicides degrade by the

action of microbes, chemical transformation and photo-degradation. This results in herbicide residues being found in the soil as well as surface or ground water.

Several studies show that herbicides residues remain in the soil and water even after the purported period of expected degradation. High levels of atrazine and metolachlor were detected in surface and ground water in the temperate regions in spite of the relatively rapid degradation rates quoted for the compounds (Mullins and Cobb, 2007). In Mali, pesticide residues of dichlorodiphenyltrichloroethane (DDT) and its breakdown products were detected in 77% of the soil samples (Mullins and Cobb, 2007). Eight pesticides were detected in water samples: lindane, endosulfan I, endosulfan II, endosulfan sulfate, dieldrin, and atrazine, (Safiatou *et al.,* 2007). Kolpin *et al.* (1995) found out that most of the pesticides found were those of the herbicides alachlor and atrazine (Sagratini *et al.,* 2007). Their concentrations were found to exceed the U.S. Environmental Protection Agency`s maximum contamination levels or health advisory levels for drinking water stated as two micrograms per liter (2 µg/L), (Sagratini *et al.*, 2007). Persistent pesticides such as DDT, aldrin and atrazine have been reported in water causing concern for the need to monitor herbicides in water. Herbicides residues of atrazine were also found in well water in Central Italy even though atrazine was commercially forbidden for years in this country, (William *et al.,* 2003).

Intensive use of herbicides has thus increased the risk connected with their presence as residues or metabolites are found in the environment and in underground water (William *et al.*, 2003). The detection of residues of pesticides in water bodies from agricultural areas raises a lot of concern and indicates that these pesticides contaminate the water bodies. Such residues thus represent unexpected or underestimated threat for consumer health, since polluted irrigating water can convey pesticides to food stuff (Barganska *et al.*, 2014).

In Zimbabwe, the Environmental Management Authority set the minimum limit of atrazine at one part per million (1 mg/L). Atrazine has been reported to have long term reproductive and endocrine disrupting effects. Reports have indicated that atrazine disrupts frog development and also causes a variety of adverse effects in fish, including reduced reproduction, kidney damage, and decreased ability to withstand warm temperatures, (Hayes *et al*., 2002). Atrazine is also reported to probably be a human carcinogen. Human health effects include low birth weights, increased numbers of breast cancer cases and low sperm counts. By their nature pesticides are designed to kill living organisms, hence present a risk to humans, animals and the environment (Hayes *et al.,* 2002). Aquatic organisms are not spared. Herbicides thus are toxic to micro-organisms which play an important role as primary producers as they cycle nutrients and aid in decomposition. N-nitrosamines, which are formed in the soils that have been treated with atrazine, have mutagenic and carcinogenic properties. Atrazine is also banned in some countries, including Angola and is restricted in South Africa (Zeljezic *et al.*, 2006). However, in Zimbabwe the herbicide is registered for use. As such, herbicide persistence in soil may affect nutrient cycling, and hence soil microbes and soil health.

People can be exposed to herbicides by drinking water from rivers and boreholes which are contaminated with the herbicides. Herbicide sprayers are also at risk. Farm workers doing land preparation and planting of sugarcane can be exposed to herbicide residues in the soil. It is evident that herbicides residues find their way in soil, surface and ground water. They present a health and environmental threat calling for monitoring of their presence and quantities. However, there is no information in the Zimbabwe Sugar Industry concerning the monitoring of herbicides in the soil and water. This project seeks to establish base line data on the occurrence of herbicides residues, in particular the commonly used herbicide atrazine.

