Degradation characteristics of metribuzin in soils within the Nzoia River Drainage Basin, Kenya

ARTICLE · JANUARY 2016

DOI: 10.1080/02772248.2015.1128938

3 AUTHORS:

Gershom Mutua

Masinde Muliro University of Science and Tech…

2 PUBLICATIONS **0** CITATIONS

SEE PROFILE

Anastasiah Ngigi

Multimedia University College of Kenya

11 PUBLICATIONS **43** CITATIONS

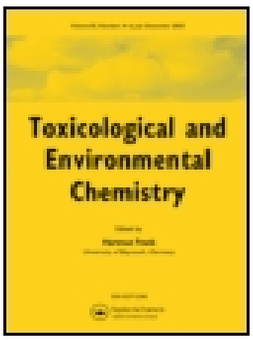
SEE PROFILE

Zachary Getenga

Masinde Muliro University of Science and Tech…

30 PUBLICATIONS **222** CITATIONS

SEE PROFILE



Toxicological & Environmental Chemistry

ISSN: 0277-2248 (Print) 1029-0486 (Online) Journal homepage: http://www.tandfonline.com/loi/gtec20

Degradation characteristics of metribuzin in soils within the Nzoia River Drainage Basin, Kenya

Gershom Kyalo Mutua, Anastasiah Njoki Ngigi & Zachary Moranga Getenga

To cite this article: Gershom Kyalo Mutua, Anastasiah Njoki Ngigi & Zachary Moranga Getenga (2015): Degradation characteristics of metribuzin in soils within the Nzoia River Drainage Basin, Kenya, Toxicological & Environmental Chemistry

To link to this article: http://dx.doi.org/10.1080/02772248.2015.1128938

Published online: 31 Dec 2015.

TOXICOLOGICAL & ENVIRONMENTAL CHEMISTRY, 2016
http://dx.doi.org/10.1080/02772248.2015.1128938

Taylor & Francis
Taylor & Francis Group

Degradation characteristics of metribuzin in soils within the Nzoia River Drainage Basin, Kenya

Gershom Kyalo Mutua[a], Anastasiah Njoki Ngigi[b] and Zachary Moranga Getenga[c]

[a]Department of Pure and Applied Chemistry, Masinde Muliro University of Science and Technology, Kakamega, Kenya; [b]Department of Physical Sciences, Multimedia University of Kenya, Nairobi, Kenya; [c]Department of Chemistry, Chuka University, Chuka, Kenya

ABSTRACT

Metribuzin, a triazinone herbicide, is heavily used within the expansive Nzoia River Drainage Basin in Kenya for the optimization of sugarcane yields. For field experiments, soils were spiked with metribuzin and amended with filter mud compost and *Tithonia diversifolia* leaves. Soils with history of metribuzin application (48 months) were also spiked with metribuzin but not amended with the organic materials. Degradation of metribuzin for the three variants was followed for a period of 102 days. Repeated exposure of metribuzin to soil and addition of filter mud compost to soil enhances the degradation of metribuzin with half dissipation times of 31 and 25 days. In soil amended with *Tithonia diversfolia* leaves, the half dissipation time was 32 days while in the control (unamended non history soil), it was 36 days. Laboratory studies showed that soil sterilization slowed the degradation of metribuzin, with a half dissipation time of 154 days. This confirmed that metribuzin was biochemically degraded in soil by an adapted community of microbes.

ARTICLE HISTORY
Received 12 May 2015
Accepted 2 December 2015

KEYWORDS
Filter mud compost; application history; herbicide; *Tithonia diversifolia* leaves; degradation

1. Introduction

The Nzoia River Drainage Basin (NRDB) is an important agricultural zone in Kenya with its middle region dominated by large-scale sugarcane (*Saccharum officinarum*) farms (Wamukoya and Ludeki 2006). The gently sloping terrain, extremely friable volcanic soils coupled with extensive use of pesticides and heavy rainfall has led to contamination of environmental matrices within and beyond the basin (Tarus et al. 2010; Muendo, Lalah, and Getenga 2012).

Metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one] is a selective systemic asymmetrical triazine herbicide that is heavily used within the basin for both pre- and post-emergence control of weeds in the sugarcane farms. It is listed as a toxic release inventory chemical whose soil half-life ranges between 14 to 60 days (USEPA 2003). Due to its low adsorption coefficient (1.12−1.25 mL g^{-1}), high water solubility (1.05 g L^{-1}, 20 °C) (Selim 2003), and high toxicity, metribuzin poses a great risk to

CONTACT Anastasiah Njoki Ngigi angigi@mmu.ac.ke

humans, terrestrial, and aquatic fauna and flora. In fact, it is more toxic than atrazine, alachlor, and metolachlor (Fairchild, Ruessler, and Carlson 1998). Metribuzin has been detected in soil and water (Pierre-Yves, Charlotte, and Allan 1996; Dores et al. 2008). Its persistence in soil is positively correlated to low organic matter, high application amounts, as well as high soil pH (Fuscaldo, Bedmar, and Monterubbianes 1999). Increasing organic matter content of soil through amendments has been reported to effectively decontaminate pesticide-polluted environments by enhancing their degradation (Lalah, Muendo, and Getenga 2009; Mutua, Ngigi, and Getenga 2015). Therefore, this study investigated the effectiveness of filter mud compost (FM) and *Tithonia diversfolia* leaves (TD) as organic amendments in enhancing the field degradation of metribuzin. The two organic materials are readily available within the basin. Over 24,000 tons of FM is produced annually within the basin (Jemutai-Kimosop, Orata, and Getenga 2012) since it constitutes about 4% of the total processed sugarcane (Jadhav 2011). TD is an annual weed with very high vegetative matter turn over. Over 10,000 tons of TD is harvestable within the watershed annually (Jemutai-Kimosop, Orata, and Getenga 2012).

Repeated exposure of pesticides to soil has been reported to modify their degradation (Getenga, Doerfler, and Schroll 2009; Mutua, Ngigi, and Getenga 2015). No information is available on the degradation characteristics of metribuzin as affected by application histories despite its intensive use within the region. Studies within the watershed have concentrated on sorption and leaching of metribuzin (Lagat et al. 2011). Therefore, the effect of repeated application of metribuzin to soil on its degradation was studied. In addition, laboratory experiments were conducted to establish the contribution of microbes to the degradation of metribuzin in soil. This was done by setting up parallel degradation experiments in non-sterile and sterile soils. Some of the metabolites formed during the degradation process of metribuzin were identified.

