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Enhanced Mineralization of Atrazine in Compost-Amended Soil in Laboratory Studies

Z. M. Getenga

Maseno University, Faculty of Science, Department of Chemistry, Post Office Box 2602, Kisumu, 333, Maseno, Kenya

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The widespread use of atrazine (2-chloro-4-ethylamino 6-isopropylamino-s-triazine) over agricultural soils has raised concern over its movement to surface and ground water (Arthur et al. 1997). Atrazine has been found to contaminate ground water (Capriel et al. 1985). The concentration level of atrazine in soil is further exacerbated by spillage during pesticide mixing, loading, rinsing at the dealer shops, spray fill sites, direct dumping into soil disposal sites, ditches and ponds (Ritter 1990). The rate of atrazine degradation has been found to be relatively slow and may be influenced by several factors such as application history, soil nutrient conditions, soil pH, organic matter content in soil and the presence of degrader micro-organisms (Assaf and Turco 1994). Various methods have been used to improve on the biodegradability of atrazine in soil. Gan et al. (1996) used manure, corn meal and ammonium phosphate as soil amendments to study their effect on the biodegradation of atrazine. Alvey and Carvey (1995) amended soils with rice husks, starch and compost to improve on the biodegradation of atrazine in soil. The use of composted grass clippings as soil amendments in the biodegradation studies of some pesticides resulted in improved biodegradation of the pesticides in soil (Vandervoort et al. 1997). Studies using light to induce the photodegradation of atrazine and therefore, accelerate the rate of degradation of atrazine in soil have been done (Konstantinou et al. 2001; Rupassara et al. 2002; Cui et al. 2002).

In the present study, soil samples from a field with no history of atrazine application were amended with compost at different concentrations, spiked with both non-labelled and ^{14}C-labelled atrazine solution and then incubated in biometer flasks. The study is intended to evaluate the effectiveness of compost made from organic solid waste as a soil amendment for enhanced biodegradation of atrazine.

MATERIALS AND METHODS

Uniformly ring-labelled ^{14}C-atrazine (International Isotopes Munich) with specific activity of 10.3mCi/mmol, and radiochemical purity >98% and atrazine liquid flowable formulation (40% active ingredient as determined by high pressure liquid chromatography (HPLC) before use), were used for laboratory experiments.

Quicksafe A, 2,5-diphenyloxazole (PPO) and 1,4-bis[5-phenyl-2-oxazolyl]-benzene; 2,2'p-Phenylene-bis[5-phenyloxazole)] (POPOP) in toluene and Harvey Carbon-14 Cocktails (Zinsser Analytic (UK) Ltd) were used in Liquid Scintillation Counting. All solvents used were re-distilled in all-glass apparatus. The clay soil (organic carbon (OC) 2.07%, pH 6.08, clay content 60%, sand content 28%, silt 12%, N 0.19%, P 80ppm, Na 0.95%, K 1.85%, Ca 10.5%, Mg 4.95%, Mn 0.51ppm, Fe 226.96ppm, Zn 3.13ppm, EC 0.62) and compost (N 1.14%, P 0.72ppm, Cu 140ppm, Mn 2217ppm, Fe 1272ppm and Zn 755ppm) were used in the laboratory experiments. A liquid Scintillation Counter (Tricarb-1000) and Biological Materials Oxidizer (OX-400 model) were used for radio-assaying.

In the incubation experiment, 50g of sieved soil samples in replicates of three were placed in biometer flasks (Bell Co. Glass Inc.) after the air-dried and homogenized soil samples were sieved through a 2-mm sieve. The soil was conditioned by being moistened to 75% of the field water capacity. The soil samples were given different treatments before they were incubated.