Materials and Methods

Atrazine standard was purchased from RESTEK (Johannesburg, South Africa). Stock standard solutions were prepared from the 500 micrograms per milliliter of the reference standard. This stock solution was kept in the freezer until such time as it was needed for use. Working standards for various concentrations were prepared by serial dilution of the stock standard solutions. Thus, a medium stock solution of 100 mg/L was prepared by dissolving 400 µL of the 500 mg/L in a two milliliter (2 mL) vial and making a total volume of 2 mL by adding 1600 µL of analytical or HPLC grade acetonitrile, which was purchased from Merck Chemicals (South Africa). Working standards in the range 10, 20, 30, 40 and 50 µg/L were prepared from the 100 ppm by appropriate serial dilution. Similarly, working standards for the calculation of atrazine (ATR) in soil extract samples from laboratory field experiments and typical soil samples from fields that were prepared in the range of 10, 20, 30, 40 and 50 ppm, respectively.

Apparatus used included variable centrifuge, vortex mixture, volumetric flasks, micro pipettes, separating funnels, syringes and general laboratory glassware.

QuEChERS (extraction salts) pre-packed salts of magnesium sulphate and sodium chloride and 2 mL micro-centrifuge tubes for dispersive solid phase extraction containing 150 mg primary secondary amine and C_{18} were obtained from RESTEK. Water for rinsing and sample preparation was obtained from Milli-Q Reverse Osmosis purification system at the Zimbabwe Sugar Association Experiment Station (ZSAES).

Planting of sugarcane and application of atrazine

This experiment was carried out in order to observe the general dynamics of atrazine after its application to soil and under a controlled irrigation cycle. Sugarcane was grown in pots. Atrazine was applied immediately after planting the sugarcane setts. Soil samples were collected from the pots before herbicide application and pH, soil texture and organic matter were determined. The pots were perforated at the bottom to allow drainage. Sugarcane setts were planted in the pots. Atrazine at zero, one, two and three liters per hectare, respectively, was applied using a previously calibrated knapsack. Atrazine is a pre-emergence herbicide hence was applied the day of planting the sugarcane setts. Nutrition management was achieved by adding a mixed nutrient solution comprising the major plant nutrients calcium, magnesium, potassium, phosphorous and nitrogen. To the nutrient solution was also added the trace nutrient elements boron, manganese, zinc, copper and molybdenum. This ensured that the sugarcane received adequate nutrition. Irrigation was carried out using 2 L measuring cylinders to which 2 L of herbicide-free water was added to each of the eight pots twice weekly, on Mondays and Fridays.

Sampling dishes were put under the perforated pots to collect the drained water. Effluent water was collected from the dishes every Friday following irrigation or watering. The effluent water was tested for pH and conductivity immediately. The collected samples were kept in a freezer until such time as they were extracted and analysed. Soil samples were collected at the same time as the water samples. They were collected after the irrigation water had drained off. The soil sample was taken at a depth of 100 mm using a fabricated auger. They were allowed to air dry at 20°C. Sampling was done throughout the growing cycle of the sugarcane for both leached water and soils in the pots.

Soil and water sampling

Soil samples were taken from fields previously treated with herbicides. Water samples were also taken from the drain and borehole. The field drain traverses M and L blocks at ZSAES, finally discharging into the main dam. Samples from the field drain were taken manually from

December 2015 to May 2016. A modified scoop-cup was used to collect the water samples. The scoop-cup was cleaned with laboratory detergent (neutral Extran) followed by Reverse Osmosis (RO) water and finally ethyl acetate. Borehole water was collected using a sampling bottle attached to a stick. The sampling apparatus was lowered and raised when full from the well. One litre sampling bottles were thoroughly cleaned and rinsed with RO water followed by ethyl acetate before use to prevent contamination. Each bottle was filled with the water to be sampled and rinsed with this water before taking a sample for laboratory analysis. The sample bottles were sealed and appropriately labelled. The samples were then kept in a freezer until analysis.