The information obtained on the degradation characteristics of metribuzin under different treatments will play a pivotal role in sustainable environmental management and environmental policy formulation. It will provide a suitable and environmentally friendly way of disposing TD and FM which are abundant in the region. The findings will also form a basis for other related studies on microbial degradation and subsequent isolation of metribuzin degraders.

2. Materials and methods

2.1. Reagents and materials

Analytical standards of metribuzin (99.0%) (CAS RN 21087-64-9), deaminometribuzin (DA) (97.5%) (CAS RN 35045-02-4), and deaminodiketometribuzin (DADK) (98.0%) (CAS RN 52236-30-3) were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). Standard stock solutions at concentrations of 100 mg L^{-1} in acetonitrile were prepared and working solutions were prepared by appropriate dilution of the stock solutions. Polyvinyl chloride (PVC) pipes, reagents, chemicals, and consumables were purchased from local reputable suppliers. All the reagents and chemicals were used without further purification.

FM and TD were obtained from the nuclear estate of the Nzoia Sugar Company within the expansive NRDB. Their elemental composition was determined; FM had 11.7% C,

2.6% N, 0.05% P, 0.11% K, and 0.4% Fe, TD contained 24.2% C, 2.94% N, 0.08% P, 0.09% K, and 0.3% Fe. Coarse and plant materials were removed from FM. TD materials were air-dried and chopped into small pieces. Both FM and TD were sieved (2 mm) in preparation for soil amendment.

2.2. Field degradation studies

Field degradation studies were conducted in sugarcane farms within the Nzoia Sugar Company nuclear estate where metribuzin is heavily used for pre- and post-emergency control of weeds. Experiments were conducted using PVC pipes of 45 cm length and an internal diameter of 6 cm which accommodated 844.6 ± 72.5 g ($n = 3$) of dry soil (Lalah, Muendo, and Getenga 2009). Background concentrations of metribuzin, DA, and DADK in soil were established before the experiments were conducted.

Four experiments were set for the study on FM, TD, application history, and control (unamended non history). The study on the effect of FM and TD was conducted in a field without prior application history of metribuzin (0° 34′ 31″ N and 34° 40′ 18″ E). The soils were sandy loam with 0.14% ± 0.01% N, 2.73% ± 0.17% C, 12.40% ± 0.05% P, and 1.30% ± 0.03% K. The fields were well prepared ready for planting by deep digging and removing all weeds and stones. The prepared fields were left to stabilize for two weeks. FM and TD were applied at the practiced field application rates of 30 and 5 tons per hectare, respectively. This translated to 8.48 g per pipe (1.004×10^4 mg kg^{-1} of soil) of FM and 1.44 g per pipe (1.71×10^3 mg kg^{-1} of soil) of TD. PVC pipes, one meter apart, were driven into the soil with about 4 cm length left protruding above the surface to prevent loss due to surface run-off. The pipes were then spiked with metribuzin at the field application rate of 1.64 kg of active ingredient per hectare giving an equivalent concentration of 42 mg kg^{-1} per pipe.

The study on the effect of application history was conducted on a cultivated field (0° 31′ 44″ N and 34° 41′ 39″ E) with a prior application history of 48 months whose residual concentrations of metribuzin, DA, and DADK were below detection limit. The soils were sandy loam with 0.13% ± 0.02% N, 2.70% ± 0.24% C, 12.38% ± 0.06% P, and 1.30% ± 0.06% K. Metribuzin was applied as described above. The control experiment for both studies was conducted on unamended soil without metribuzin application history. All experiments for the different treatments in triplicates lasted for 102 days. Each treatment had a total of 21 pipes. Immediately after setting up the experiment, samples were collected, and further sampling was done on the 1st, 7th, 21st, 35th, 63rd, and 102nd day. For each sampling time, the soils in the whole pipe (45 cm, in triplicate) were taken to obtain a representative sample. The dry weight of soil sample in each pipe ($n = 3$) was 844.6 ± 72.5 g.

During the study period, the weather was monitored. The average relative humidity and evaporation rate were 49.5% ± 14.6% and 6.1 ± 1.4 mm day^{-1}, respectively. The mean air temperature and soil temperature to a depth of 30 cm below the earth's surface were 21.2 ± 1.0 °C and 26.6 ± 1.1 °C, respectively. The average dew point temperature was 11.5 ± 4.0 °C. Rainfall of 250.7 and 63.8 mm was recorded during the first and second months of the study. In the last month, a total of 2.6 mm of rainfall was recorded. A mean daily wind run of 119 ± 29 km day^{-1} and sunshine of 7.2 ± 1.4 hours day^{-1} was recorded.

2.3. Laboratory degradation studies

The study was conducted using soil with a metribuzin application history of 48 months. The residual levels of metribuzin, DA, and DADK in this soil was below detection limit. Soil samples were obtained from the upper layer (0−10 cm) using soil auger. The moisture content was gravimetrically determined by heating 1 g of the soil at an oven temperature of 105 °C for 24 hours. The experiments were conducted using 250 mL conical flasks (18 in total) into which aliquots of 3.5 g of dry soil were added. Then, metribuzin dissolved in acetonitrile was added drop-wise at the field application rate of 1.64 kg ha^{-1} (42 mg kg^{-1}). The conical flasks were then placed in a fume hood for 24 hours for the solvent to evaporate. The moisture content of the samples was restored to 60% (gravimetric water content of soil at 100% of water holding capacity) of the water holding capacity by addition of deionized water. Additional aliquots of 46.5 g of dry soil equivalent were then added to each flask, homogenized and compressed to a volume of 38.5 mL (Getenga, Doerfler, and Schroll 2009). All the flasks were then loosely covered with perforated aluminum foils and the moisture content maintained at 60% throughout the study period. A sterile set (18 flasks) was used as a control; microbial inhibition was achieved by addition of 1% (w/v) of sodium azide to the soil samples (Rastegarzadeh, Nelson, and Ririe 2006). The pesticide was applied as described above. All experiments were conducted in triplicate at room temperature and monitored for 102 days. Sampling was done immediately after setting the experiment and on 1st, 7th, 35th, 63rd, and 102nd day.