The first set of the soil samples was autoclaved at 121°C and at pressure of 1.2 bars for 45 minutes for three days. After autoclaving the soil samples were further mixed with mercury chloride solution at a concentration of 1000ppm of soil to ensure there was no growth of microbes in the course of experiment. The second set of the soil samples were neither sterilized nor received compost. The third set of the soil samples were spiked with compost concentrations of 1000ppm, 2500ppm and 5000ppm. Atrazine solution (both labelled and non-labelled) in water was added to the 50g-soil sample in each flask giving an initial pesticide concentration of 100ppm and radioactivity of 2μCi. The soil was thoroughly mixed to ensure uniform distribution of the pesticide in soil. The side arm of each biometer flask was filled with 10ml of 1.0M NaOH to trap the $^{14}CO_2$ gas released during mineralization by soil microorganisms. The inlet of the biometer flask was filled with Ascarite to exclude carbon dioxide from entering the system. The biometer flasks were placed in an incubator at 30°C in darkness under aerobic conditions in order to monitor the progress of mineralization. At different time intervals, the solution from the side arm was sampled from which one ml of the solution aliquot was taken and mixed with 5ml of Quicksafe A cocktail in a 20-ml scintillation vial before it was radio-assayed. After sampling the side arm of the biometer flask was filled with fresh solution of NaOH. The experiment was run for 112 days. At the end of the incubation period, soil samples from the biometer flasks were removed and air-dried. A sub-sample of 20g was taken and extracted with 200ml of methanol for four hours. An aliquot of 1ml of the methanol extract was taken and mixed with 5ml of a mixture of PPO and POPOP in toluene and radioassayed to quantify extractable residues. The readings from the Liquid Scintillation Counter were corrected by external standardization. The extracted soil samples were air-dried and 1.5g of the soil sample was combusted in a Biological Materials Oxidizer. The $^{14}CO_2$ released during combustion of the extracted soil samples was trapped in 15ml of Harvey Carbon-14 cocktail and then radio-assayed to quantify non-extractable residues of atrazine. 1.5g of soil

spiked with known amount of radioactivity was combusted to determine the efficiency of the Biological Materials Oxidizer. All the readings were corrected according to the efficiency of the oxidizer.

Adsorption studies were also carried out to determine the proportion of the pesticide sorbed to the soil during mineralization in non-amended soil. 4g of homogenized soil samples were weighed and placed in 40ml-centrifuge tubes. 10ml of the pesticide solution prepared in 0.01M $CaCl_2$ at different concentrations of 0.1, 0.5, 1.0, 5.0 and 10.0ppm with initial radioactivity of 0.56μCi was added to 10g of soil in the centrifuge tubes. The resulting pesticide concentration in soil in each centrifuge tube was determined after adsorption at different periods of shaking. The centrifuge tubes were shaken on an orbital shaker at 200rpm for 4 hours, 8 hours and 24 hours respectively. Thereafter, the centrifuge tubes were transferred to a centrifuge machine and centrifuged at 3600rpm for 30 minutes. 1ml of the supernatant was taken and mixed with 5ml of the Quick safe A cocktail solution and radio assayed. The difference between the amounts of pesticide in the 0.01M $CaCl_2$ solution before and after shaking was the amount of pesticide adsorbed by soil. At equilibrium, all the 0.01M $CaCl_2$ solution was removed from the centrifuge tube and replaced with a fresh pesticide-free solution of 0.01M $CaCl_2$. Desorption was carried out for 24 hours when the pesticide in the two phases (soil and 0.01M $CaCl_2$ solution) was assumed to have attained equilibrium. The amount of pesticide desorbed from the soil was computed at different concentrations.

To study the effects of natural light on the degradation of atrazine in soil, ^{14}C-atrazine-pesticide solution was applied to moist soil samples in quartz tubes. Another set of quartz tubes was covered with aluminium foils to shield them from natural light. To 5g of soil in each tube a solution of both non-labelled and labelled ^{14}C-atrazine in methanol was applied giving an initial pesticide concentration of 10ppm and radioactivity of 0.25μCi. The tubes were taken to the rooftop of a two-storey building where the average daily temperature was 27°C. At a one-week interval, the soil samples in replicates of three from the quartz tubes were taken and analysed for both extractable and non-extractable residues of atrazine. The soil from the quartz tube was removed and placed in 40-ml centrifuge tubes and extracted with 20ml of methanol by continuously shaking on an orbital shaker for 4 hours. One ml of the extract was mixed with 5 ml of PPO/POPOP in toluene cocktail and radio-assayed.