*Extracting herbicides from soil samples (dSPME***)**

A mass of ten grams of well mixed soil sample was weighed using an analytical balance and transferred to QuEChERS extraction tubes. Five millilitres of RO water was added to the extraction tube to bring the soil to saturation point. To the moistened or wetted soil, 10 mL of acetonitrile was added. Pre-weighed and packed buffer-salt mixture (4.0 g of anhydrous magnesium sulphate, 1.0 g of sodium chloride and 1.0 g of tri-sodium citrate) was added. The contents of the extraction tube were shaken thoroughly for a minute and centrifuged for five minutes at 3 000 rpm. A 1 mL aliquot of the clearly separated acetonitrile phase was transferred to a capped centrifuge tube containing Primary Secondary Amine (PSA) for clean-up. This was vortexed for one minute to allow for mixing, and subsequently centrifuged for five minutes at 3 000 rpm. The supernatant of the extract was transferred to an auto-sampler vial for injection on the GC-MS column.

Methods for GC-TOF-MS

Auto-sampler method

The GC-TOF-MS instrument is equipped with a Rail System CTC (GERSTEL) for sample introduction. The auto-sampler was controlled using the computer for communication with the GC. Settings were introduced for the syringe volume, solvent pre-wash, sample pre-wash, viscosity delay, injection rate and post injection wash. The auto-sampler settings ensured that there was reproducibility in all the injections.

Gas chromatography (GC) method

An Agilent 7890 series GC hyphenated to a Pegasus HT Time of Flight MS was used with helium as a carrier gas. Injections were done on the front inlet in the split-less mode onto a fused silica column (30 m x 0.25 mmid x 0.25 µm), and an injection volume of 1 µL . The column front inlet pressure was set to 14.20 psi, with front inlet purge at 3 mL/min. The inlet temperature was set at 50°C and holding the temperature constant for one minute. Temperature was then ramped at 20°C per minute up to 170°C to allow for the volatilisation of atrazine. Further temperature ramping at 8.75°C per minute was done to allow for other compounds to separate and lastly holding the oven temperature at 240°C for three minutes, thus eluting all the compounds in the capillary column.

Mass spectrometer (MS) method

A Pegasus HT Leco MS in tandem with the GC and had the following settings. An acquisition delay time of 6 min was selected from previous trials, as the retention time for the solvent was around 5 min. The mass range of 50 to 500 covered the molecular weight of atrazine (215.6). A detector voltage of 70 volts was used to allow for library comparison and matching.

Data processing method

Data processing was done using the Leco ChromaTOF software. The software allowed for the quantification of atrazine as well as giving similarity matches to the NIST library. A similarity match of above 80% was chosen as the minimum for the acceptance of atrazine.

Quality control method

The GC-MS was programmed such that before analysis of the samples a check was made for leaks, tuning and calibration. The acceptable values of nitrogen, water and oxygen in reference to reference mass 69 was used as the basis for the correct function of the GC-MS. The tune and leak checks were done once every day and after 12 hours of continuous use.

Quality control

Field soil samples without any herbicide treatment were subjected to Dispersive Solid Phase Micro-Extraction, and recovery studies were also conducted by spiking a known concentration of atrazine followed by extraction. Standard calibration curves were generated from the injection of standards.

Results and Discussion

Atrazine in the soil, leached water, borehole and surface drainage water was analysed using the GC-MS following extraction using Solid Phase Extraction. Water and soil samples from the laboratory experiment were collected from 22 January to 15 April 2016. Drainage water samples from the Centre Pivot sampling station were collected from December 2015. An inspection well/borehole close to field L1 and the Centre Pivot sampling station was sampled to check for incidence of ground water contamination. Soil samples from fields that had been harvested, ploughed and disced were also sampled to check for residual atrazine, although a fairly long time had elapsed since ATR had been applied.

Gas chromatography mass spectrometer method performance evaluation.

The GC-MS method used for the determination of atrazine in soil and water samples was reproducible. The linearity of the method gave a correlation coefficient value for R^2 of 0.9972 and 0.9980 for the soil and water extracts, respectively. The recoveries for the 10 ppm spiked atrazine was 87.9% and RSD 4.6% (mean=8.79, standard deviation=0.36 and n=13). Recoveries of 20 ppb atrazine following extraction was 85% and RSD 1.13% (mean=17.00, standard deviation=1.00 and n=13).