2.4. Extraction and analysis

After sampling, soil in the whole pipe (in triplicate) was taken to the laboratory, air dried in the shade and homogenized. Then, soil sub-samples of 50.0 g (in triplicate) were transferred to single-layer cellulose thimbles and extracted by Soxhlet method using 150.0 mL methanol for 5 hours, a slight modification of the method by Johnson and Pepperman (1995). The extracts were concentrated to about 2.0 mL at reduced pressure using a rotary evaporator (N-1000 model, Eyela, Tokyo, Japan) and then diluted with 250 mL of deionized water. Clean-up was done by solid-phase extraction (SPE). The SPE cartridges (Strata C-18 E 55 μm, 70 A, 1000 mg 6m L^{-1}, Kobian, Nairobi, Kenya) were mounted on a 24-position vacuum operated SPE manifold set (Phenomenex, Cheshire, United Kingdom) and pre-conditioned with 10.0 mL methanol and 10.0 mL deionized water. The extracts were then loaded immediately and analytes eluted with 4.0 mL methanol. A high-performance liquid chromatograph (HPLC) (Prominence LC-20AT, Shimadzu, Kyoto, Japan) with an ultra-violet detector (Prominence, SPD-20A, Shimadzu), degasser (DGU-20A prominence, Shimadzu), and C_{18}-column (250 × 4.6 mm, 5 micron, 130 A, Phenomenex) was used for the analysis of metribuzin and its metabolites. Detection wavelength was 254 nm. The mobile phase consisted of methanol and 0.05 mol L^{-1} acetic acid (62:38, v/v) at a flow rate of 0.5 mL min^{-1} (Pavel et al. 1999). A 10 μL syringe (Hamilton, Bonaduz, Switzerland) was used for sample injection. The limits of detection based on a noise signal ratio of 1:3 and limits of quantification based on a ratio of 1:10 for both metribuzin and DADK were 0.01 ± 0.00 and 0.04 ± 0.02 mg L^{-1}, respectively, for DA 0.01 ± 0.00 and 0.05 ± 0.02 mg L^{-1}.

Before sample analysis, standard solutions were run to check column performance, peak height, and resolution. With each set of samples to be analyzed, a solvent blank and a standard mixture were run in sequence for peak identification. Standard calibration curves were used for pesticide residue quantification. Linearity of the ultraviolet detector used was assessed by plotting a curve of peak areas against concentration (0−25 mg L^{-1}). Linearity was observed for all the three compounds with R^2 = 0.9987 (metribuzin), R^2 = 0.9992 (DA), and R^2 = 0.9987 (DADK). Recovery studies and relative standard deviations (RSD) done on soil free of the analytes were used to evaluate the precision and accuracy of the extraction method adopted (Huertas-Pérez et al. 2006). Soil samples were fortified with standard solutions at two different concentration levels (0.5 and 1 mg L^{-1}). Four replicates were prepared at each concentration level, extracted and analyzed as described above. High-percentage recoveries and low RSD values were obtained indicating a good accuracy and precision of the method adopted. Recoveries were 85.0 ± 7.9 for metribuzin, 87.0 ± 2.4 for DA, and 88.5% ± 2.2% for DADK. RSD values were 5.9% ± 2.0% for metribuzin, 5.0% ± 0.1% for DA, and 8.1% ± 1.9% for DADK.

The decrease of concentrations of methanol extractable metribuzin and concentrations of metabolites formed was analyzed, not the degradation by analyzing the production of CO_2. Despite this, we assume that the disappeared metribuzin portion was degraded, and referred this part as "degraded metribuzin." Degradation curves for metribuzin in the different treatments were fitted in first-order degradation kinetics ($C_t = C_o\, e^{-kt}$). Statistical treatment of degradation data obtained was done using SPSS 17.0 version software. Significance of differences in degradation of metribuzin under the various treatments was done using paired *t*-test at 95% confidence level.

3. Results and discussion

3.1. Degradation of metribuzin in soils amended with FM and TD

The disappearance of metribuzin in soils amended with FM and TD were compared with unamended (control) soil (Figure 1(a)).

Soil concentrations of metribuzin in the three treatments showed an initial rapid derease up to the 21st day followed by a slower rate reaching a plateau on the 63rd day. The decreased rate of degradation over time could be due to adsorption of metribuzin hence slowing the degradation processes. The plateau indicates lack of extractability or bioavailability of metribuzin residues to microbes (Getenga, Madadi, and Wandiga 2004).

From Figure 1(a), degradation of metribuzin was fastest in the FM-amended soil and slowest in the unamended soil. At the end of the experiment, metribuzin residue levels were 4.0% ± 1.5% in the FM-amended, 9.2% ± 1.3% in TD-amended, and 10.0% ± 1.5% of applied amount in the unamended soil. The degradation half-life values were 25 days (k = 28 μg kg^{-1} day^{-1}, R^2 = 0.89) in soil amended with FM, 32 days (k = 22 μg kg^{-1} day^{-1}, R^2 = 0.88) in soil amended with TD and 36 days (k = 19 μg kg^{-1} day^{-1}, R^2 = 0.92) in the control. The results obtained in this study are comparable to those obtained by Lechon et al. (1997) in which field half-life values of between 16 and 52 days were reported. Amending soil with FM significantly enhanced (ρ = 0.014) the field degradation of metribuzin. In addition, degradation of metribuzin in the two organic

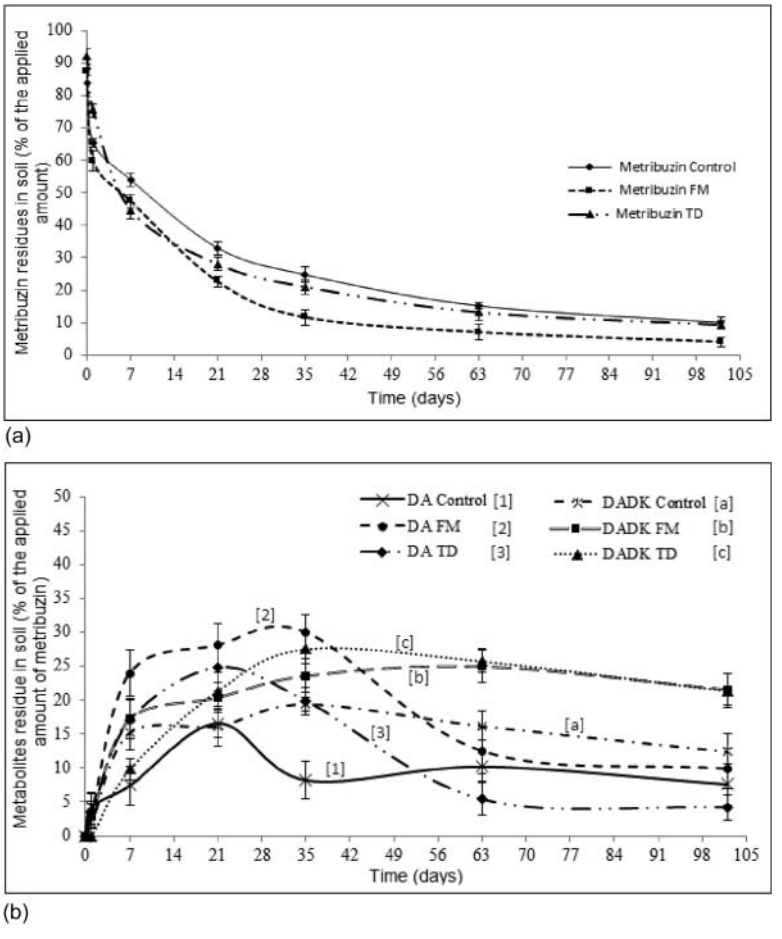


Figure 1. (a) Degradation of metribuzin under field conditions in soil with two organic amendments and in unamended soil. (b) Formation of methanol extractable metabolites under field conditions in soil with two organic amendments and in unamended soil.