RESULTS AND DISCUSSION

The results of mineralization of atrazine in the different sets of soil samples are shown in figure 1. All the different sets of soil samples except the sterile set in which no $^{14}CO_2$ was evolved, showed mineralization curves with three distinct phases. There was an initial lag phase, which lasted for 15 days. During this phase, although the extractable residue of atrazine was high, the microbes had not adapted to the utilization of atrazine as a source of energy. In the next phase the rate of $^{14}CO_2$ production was the highest. During this period, the extractable

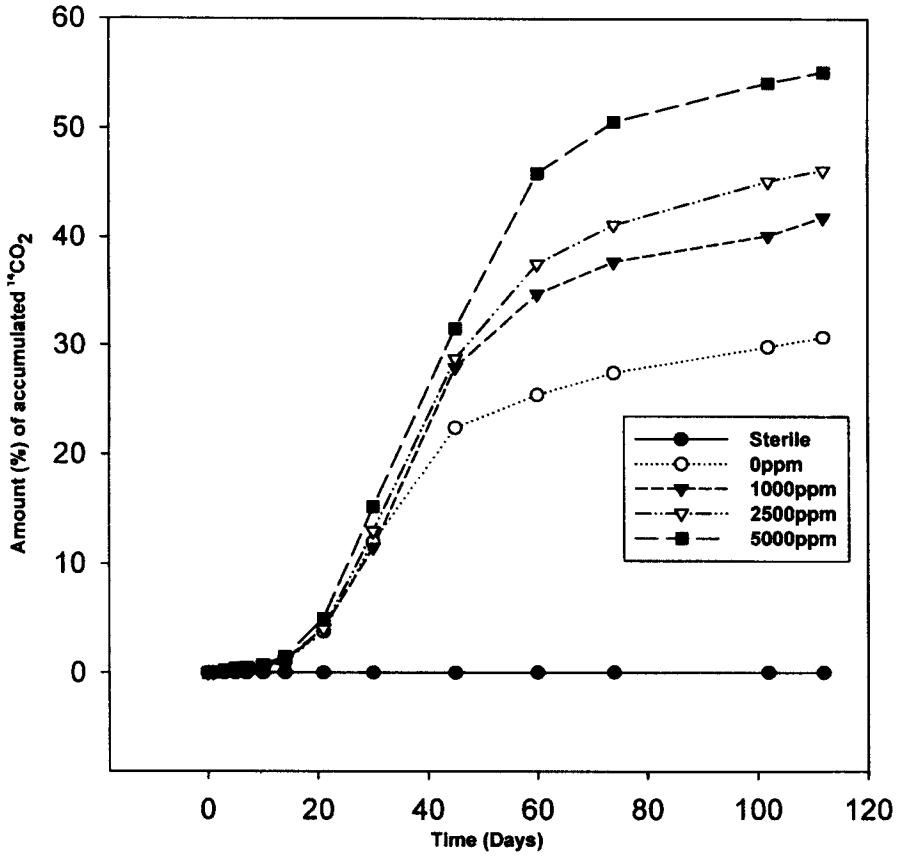

Figure 1. Mineralization of ^{14}C-Atrazine at different compost concentrations in soil.

residue of atrazine was highest and the proliferation of microbes was high. The phase lasted for 45 days in the set of soil samples with the highest compost concentration (5000ppm). However, for the set of soil samples without compost the phase lasted for 25 days. In the final phase the curves levelled off, attaining plateaus. There was no more increase in the rate of production of $^{14}CO_2$. The limiting factor at this phase was the available extractable residues, which had actually diminished. At the end of the experimental period of 112 days, the amount of extractable residue of atrazine was 10%, 2%, 2.4%, 2.6% and 2.6% for sterile, soil with no compost, soil with 1000ppm, soil with 2500ppm and soil with 5000ppm of compost, respectively. The highest amount of extractable residue of atrazine was recorded at the end of the experiment in sterile soil samples because no mineralization took place. The study showed that we could still apply higher concentration of compost than the 5000ppm applied and realize higher production of $^{14}CO_2$. After 112 days of the experiment, only 30.7% of the pesticide was