As shown in Figure 1 atrazine at three different rates of application declined proportionately from 22 January to around 25 March 2016, when it was no longer being significantly detected. The standard recommendation rate for atrazine application in soil for weed control is 2 L/ha. The initial atrazine applied corresponding to the 2 L/ha was 563 ppm. However, in the first week of sampling the soil the amount of atrazine detected for the standard 2 L/ha was 29.00 ppm, giving a percentage reduction of 94.85%. Thus only 5% of the applied herbicide was detected after one week from initial application. The explanation could be that when a herbicide is applied to the soil surface, its surface initial concentration diminishes immediately due to degradation by microbes, chemical and photochemical action as well as by volatilisation (Gianni *et al*, 2007). The decrease in residual atrazine was within the first nine weeks, after which no significant levels were detected. There was a general decrease of the atrazine in the soil samples. This trend shows that every week the levels of atrazine were reducing systematically, probably due to the controlled nature of irrigation events and management of the trial. The explanation for not finding atrazine by the 9th week is

attributed to the herbicide being taken up by weeds and also due to adsorption on clay and organic matter particles.

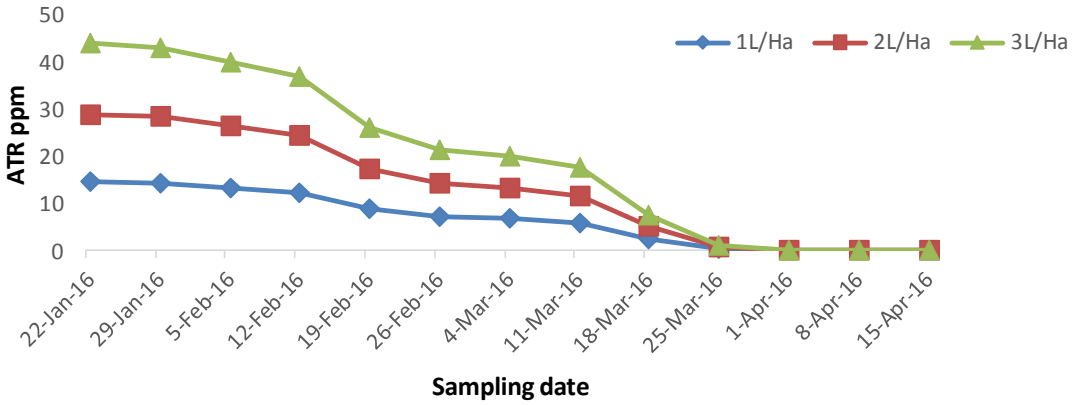

Figure 1. The rate of degradation of atrazine in soil over time at different rates of initial application (1, 2 and 3 L/ha). Combined graph showing residual atrazine in soil from the 1, 2 and 3 L/ha.

The three levels of atrazine treatments are plotted individually in Figure 2, showing similar trends in the decline of atrazine.