amendments were significantly different ($\rho = 0.022$). However, the degradation of metribuzin in TD-amended and unamended soil were not significantly different ($\rho > 0.05$). The higher degradation rates of metribuzin observed in FM- and TD-amended soils compared to the unamended is as a result of increased nutrients in soil. Addition of organic matter to soil increases the rate of degradation by stimulating microbial growth and activities (Getenga, Madadi, and Wandiga 2004). Improved organic content promotes the activities of soil enzymes leading to improved microbial populations (Tejada et al. 2010). It may also be as a result of upset of carbon−nitrogen ratio of the soil leading to the use of metribuzin to regain the optimal nutrient balance (Lalah, Muendo, and Getenga 2009). Soils from the study site had a nitrogen content of 0.14%, and a C/N ratio of 20. Therefore, addition of FM and TD with nitrogen content of 2.6% and 2.9%, respectively, decreased the carbon−nitrogen ratio (≈ 5 and ≈ 8 for FM and TD, respectively) creating a scarcity in soil carbon. Thus, soil microbes utilized metribuzin as a carbon and energy source resulting in observed enhanced degradation (López-Piñeiro et al. 2011).

The higher degradation rate observed in the FM-amended soil compared to the TD-amended soil may have been due to the fact that the added FM was already decomposing and therefore easily released nutrients to soil. In addition, a small amount of TD (1.71×10^3 mg kg^{-1} of soil) was used compared to FM (1.004×10^4 mg kg^{-1} of soil), therefore the nutrients supplied by TD were less compared to that supplied by FM. Accordingly, a slower degradation rate was realized as degradation is positively correlated to the amount of organic matter applied (Getenga, Madadi, and Wandiga 2004). This implies that, increasing the amount of TD applied to soil would significantly enhance the degradation of metribuzin.

The rate of degradation was slowest in the unamended soil because there was no stimulation of microbial activities. This is because of inadequate decomposable organic matter in study soil, making metribuzin recalcitrant in soil for a long time.

The enhanced degradation reported corroborates with other studies in which addition of organic matter increased degradation of metribuzin (Khoury, Camille, and Kawar 2006). Soils amended with composited and field-aged olive mill waste recorded higher rates than unamended soil (López-Piñeiro et al. 2013) as well as those amended with compost (Getenga, Madadi, and Wandiga 2004).

The study shows that addition of both FM and TD enhanced metribuzin degradation in soils. Therefore, this strategy can be effectively used in cleaning polluted environment by utilizing and enhancing the activities of indigenous microbes.

3.1.1. Metabolites formation in soils amended with organic materials

Metabolites DA and DADK were observed in the three treatments and quantified over the study period of 102 days as expressed in Figure 1(b).

The concentration of metabolite DA was highest in the FM-amended soil recording a value of 30.0% ± 2.6% on the 35th which decreased to 9.9% ± 2.9% at the102nd day. In the TD-amended soil and the control, the highest concentrations of metabolite DA were observed on the 21st day which were 24.8% ± 3.5% and 16.7% ± 3.1%, respectively. At the close of the experiment, the levels were 4.2% ± 1.8% and 7.6% ± 3.0% in the TD-amended and control, respectively. The concentration of DADK obtained in the FM-amended soil reached a maximum value of 25.0% ± 2.4% on the 63rd day. The highest concentration of DADK in the TD-amended soil was 27.6% ± 2.2% on the 35th day which reduced to 21.4% ± 3.4% on the 102nd day while the control had a maximum value of 19.4% ± 2.3% on the 35th day. Metabolite, DADK was very stable in both FM-amended and unamended soils.

From the results, amending soil with organic materials favours the degradation of metribuzin and the subsequent formation of metabolites. FM favored significantly ($\rho = 0.019$) the deamination of metribuzin resulting in high amounts of DA compared to soil amended with TD and the control. The enhanced formation of metabolite DA is consistent with the enhanced degradation of metribuzin in FM-amended soil compared to the TD-amended soil and the control. FM was already decomposing and therefore easily released nutrients to soil leading to enhanced degradation of metribuzin.

The levels of DADK in both FM- and TD-amended soils were not significantly different throughout the experiment; however, its levels in the two treatments were higher compared to the control. This indicates that, addition of organic materials promotes both deamination and oxidative desulfuration of metribuzin.

3.2. Degradation of metribuzin in soils with previous application of metribuzin

Degradation curves of metribuzin in soils with and without prior metribuzin application history is shown in Figure 2(a). The residue levels in soil with application history were 45.0% ± 0.9% on the 7th day reducing to 6.0% ± 1.2% of the applied amount on the 102nd day. On the other hand, the levels in the soil without metribuzin application history were 54.0% ± 2.0% on the 7th day and decreased to 10.0% ± 1.5% of the applied amount at the close of the experiment.