mineralized to $^{14}CO_2$ gas in un-amended soil. In the soil with the highest compost concentration 55.1% of the pesticide was mineralised, while at the concentrations of 1000 and 2500ppm of compost, 41.8% and 46.1% of the pesticide was mineralised, respectively. The sterile soil had the highest non-extractable (bound) residue of atrazine of 57% of the total pesticide applied. Other soils had 53%, 40%, 26% and 25 % for non-amended soil, soil with 1000ppm, 2500ppm and 5000ppm of compost concentration, respectively. The amount of non-extractable residues formed in the soil inversely related to the amount of $^{14}CO_2$ evolved. The sterile soil had the highest amount (33%) of pesticide residue, which could not be accounted for. The other soils, apart from the soil with 2500ppm of compost, had relatively low amount of unaccountable pesticide residue ranging from 15% to 18% of the total amount of the pesticide applied. This is because mineralization took place in these sets of soil samples. The soil treated with compost at 2500 ppm had lost 25% of the pesticide residue, which could not be accounted for. The unaccounted for pesticide residues from the soil is the portion of the pesticide which may have been converted to the volatile material, which could not be quantified in the study.

The soil used in this study was characterized with nitrogen deficiency (0.19%). In this condition, the soil was able to mineralise 30.7% of the atrazine. The addition of compost increased the rate of mineralization of atrazine because compost with nitrogen content of 1.14% probably decreased C/N ratio creating a shortage of C in the soil so that microbes resorted to the use of atrazine as an energy source. Another probable explanation is that the compost contained a consortium of microbes, which were capable of metabolising atrazine. This resulted in the increase in the rate of production of $^{14}CO_2$ from soils treated with compost at all concentrations applied. The atrazine was uniformly ^{14}C-ring labelled. Therefore, the $^{14}CO_2$ released may have resulted from the full oxidation of atrazine ring by the microorganisms to release energy in the soil. Radosevich et al. (1995) showed that an atrazine-degrading bacterial culture isolated from an agricultural soil previously impacted by herbicide spills was capable of causing atrazine-ring cleavage and mineralizing it, which was confirmed in $^{14}CO_2$ evolution experiments with [U-^{14}C-ring] atrazine as a sole source of C. There was between 40 and 50% of ring-^{14}C, which was mineralised to $^{14}CO_2$ while cellular assimilation of ^{14}C was negligible, in keeping with the fully oxidized valence of the ring carbon. The observation of negligible cellular assimilation of ^{14}C by Radosevich et al. (1995) is also supported in this study in that there was no substantial increase in ^{14}C in the non-extractable (bound) residue in soil where microbes thrived. ^{14}C-atrazine was only used for energy production by the microorganisms. Studies by Yassir et al. (1999) showed that ring-labelled atrazine resulted in only 5% of the initial radioactivity incorporated in microbial cells while 20-25% of the chain-labelled atrazine was incorporated in the microbial cells. The short lag period of 15 days for soil with no history of atrazine application is really remarkable. Gan et al. (1996) reported a lag phase of 35 days in mineralization studies carried out in a clay soil (organic carbon content of 4.09% and pH of 7). In other studies carried out by Alvey and Crowley (1995) a lag phase of between 20 and 25 days was observed in different organic