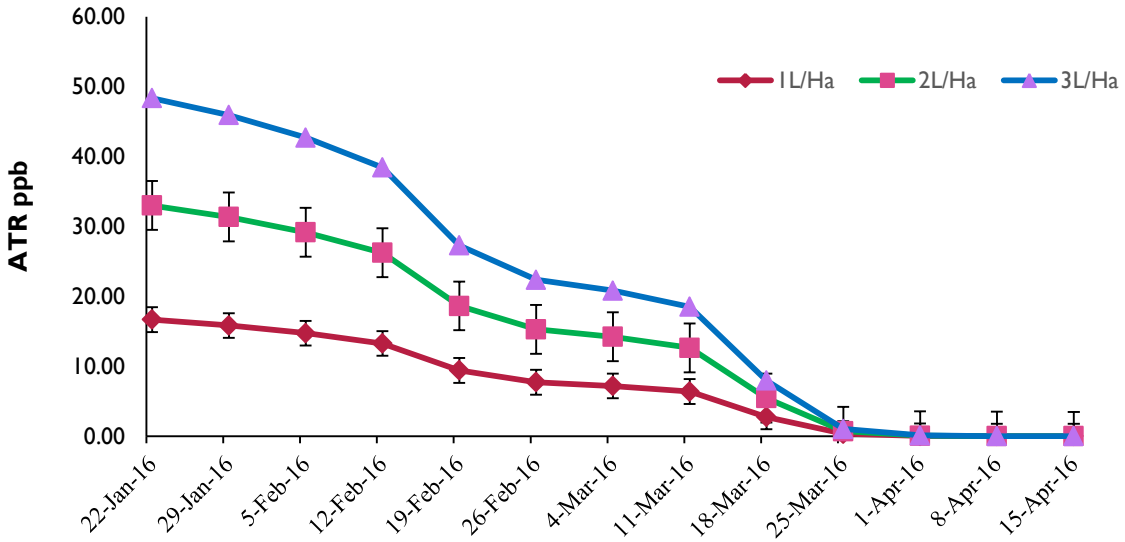

Figure 2. Residual atrazine in water over time when the initial applied herbicide rates were 1, 2 and 3 L/ha.

The concentration for the 2 L/ha atrazine application in the first week of sampling had a mean value of 33.17 ppb atrazine that was detected in the sample. This level is by far much higher compared to the WHO minimal levels of atrazine in surface water set at 2 ppb. Assuming this water was going to the drain, then there is cause for concern for possible contamination. However, when Zimbabwean Environmental Management Act regulations are applied there is no cause for concern, as the Act sets the limit at 1 ppm. It is best to work with stringent regulations; hence the WHO regulations are referred to in this work. Considering that this result was a snap short on that particular day following irrigation in the

morning, there is possibly a lot more of the atrazine that had leached and this result could be an underestimation of typical fluxes of the herbicide into the surface waters. The other two atrazine treatments had a similar effect, with the 3L/Ha leaching out more of the herbicide. The results show that even the lower atrazine application rate had levels that exceeded the WHO guidelines as this treatment released mean atrazine of 16.67±0.58 ppb. By the 10th week, 1, 2 and 3 L/ha had residual atrazine of 0.36, 0.71 and 1.04 ppb, respectively. This indicates that irrespective of lower atrazine application, contamination of the surface waters could occur. The lower levels could be a result of corresponding lower residues in the soil due to degradation and uptake by the weeds. The results indicate that it is within the 9th week when pollution of surface waters is imminent. Hence, strategies need to be put in place to mitigate surface water contamination following herbicide application. Such interventions can be in the form of growing grasses such as *vetiver* to act as buffer zones or herbicide sinks (Popov and Cornish, 2006). Without this intervention, there will be serious herbicide pollution of receiving waters.

The highest amount of atrazine was 109.21±22.10 ppb. This result is far much higher than the WHO guidelines of 2 ppb ATR in surface and ground water samples. The result is 55 times higher than the permissible limit, presenting a big environmental threat. These pulses in the occurrence of atrazine in the drainage water indicate the relative intensities of the herbicide in the surface water. The high levels were probably due to flushes of atrazine from neighbouring fields from where the drain collects its water (Figure 3). The result shows that export of atrazine to surface waters is influenced by soil dynamics of absorption and desorption. In particular, the fields from where this drain collects its waters had long since been applied with the herbicide. Occurrence of atrazine residues in the drainage water can also be explained as due to the presence of atrazine in the soils from fields L1, M2 and M3 from which the drain collects some of its water. Despite there being no atrazine applied in the previous years to these fields, atrazine was found in the drain. This implied the soil from which atrazine had long since been applied released the atrazine slowly. Atrazine had been used in the industry for the past three decades; hence the soils could be having banks of atrazine below the surface, which is released to the surface waters. According to the WHO on levels of atrazine in surface water the results present a real challenge to the health of the consumers who use this untreated water for irrigating vegetables and subsequently eating the vegetables. However, the Zimbabwean EMA regulation considers this level safe.