Pair-wise multiple comparisons showed that metribuzin degradation in soil with prior application history was significantly different ($\rho = 0.021$) from that in soil without application history. This may be due to the presence of native adapted microbes that have developed resistance to the toxicity of metribuzin as a result of continuous exposure of metribuzin to soil. It can also be due to the emergence of opportunistic microbes which utilize metribuzin as carbon and energy source making them to multiply significantly (Tamilselvan et al. 2014). A fast degradation rate of metribuzin was noted in soil with prior application history during the first 21 days of the experiment compared to that in soils without application history. The half-life of metribuzin in soil with application

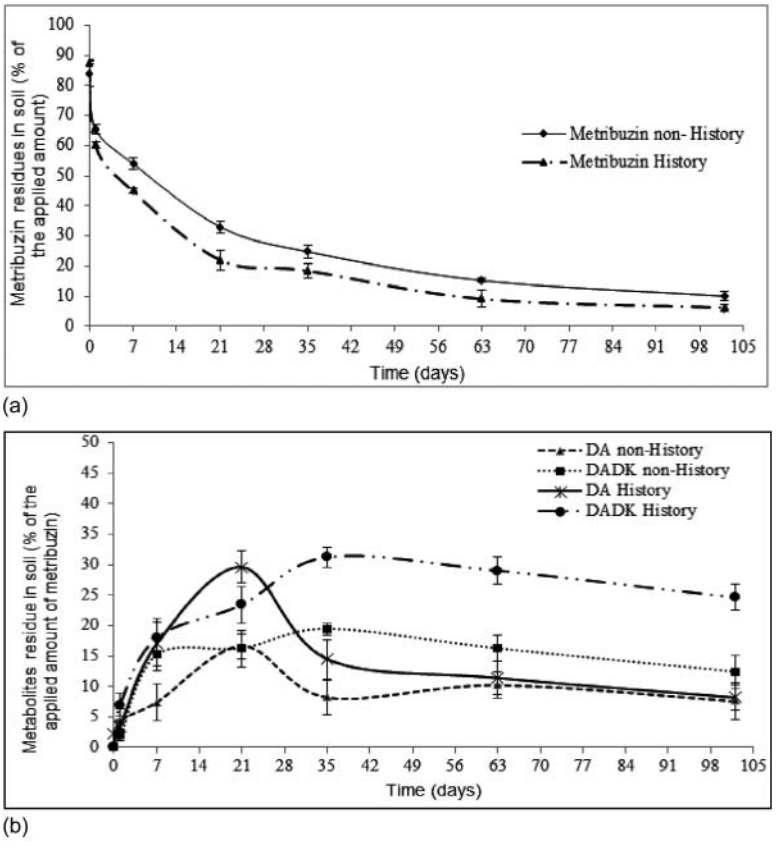


Figure 2. (a) Degradation of metribuzin under field conditions in soil with and without metribuzin application history. (b) Formation of methanol extractable metabolites under field conditions in soil with and without metribuzin application history.

history was 31 days ($k = 23$ μg kg^{-1} day^{-1}, $R^2 = 0.89$). In the control treatment, the half-life was 36 days ($k = 19$ μg kg^{-1} day^{-1}, $R^2 = 0.92$). Other studies have also reported enhanced degradation of metribuzin as a result of long history application of metribuzin to soil (James and Rahman 2010) hence reduced efficacy of metribuzin to control weeds. Enhanced degradation of other triazine herbicides in soil with prior pesticide application history or its analog has been reported. For example, enhanced degradation of atrazine has been reported in soils with prior application history of atrazine, ametryn, and propazine (Krutz et al. 2008; Getenga, Doerfler, and Schroll 2009).

Enhanced degradation of the herbicide in soil may lead to reduced efficacy to control weeds when used as a post emergence herbicide and some weeds becoming resistant to metribuzin. However, it is a natural way of detoxifying contaminated environment.

3.2.1. Metabolites in soils with and without application history

Figure 2(b) shows the concentrations of metabolite DA and DADK in soils with and without prior application history of metribuzin. By the end of the experiment, in soil with prior application history of metribuzin, DA had degraded by more than 72% of its highest concentration while in soil without application history, 54% of DA had degraded. This implies that, in addition to the presence of metribuzin-degraders in the soil with pesticide application history, there also existed microbes that degraded DA. On the 21st day, the levels of DA were maximum in both treatments with concentrations of 29.6% ± 2.7% and 16.6% ± 3.0% in soils with and without application history of metribuzin, respectively. However, from the 63rd day, the concentrations were not different for the two variants. There was a significant difference ($\rho = 0.004$) in the levels of metabolite DADK in soil for the two treatments. On the 102nd day, DADK residues were 24.6% ± 3.2% and 12.4% ± 2.7% in soils with prior history and those without history, respectively. The enhanced levels of DADK in soil with application history implies that repeated exposure of soil to metribuzin favored the degradation of metribuzin and DA. However, the persistence of DADK was not affected by the application history of metribuzin.

3.3. Laboratory degradation studies of metribuzin

Figure 3(a) shows degradation characteristics of metribuzin in sterile and non-sterile soil with previous exposure of metribuzin. The moisture content (±SD) of the soil used in this study was 17.2% ± 0.1%. The concentration of metribuzin residues in both sterile and non-sterile soils decreased over time. On the 35th day, the level of metribuzin residues in the non-sterile set was 33.3% ± 3.3% of the applied amount and decreased further to 13.0% ± 2.4% on the 63rd day. In the sterile set, the concentration of metribuzin residues were 71.9% ± 3.8% and 63.0% ± 4.3% of the applied amount on the 35th and 63rd day, respectively. At the close the experiment, 93.6% of the applied metribuzin had degraded in the non-sterile set while the percentage degradation in the sterile set was 41.4%.

Metribuzin was more rapidly degraded ($\rho = 0.027$) in non-sterile soil compared to the sterile set recording a DT_{50} value of 32 days ($k = 21.7$ μg kg^{-1} day^{-1}, $R^2 = 0.96$). This DT_{50} value compares closely to that obtained by other studies. Lechon et al. (1997) reported laboratory half-life range of between 19 and 106 days at 25 °C depending on moisture content. At an average temperature of 28 °C, the laboratory half-life ranged