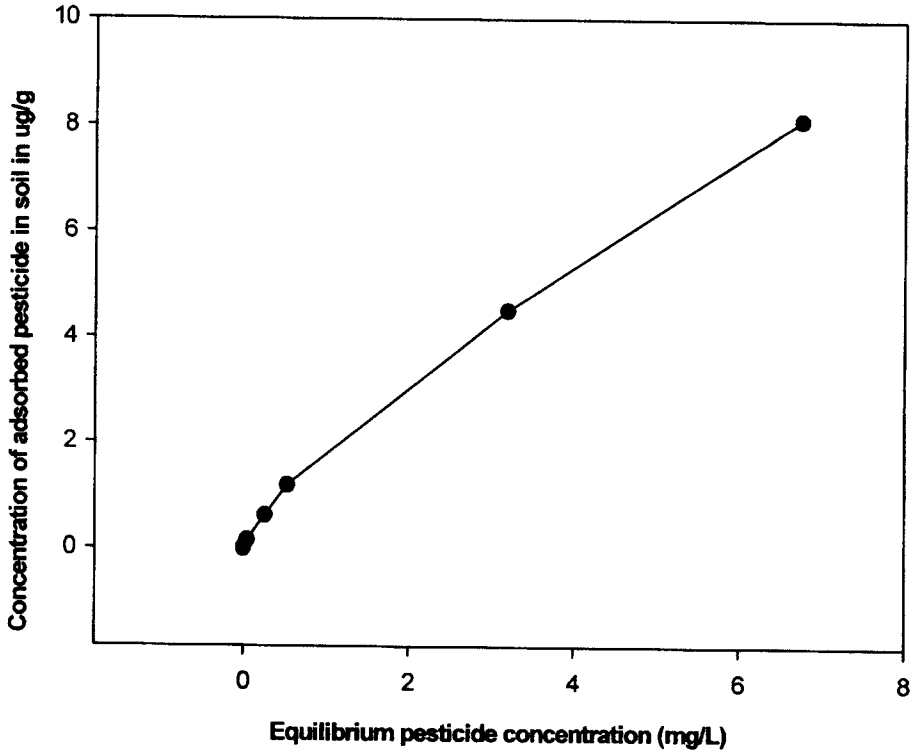

Figure 2. Adsorption curve for atrazine in non-amended soil.

amendments applied at a concentration of 50000ppm to soil with a history of atrazine application. At a concentration of 50000ppm of compost, which was 10 times as much as the compost concentration used in the present study, only 59% of the atrazine was mineralized.

Figure 2 shows the amount of pesticide adsorbed in the soil at different equilibrium solution concentrations. The adsorption isotherm was described using the Freundlich equation: $x/m = K_f C^n_{eq}$, where x/m is the amount of the adsorbed pesticide per unit mass of soil (mg/kg), C_{eq} is the equilibrium pesticide concentration in solution (ppm), K_f and n are empirical constants. Applying the Freundlich equation and transforming both axes of the data into logarithmic scales, the K_f and n values were found to be 1.545 and 0.9457, respectively in non-amended soil. The correlation coefficient (R^2) for the data was 0.95. The slope ($n<1$) of the isotherm indicated that as the initial concentrations of the pesticide solution increased, the percentage adsorbed by soil decreased. The actual values decreased from 59% of the pesticide adsorbed from 0.1ppm pesticide