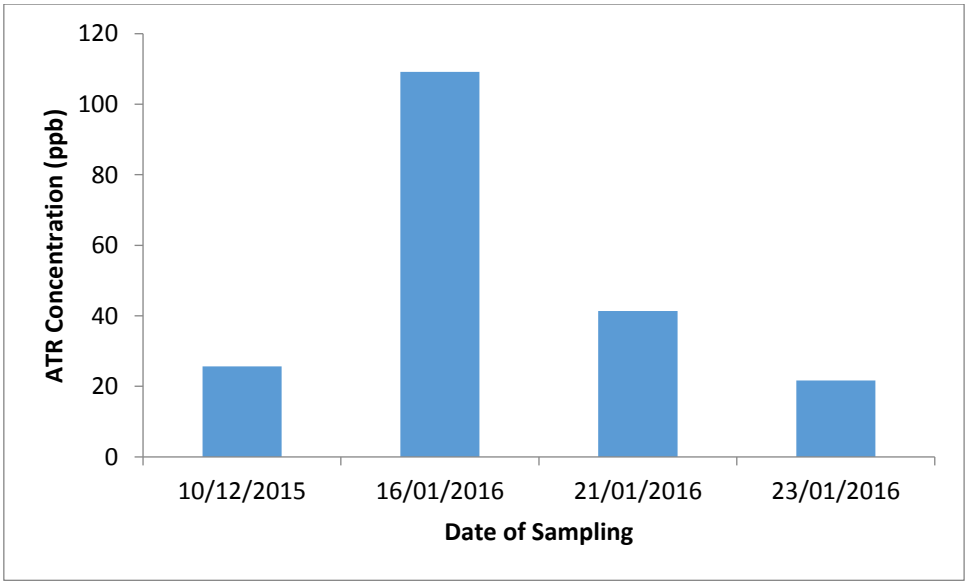

Figure 3. Atrazine in drainage water, samples collected when there was flow.

The declining levels of atrazine, 48.76 and 49.00 ppb with no subsequent detection in the remainder of the study period could be explained as due to adsorption and the effect of vetiver grass that was grown in the drainage to reduce soil erosion. The vetiver grass probably acted as a filtering media for the atrazine. The distance between the drainage and the previously herbicide treated fields also affected herbicide export to the drain. Fields L3, M2 and M3 are far away from the drain hence this distance meant there are greater chances of the herbicide being adsorbed from the soil before it finds its way to the drain. This herbicide sink could then be released only when underground conditions are permissible for the desorption of the herbicide. There is need to continuously monitor this drainage water over different seasons to find out the actual mechanism of the release of the herbicide. Monitoring of the herbicide during rainy season may possibly explain the presence of atrazine in the soil sink.

Occurrence of atrazine in well or borehole water.

The levels of atrazine in the borehole were above the WHO limits for atrazine in water. This important discovery of the presence of atrazine in ground water clearly demonstrates that herbicides persist in the environment and can leach into wells and underground water reserves (Figure 4). This is probably due to the long half-life of atrazine and microbial and environmental conditions that may not promote its metabolism and degradation.