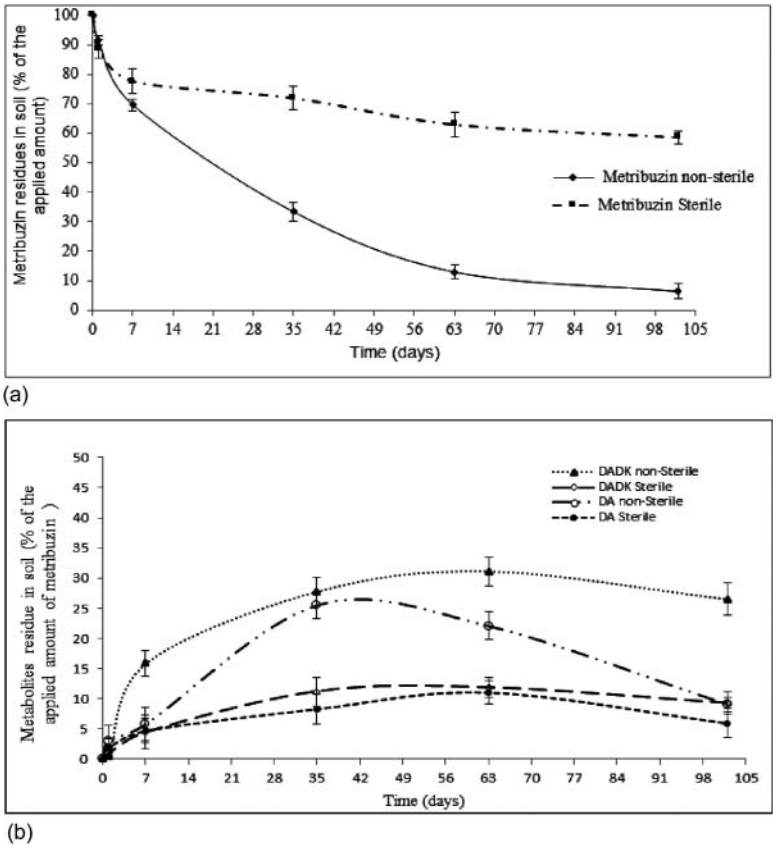


Figure 3. (a) Laboratory degradation of metribuzin in a soil material after application of metribuzin and incubated in vitro after sterilization with sodium azide and in non-sterilized soil. (b). Development of methanol extractable metabolites in a soil material after application of metribuzin and incubated in vitro after sterilization with sodium azide and in non-sterilized soil.

between 26 and 36 days (Perceval et al. 2006). The half-life of metribuzin in the sterile soil was 154 days ($k = 4.5$ μg kg^{-1} day^{-1}, $R^2 = 0.84$), about five times longer than in the non-sterile soil. The difference in degradation rates show that soil microbial activities play a key role in the degradation process of metribuzin. Since the soil had been previously applied with metribuzin, there appears to have developed microbes that are able to degrade metribuzin. The degradation of metribuzin in the sterilized soil is attributable to chemical degradation.

Singh, Raunaq, and Singh (2013) showed that there was no degradation of metribuzin in sterilized soil samples implying that the degradation of metribuzin is microbial in nature. Metribuzin is reported to be degraded by soil fungi *Botrytis cinerea*, *Sordaria superba* and *Absidia fusca* (Bordjiba et al. 2001), white-rot fungus *P. chrysosporium* (BKM-F-1767) (Castillo and Torstensson 2007), *Burkholderia cepacia* bacterial strains (Madhuban et al. 2011), *Pseudomonas aeruginosa*, *Staphylococcus aureus* (Tamilselvan et al. 2014), and *Bacillus* sp. N1 (Zhang et al. 2014). Likewise, streptomycetes utilize metribuzin as a nitrogen source (Mohamed et al. 2012).

3.3.1. Metabolites in both sterile and non-sterile soils

The concentrations of DA and DADK were higher in the non-sterile soil (Figure 3(b)). In the non-sterile soil, the levels of DA were 25.5% ± 3.8% and 9.1% ± 3.2% on the 35th and 102nd day, respectively, while in the sterile set, the levels of DA were 8.3% ± 3.6% and 6.0% ± 2.4%, respectively. The concentrations of metabolite DADK were 31.1% ± 2.5% and 26.5% ± 3.3% on the 63rd and 102nd day, respectively, for the non-sterile soils. Whereas in the sterile set, the levels of DADK were 11.2 ± 2.9% on the 35th day and reduced to 9.3% ± 2.7% on the 102nd day.

The higher concentrations of metabolite, DA observed in non-sterile soil indicates that the degradation rate of metribuzin in non-sterile soil was higher compared to sterile soil. It further confirms that the degradation of metribuzin is mainly through microbial activities as opposed to abiotic processes. Degradation of DA in the two sets was also observed. In the sterile soil, DA reduced by 46.9% of its highest value of 11.1% ± 2.9% while in the non-sterile soil it decreased by 58.8% of its highest concentration of 25.5% ± 3.8%. This difference may be attributed to biotic degradation in the non-sterile soil.

In this study, three metabolites of metribuzin were observed throughout the study period of 102 days for all the different treatments. Two of them were positively identified as DADK and DA with retention time of 15.1 and 21.1 min, respectively. The peak of the parent metribuzin appeared at 18.5 min while unidentified metabolite appeared at 24.3 min.

In Figure 4, the degradation pathway of metribuzin is shown. Metabolite DA is formed through the deamination of metribuzin (Rasche et al. 1998; Khoury, Camille, and Kawar 2006) as a result of microbial and photochemical processes (Rasche et al. 1998). On the other hand, metabolite DADK is a product of reductive deamination of metribuzin. Metribuzin undergoes two different pathways to yield DADK. One route involves the deamination of metribuzin to form DA which then undegoes oxidative desulfuration to produce DADK. The other pathway involves oxidative desulfuration of metribuzin

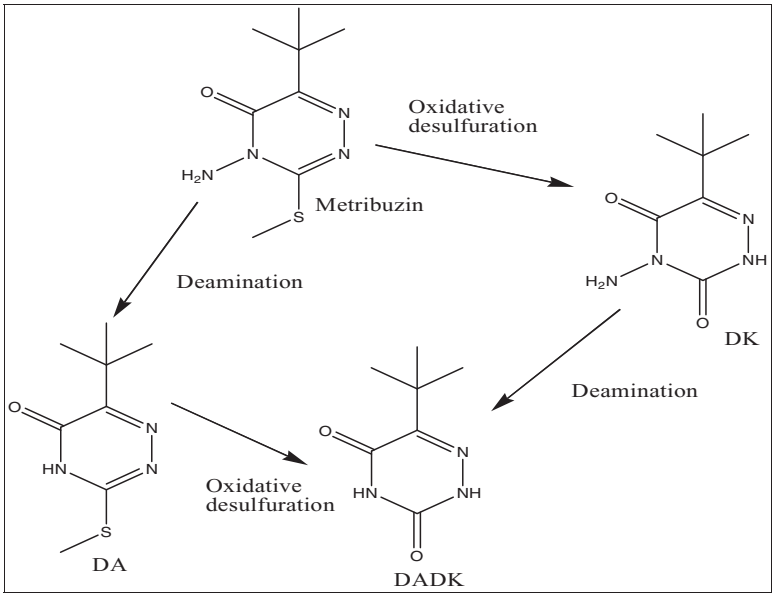


Figure 4. Degradation pathways of metribuzin (Huertas-Pérez et al. 2006).

yielding diketometribuzin (DK) which then undergoes deamination (Henriksen, Svensmark, and Juhler 2002). In this study, oxidative desulfuration of DA may have greatly contributed to the observed concentrations of DADK. This is because the soil had been freshly cultivated and therefore well aerated (Khoury et al. 2003). Both DA and DADK are polar than the parent metribuzin, therefore they poses more risk to water contamination than metribuzin. Total degradation of metribuzin is reported to occur through DADK (Henriksen, Svensmark, and Juhler 2002; Khoury et al. 2003). Thus, the detection of DADK implies possible complete mineralization of metribuzin in these soils. However, this would need to be investigated.