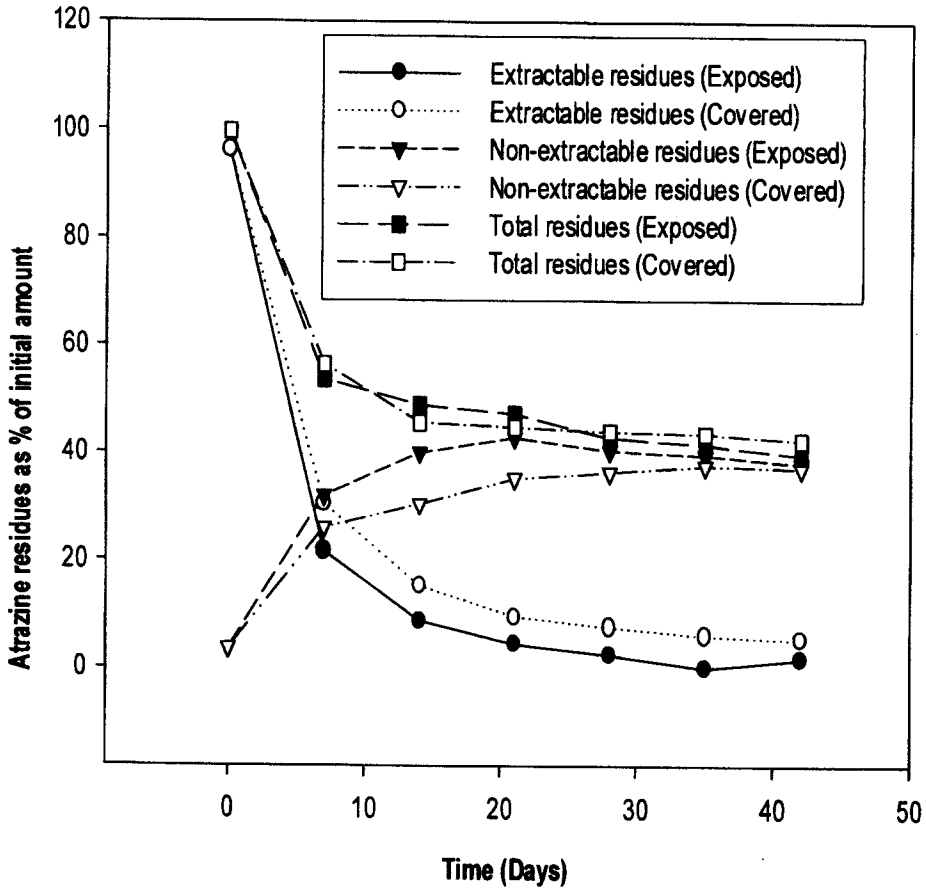

Figure 3. Dissipation curves for atrazine in covered and exposed soils.

solution to 32.5% of the pesticide adsorbed from 10ppm pesticide solution. This has been explained by an increased difficulty to access the adsorption sites when pesticide concentrations are elevated (Konda et al. 2002). In desorption studies, the pesticide was readily taken back into solution. The extent of pesticide removal by the 0.01M $CaCl_2$ solution from the soil depended directly on the amount of the pesticide, which had been adsorbed. The amount of pesticide desorbed back increased from 23.7% of the adsorbed soil to 64% in the soil with the highest amount of the adsorbed pesticide. Both the adsorption and desorption studies showed that a higher proportion of atrazine remained in the desorbed state than in the adsorbed state. In the mineralization experiment in which the pesticide was applied to soil at a concentration of 100ppm, most of atrazine remained in the desorbed state, which was accessed easily by microorganisms.

Figure 3 shows how atrazine dissipated from covered soils and soils exposed to natural light. The extractable residue of atrazine was higher in covered soil than in the exposed soil. However, the non-extractable residue was higher in the exposed soil than in the covered soil. After 21 days in the soil the non-extractable residue, which had been accumulating started decreasing in the exposed soil. The dissipation curves for the total residues in both conditions showed same patterns of dissipation. From first order kinetics, the half-life periods ($t_{1/2}$) for the total pesticide residues in the covered and exposed conditions were found to be 100.4 and 92.4 days respectively. From the results, the difference between the rates of dissipation of the extractable residue in the covered soil and in the exposed soil was clearly due to photo-degradation in the exposed soil. The non-photochemical processes, which entirely controlled the degradation of the pesticide in the covered soil, could be biotic, hydrolysis, volatilisation and adsorption by soil. In exposed soil, photo-degradation increased the rate at which both the extractable and non-extractable residues dissipated from the soil. The photo-degradation products of atrazine, which have been identified in soil by Konstantinou et al. (2001), are the hydroxy- and dealkylated-derivatives. The derivatives of atrazine quickly got adsorbed by soil thus increasing the rate at which the non-extractable residue formed in exposed soil. At the same time the adsorption of the derivatives led to the decline in the amount of the extractable residue in soil. The abiotic processes in exposed soil were enhanced by ambient temperature, which on average was 27°C. This may explain why the dissipation rate of atrazine residues in exposed soil was higher than in the covered soil.

The study showed that microbial degradation of atrazine in soil was enhanced by compost at all the concentrations in the soil. At high concentration atrazine in soil remained in the non-adsorbed state, which became accessible to microorganisms. No study was carried out to find out if the application of compost to soil affected the extent of adsorption of the pesticide by soil. From the results of mineralization in which the rate of mineralization increased as the concentration of compost increased in soil, we can conclude that there was no appreciable effect on the adsorption of the pesticide by compost if we assume only the non-adsorbed pesticide was mineralised. In the tropical conditions, the high temperatures and intensity of natural light play a significant role in the degradation of atrazine and hence enhance its rate of dissipation from soil.

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