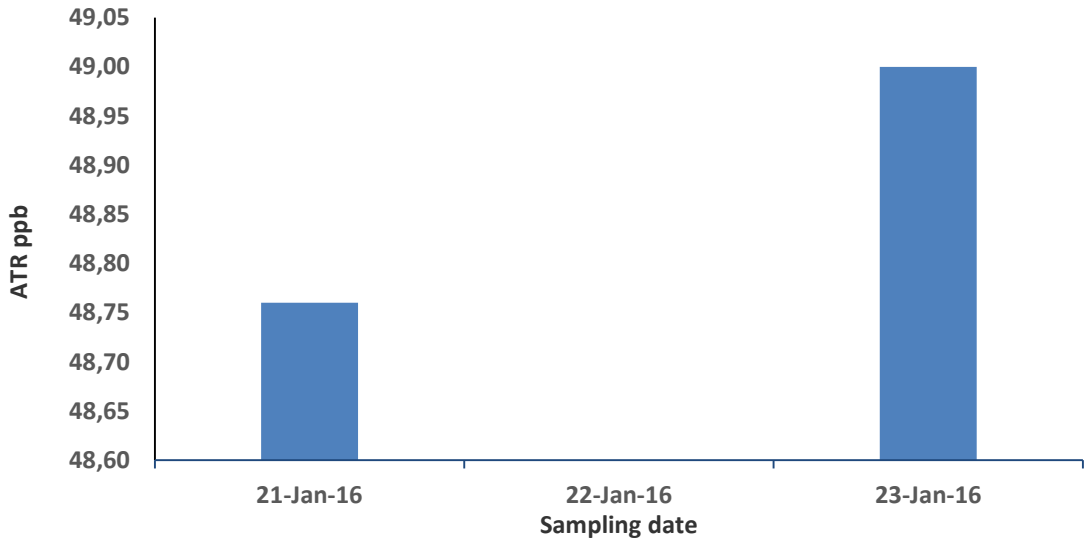

Figure 4: Occurrence of atrazine in borehole water, only on occasions it was detected.

The occurrence of such pulses was attributed to seepage of atrazine from fields where the drain collects the water. The location of the borehole is below fields J4 and M3, and it is probable that underground seepage could have conveyed the herbicide to the borehole (Figure 5).

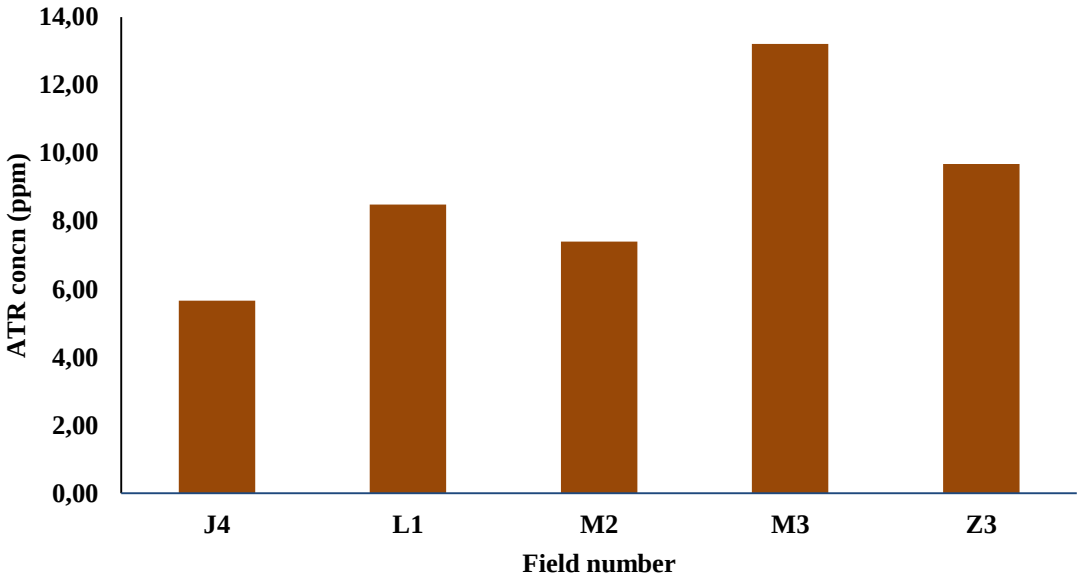

Figure 5. Atrazine in soils taken from field numbers J4, L1, M2, M3 and Z4.

The levels of atrazine range from 5.66 to 13.21 ppm. For a long period these soils had not received treatment with atrazine. These levels could be due to the effect of ploughing that could have brought to the surface residual atrazine bound in the soil. Ploughing and disking operations usually go to depths of more than a metre. Thus this process could be responsible for bringing atrazine buried in the lower soil strata to the surface.