4. Conclusion

The study established that field degradation of metribuzin can be enhanced by addition of FM compost and *Tithonia diversifolia* leaves thus reducing its concentration in soil and water within the NRDB and beyond. It was also established that prolonged use of metribuzin for weed control leads to enhanced degradation due to the development of adapted native microbes. Sterilization of soil significantly decreased the degradation of metribuzin proving that microbial degradation contributes greatly in the degradation of metribuzin. The study recommends that the effects of varying amounts of FM and TD be investigated and the isolation of microbes responsible for the degradation of metribuzin be carried out.

Acknowledgements

This work was supported by the National Commission of Science, Technology and Innovation, Kenya, under Grant number (NCST/5/003/3rd CALL MSc/026). We recognize the contribution of Selly Kimosop and Agnes Muyale towards the completion of this work.

Disclosure statement

We authors declare that we have no conflict of interest.

Funding

National Commission of Science, Technology and Innovation, Kenya [grant number NCST/5/003/3rd CALL MSc/026].

References

Bordjiba, O., R. Steiman, M. Kadri, A. Semadi, and P. Guiraud. 2001. "Removal of Herbicides from Liquid Media by Fungi Isolated from a Contaminated Soil." *Journal of Environmental Quality* 30: 418–426. doi:10.2134/jeq2001.302418x.

Castillo, M.P., and L. Torstensson. 2007. "Effect of Biobed Composition, Moisture and Temperature on the Degradation of Pesticides." *Journal of Agriculture and Food Chemistry* 55: 5725–5733. doi:10.1021/jf0707637.

Dores, E.F., L. Carbo, M.L. Ribeiro, and E.M. De-Lamonica-Freire. 2008. "Pesticide Levels in Ground and Surface Waters of Primavera do Leste Region, Mato Grosso, Brazil." *Journal of Chromatographic Science* 46: 585–590. doi:10.1093/chromsci/46.7.585.

Fairchild, F., D. Ruessler, and A. Carlson. 1998. "Comparative Sensitivity of Five Species of Macrophytes and Six Species of Algae to Atrazine, Metribuzin, Alachlor and Metolachlor." *Environmental Toxicology and Chemistry* 17 (9): 1830−1834. doi:10.1002/etc.5620170924.

Fuscaldo, F., F. Bedmar, and G. Monterubbianes. 1999. "Persistence of Atrazine, Metribuzin and Simazine Hherbicides in Two Soils." *Pesquisa Agropecuaria Brasileira Brasília* 34 (11): 2037−44.doi:10.1590/S0100-204x1999001100009.

Getenga, Z.M., U. Doerfler, and R. Schroll. 2009. "Study of Atrazine Degradation in Soil from Kenyan Sugarcane-Cultivated Fields in Controlled Laboratory Conditions." *Toxicological and Environmental Chemistry* 91 (2): 195−207. doi:10.1080/02772240802144497.

Getenga, Z.M., V. Madadi, and S.O Wandiga. 2004. "Studies on Biodegradation of 2, 4-D and Metribuzin in Soil under Controlled Conditions." *Bulletin of Environmental Contamination and Toxicology* 72: 504−513. doi:10.1007/s00128-004-0273-8.

Henriksen, T., B. Svensmark, and R.K. Juhler. 2002. "Analysis of Metribuzin and Transformation Products in Soil by Pressurized Liquid Extraction and Liquid Chromatographic−Tandem Mass Spectrometry." *Journal of Chromatography A* 957: 79−87. doi:10.1016/S0021-9673(01)01453-4.

Huertas-Pérez, J.F., M.O. Iruela, A.M. García-Campaña, A. González-Casado, and A. Sánchez-Navarro. 2006. "Determination of the Herbicide Metribuzin and its mMjor Conversion Products in Soil by Micellar Electrokinetic Chromatography." *Journal of Chromatography A* 1102: 280−286. doi:10.1016/j.chroma.2005.10.052.

Jadhav, R.T. 2011. "Environmentally Safe Renewable Energy Technology Uusing Sugar Cane." *Universal Journal of Environmental Research and Technology* 1 (2): 216−218.

Jemutai-Kimosop, S., F. Orata, and Z. Getenga. 2012. "The Influence of Filter Mud Compost and *Tithonia diversfolia* Leaves on the Dissipation of Diuron in Soils within the Nzoia River Drainage Basin, Kenya." *Bulletin of Environmental Contamination and Toxicology* 89 (2): 328−333. doi:10.1007/s00128-012-0685-9.

Johnson, R.M., and B.A. Pepperman. 1995. "Analysis of Metribuzin and Associated Metabolites in Soil and Water Samples by Solid Phase Extraction and Reversed Phase Thin Layer Cchromatography." *Journal of liquid chromatography* 18 (4): 739−753. doi:10.1080/10826079508009269.

Khoury, R., A. Geahchan, C. Coste, J. Coopers, and A. Bobes. 2003. "Retention and Degradation of Metribuzin in Sandy Loam and Clay Soils of Lebanon." *European Weed Research Society* 43: 252−259. doi:10.1046/j.1365-3180.2003.00341.x.

Khoury, R., M. Camille, and S. Kawar. 2006. "Degradation of Metribuzin in Two Soil Types of Lebanon." *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants and Agricultural Wastes* 41 (6): 795−806. doi:10.1080/03601230600805790.

Krutz, L.J., D.L. Shaner, C. Accinelli, M. Zablotowicz, and W. Henry. 2008. "Atrazine Dissipation in *s*-triazine-adapted and Nonadapted Soil from Colorado and Mississippi: Implications of Enhanced Degradation on Atrazine Fate and Transport." *Journal of Environmental Quality* 37: 848−857. doi:10.2134/jeq2007.0448.

Lagat, S.C., J.O. Lalah, C.O. Kowenje, and Z.M. Getenga. 2011. "Metribuzin Mobility in Soil Column as Affected by Environmental and Physico-Chemical Parameters in Mumias Sugarcane Zone, Kenya." *ARPN Journal of Agricultural and Biological Science* 6 (3): 27−33. ISSN: 1990-6145.