Conclusion

This initial review informs the sugarcane industry stewards that there is a need to establish baselines for the amount and presence of herbicides residues in the soil and water. A laboratory experiment showed that in about 10 weeks after herbicide application low levels of the residual atrazine were detected both in the soil and in leached water. It is within this time that herbicides need to be monitored. Under the conditions of this study, the results show potential herbicide pollution as the herbicide was detected in the borehole and open drain. The highest amount of atrazine in the borehole water was found to be 49.00±3.32 ppb. Residual atrazine in the open drain was found to be in the range 21.71 to 109.21 ppb. Typical soil samples had residual atrazine covering the range 5.66 to 13.21 ppm for the fields J4, L1, M2, M3 and Z3. This was even after years of not using atrazine as an herbicide in these fields.

Recommendations

There is a need to continue with this project so that it covers all the pesticides previously used and still being used in sugarcane production for the control of weeds. The project needs to be extended to cover all areas under sugarcane production. These areas are to include Out-growers, Mwenezana Estate, Triangle and Hippo Valley Estates. Fields with subsurface drains or tile drains cutting through them are good candidates since they allow for the collection of drainage water. There is also the need to identify fields which have not been previously been treated with herbicides, and sink lysimeters to collect water samples at pre-defined depths. Accurate and undisputed results of herbicides need to be conducted using radio-labeled herbicides to clearly show the dynamics and movements of atrazine from the soil to underground and surface water reserves.

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REFERENCES

Barganska Z, Slobodan M and Nameisnik J (2014). Determination of pesticide residue in honeybees using modified QuECheRS and liquid chromatography – tandem mass spectrometry. *Molecules* Vol No: 2911-2924.

Gianni S, Mirco A, Maddalena C, Cristalli G, Franoletti E, Giardina D and Vittori S (2007). Well water in central Italy: Analysis of herbicide residues as potential pollutants of untreated crops. *Fresenius Environmenta Bulletin* Vol No: 973-979.

Gupta OP (2011). *Weed Management Principles and Practices*. Third Edition. AGROBIOS, India.

Hayes TB, Collins A, Lee M, Mundoza M, Noriega N, Stuart AA and Vonk A (2002). Hermaphroditic demasculined frogs after exposure to herbicide atrazine at low ecological relevant doses*. Proc National Academy Science,* Vol No: 5476-5480.

Kolpin DW, Kalkhoff SJ, Goolsby DA, Fahre DA and Thurma EM (1995). Occurrence of selected herbicides and herbicide degradation products in Iowa ground water. *Journal of Ground Water* Vol No: 679-688.

Monaco TJ, Weller SC and Ashton FM (2002). *Weed Science: Principles and Practices*. New York: John Wiley and Sons.

Mullins DE and Cobb JM (2007). Pesticide residue in soil and water from four cotton growing areas of Mali, West Africa. *Agricultural, Food and Environmental Sciences*. Vol1. ISSN 1934-7235.

Popov VH and Cornish PS (2006). Atrazine degradation in soils: The role of microbial communities, atrazine application rate, and soil carbon. *Australian Journal of Soil Research*. 43: 861.

Sagratini G, Ametisti M, Cannella M, Cristalli G, Francoletti E, Giardina D, Luminari M, Paparelli G, Pico Y, Volpini R, Vittori R and Camerino (2007). Analysis of herbicide residues as potential pollutants of untreated crops: *Fresenius Environmental Bulletin* Vol 16.

Safiatou BD, Jean MC and Donald EM (2007). Pesticide residues in soil and water from four cotton growing areas of Mali, West Africa. *J Agric Food Environ Sci* 1(1): 1-17.

William A, Battaglin, Earl MT and Stephen DP (2003). Herbicides and transformation products in surface waters of Midwestern United States. *Journal of the American Water Resources Association*, Colorado.

Zeljezic D, Garaj V and Perkovic P (2006). Toxicology in vitro. *Journal of Toxicology* pp. 923-935.