Lalah, J.O., B.M. Muendo, and Z.M Getenga. 2009. "The Dissipation of Hexazinone in Tropical Soils Under Semi Controlled Field Conditions in Kenya." *Journal of Environmental Science and Health, Part B* 44 (7): 690−696. doi:10.1007/s00128-003-0065-6.

Lechon, Y., A.I. Garcia-Valcrcel, T. Matienzo, C. Snchez-Brunete, and J. L. Tadeo. 1997. "Laboratory and Field Studies on Metribuzin Persistence in Soil and its Prediction by Simulation Models." *Toxicological and Environmental Chemistry* 63 (1 and 4): 47−61.doi:10.1080/02772249709358516.

López-Piñeiro, A., D. Cabrera, A. Albarrán, and D. Peña. 2011. "Influence of Two-phase Olive Mill Waste Application to Soil on Terbuthylazine Behaviour and Persistence Under Controlled and Field Conditions." *Journal of Soil Sediments* 11: 771−782. doi:10.1007/s11368-011-0362-3.

López-Piñeiro, A., D. Peña, A. Albarrán, D. Becerra, and J. Sanchez-Lierena. 2013. "Sorption, Leaching and Persistence of Metribuzin in Mediterranean Soils Amended with Olive Mill Waste

of Different Degrees of Organic Matter Maturity." *Journal of Environmental Management* 15 (122): 76–84. doi:10.1016/j.jenvman.2013.03.006.

Madhuban, G., D. Debashis, S. Jha, K. Shobhita, S. Bandyopadhyay, and S. Das. 2011. "Biodegradation of Imidacloprid and Metribuzin by *Burkholderia cepacia* Strain CH9." *Pesticide Research Journal* 23 (1): 36–40. ISSN: 2249-524X.

Mohamed, H., A. Rahal, M. Zaki, E. Saleh, and A. Sadik. 2012. "Biodegradation of Sencor Herbicide by Some Streptomycetes in Liquid Culture." *Pakistan Journal of Biotechnology* 9 (2): 49–56. ISSN 1812-1837.

Muendo, B., J. Lalah, and Z. Getenga. 2012. "Behaviour of Pesticide Residues in Agricultural Soils and Adjacent River Kuywa Sediment and Water Samples from Nzoia Sugarcane Belt in Kenya." *The environmentalist* 32 (4): 433–444. doi:10.1007/s10669-012-9407-4.

Mutua, G.K., A.N. Ngigi, and Z.M. Getenga. 2015. "Chlorpyrifos Degradation in Soils with Different Treatment Regimes within Nzoia River Drainage Basin, Kenya." *Bulletin of Environmental contamination and Toxicology* 94 (3): 387–392. doi:10.1007/s00128-015-1465-0.

Pavel, W., R. Lopez, F. Berry, P. Smith, B. Reneau, and S. Mostaghim. 1999. "Anaerobic Degradation of Dicamba and Metribuzin in Riparian Wetland Soils." *Water Resources* 33 (1): 87–94. doi:10.1016/S0043-1354(98)00181-X.

Perceval, J., P. Benoit, M. Stenrød, O.-M. Eklo, M.-P. Charnay, and E. Barriuso. 2006. "Availability and Biodegradation of Metribuzin in Sandy Soils as Affected by Temperature and Soil Characteristics." SCI Pest Management Group, Proceedings of the Pesticide Behaviour in Soils, Water and Air Conference, Warwick, March 27–29.

Pierre-Yves, C., B. Charlotte, and C. Allen. 1996. "Fate and Impact of Pesticides Applied to Potato Culture: The Nicolet River Basin." Ecotoxicology and Environmental Safety. 33 (2): 175–185.

Rahman, A., and T.K. James. 2010. "Herbicide Efficacy on Three Annual Grass Weeds in Soils with Different Cropping Histories." *New Zealand Plant Protection* 63: 66–71.www.nzpps.org/journal/63/nzpp_630660.

Rasche, U., G. Werner, H. Wilde, and U. Stottmeister. 1998. "Photolysis of Metribuzin in Oxygenated Qqueous Solutions." *Chemosphere* 36 (8): 1745–1754. doi:10.1016/S0045-6535(97)10069-8.

Rastegarzadeh, L., Y. Nelson, and G.T. Ririe. 2006. "Bio-Treatment of Synthetic Drill-Cutting Waste in Soil in: Remediation of Chlorinated and Recalcitrant Compounds." Proceedings of the Fifth International Conference on Remediation of Chlorinated and Recalcitrant Compounds Monterey, CA, May 2006. ISBN 1-57477-157-4. Columbus, OH: Battelle Press. www.battelle.org/bookstore.

Selim, H.M. 2003. "Modelling Kinetic Retention of Atrazine and Metribuzin in Soil." *Soil Science* 169 (1): 25–34. doi:10.1097/01.ss.0000112017.97541.61.

Singh, N., Raunaq, and B. Singh. 2013. "Effect of Fly Ash Amendment on Persistence of Metribuzin in Soils." *Journal of Environmental Science and Health B* 48 (2): 108–113. doi:10.1080/03601234.2013.726893.

Tamilselvan, C., S.J. Joseph, G. Mugunthan, A.S. Kumar, and S.S.M. Ahamed. 2014. "Biological Degradation of Metribuzin and Profenofos by Some Efficient Bacterial Isolates." *International Letters of Natural Sciences* 9: 26–39. ISSN 2300-9675.

Tarus, S., E. Nyambati, J. Kituyi, F. Segor, and F. Chebii. 2010. "Pesticide Residue Levels in Nzoia River Catchment Area." Proceedings of 12th KARI Biennial Scientific Conference, Nairobi.

Tejada, M., A.M. García-Martínez, I. Gómez, and J. Parrado. 2010. "Application of MCPA Herbicide on Soils Amended with Biostimulants: Short-Time Effects on Soil Biological Properties." *Chemosphere* 80 (9): 1088–1094. doi:10.1016/j.chemosphere.2010.04.074.

USEPA (United States Environmental Protection Agency). 2003. *Health Effects Support Document for Metribuzin*. www.epa.gov/safewater.

Wamukoya, G., and V. Ludeki. 2006. *Nzoia River Basin Management Initiative (NRBMI) 2006–2011: A Public Private Partnership Programme between Water Resources Management and Civil Society, Learning Institutions and Communities*. www.unep.org/training/downloads/pdfs/nrbmi.

Zhang, H., Y. Zhang, Z. Hou, X. Wu, H. Gao, F. Sun, and H. Pan. 2014. "Biodegradation of Triazine Herbicide Metribuzin by the Strain *Bacillus* sp. N1." *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes* 49 (2): 79–86. doi:10.1080/03601234.2014.844610.