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ATRAZINE PERSISTENCE AND EFFECT ON SOIL MICROBIAL POPULATION IN SOUTHERN GUINEA SAVANNAH OF NIGERIA

Adelasoye, K.A and *Adesina, G.O
Department of Crop and Environmental Protection,
Ladoke Akintola University of Technology, PMB. 4000, Ogbomoso, Nigeria

ABSTRACT

The persistence of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] and its effect on microbial population in southern guinea savannah soil was studied using spectrophotometry. The treatments applied were 2.2, 3 and 3.75 L/ha atrazine.The initial soil sample was observed to have higher and significantly different bacterial population (21.30 and 21.63 $Cfug^{-1}$) than soil from all the rates of atrazine for the two years. Fungal populations were highest (3.7 $Cfug^{-1}$) in the initial soil samples compared with the rates of atrazine and throughout the duration of the sampling for both seasons. Bacterial population increased from the fourth to the sixth week of sampling and the highest rate of atrazine had the highest bacterial colony for the three consecutive samples.There was steady increase in fungal populations from two to six weeks after atrazine application during the two years.The rate of disappearance of atrazine within the first 28 days was higher (1.33, 1.05 and 0.66 mg/kg for 2.2, 3.0, and 3.75 L/ha respectively) than the second 28 days (1.16, 0.91, and 1.47 mg/kg for 2.2, 3.0, and 3.75 L/ha respectively). The last 28 days recorded the highest rates 2.26, 1.81 and 1.53 mg/kg for 2.2, 3.0, and 3.75 L/ha respectively. The disappearance time for 50% atrazine (DT_{50} or half-life) irrespective of the rate applied was less than 50 days.Atrazine was observed to be moderately persistent in this zone irrespective of the rates and microbial population increased as the quantity of atrazine residue in the soil decreased considering the duration of the experiment.

Keywords: Atrazine, bacterial population, disappearance time, fungal population, persistence.

INTRODUCTION

Atrazine is a selective herbicide belonging to the family of the s-triazines (Abigail and Nilanjana, 2012). Atrazine kills susceptible plants by binding to the quinone-binding protein in photosystem II, thus, inhibiting the photosynthetic electron transport (Wang *et al*., 2011). Atrazine is naturally non-volatile herbicide (Feria-Reyes *et al*., 2011) which is used worldwide either alone or in combination with other herbicides (Chan and Chu, 2005), to get rid of broad leafy and grassy weeds mainly from corn, sorghum, sugarcane, maize crops, pineapple and also in conifer reforestation plantings (Sene *et al*., 2010).

Atrazine is considered persistent due to its moderate water solubility (33 mg l^{-1} at 20ºC) and low soil sorption partition coefficient (K_d = 3.7 l kg^{-1}). Although the halogen, methylthio ether, and N-alkyl substituents on the s-triazine ring of this group of herbicides hinder the microbial metabolism (Wackett *et al.*, 2002), some reports have demonstrated the ability of some soil microorganisms to degrade atrazine partially or totally directing it to carbon dioxide and ammonia formation (Mandelbaun *et al*., 1995; Rosseaux *et al*., 2003; Singh *et al*., 2004). Atrazine is an active environmental pollutant due to its low biodegradability, having high potential to contaminate both surface as well as ground water (Chan and Chu, 2005). Its continuous use has resulted in environmental pollution in soil, ground and aquatic water (Wang *et al*., 2011). Many microorganisms have been reported to be endowed with the ability to utilize Atrazine as their sole carbon and nitrogen source, and can thus degrade atrazine and their derivatives (Zhang *et al*., 2009) which include bacteria genera: *Pseudomonas*, *Arthrobacter*, *Acinetobacter*, *Rastonia*, *Agrobacterium* and Norcardioides) and fungi: genera *Penicillium*, *Rhizopus*, *Aspergillus*, *Trichoderma*, *Phanerochaete* and *Fusarium* (Abigail and Nilanjana, 2012). Various pathways were used consisting steps like N-dealkylation, dechlorination, dehalogenation, Ring-cleavage and deamination (Gebendinger and Radosevich, 1999). Reports on atrazine effects on soil microbial population in relation to its degradation in the field in the southern Guinea Savannah were either scanty or non-existing. This work was designed to monitor the persistence of atrazine and its effect on microbial population in a maize-based cropping system.

*Corresponding author e-mail: olulakinadesina@gmail.com

Table 1. Bacterial population isolated from the soil treated with atrazine during 2012 and 2013 seasons.

Treatment ↓	Bacteria count ($Cfug^{-1}$) 2012			Bacteria count ($Cfug^{-1}$) 2013		
	2WAS	4WAS	6WAS	2WAS	4WAS	6WAS
Pre-spray soil	21.3×10^6	21.3×10^6	21.3×10^6	21.63×10^6	21.63×10^6	21.63×10^6
2.2 L/ha	1.43×10^6	2.43×10^6	12.0×10^6	1.53×10^6	2.2×10^6	12.63×10^6
3.0 L/ha	1.4×10^6	3.3×10^6	14.6×10^6	1.5×10^6	3.47×10^6	15.63×10^6
3.75 L/ha	1.47×10^6	4.0×10^6	16.6×10^6	1.67×10^6	$4,17 \times 10^6$	17.23×10^6
LSD	0.59	1.07	1.47	1.48	1.72	2,47

SBS = Soil Before Spraying WAS = Week After Spraying Cfu = Colony forming unit

Table 2. Fungal population isolated from the soil treated with atrazine during 2012 and 2013 seasons.

Treatment ↓	Fungi count ($Cfug^{-1}$) 2012			Fungi count ($Cfug^{-1}$) 2013		
	2WAS	4WAS	6WAS	2WAS	4WAS	6WAS
Pre-spray soil	3.7×10^6	3.7×10^6	3.7×10^6	3.7×10^6	3.7×10^6	3.7×10^6
2.2 L/ha	0.53×10^6	0.67×10^6	1.67×10^6	0.57×10^6	0.67×10^6	1.73×10^6
3.0 L/ha	0.43×10^6	0.77×10^6	1.47×10^6	0.47×10^6	0.77×10^6	1.53×10^6
3.75 L/ha	0.37×10^6	0.73×10^6	1.67×10^6	0.4×10^6	0.73×10^6	1.7×10^6
LSD	0.26	0.24	0.27	0.09	0.24	0.24

SBS = Soil Before Spraying WAS = Week After Spraying Cfu = Colony forming unit

MATERIALS AND METHODS

The experiment was conducted at Ladoke Akintola University of Technology, Ogbomoso, Teaching and Research Farm, Latitude 80^0 $10^{1,}$ Longitude 4^0 10^1E, Altitude of 700 m, during the 2012 and 2013 cropping seasons. The site was ploughed twice and levelled; divided into 5 plots of 3 m by 3 m sizes. The experimental design used was Randomised Complete Block Design with three replications. Soil samples were taken initially using soil auger at 0 - 15 cm depth before spraying the atrazine to take initial microbial population. Maize was planted and the atrazine was sprayed pre-emergent at 2.2, 3 and 3.75 L/ha. Soil samples were taken seven times with the first immediately after herbicide application, subsequently at two weekly intervals using soil auger as stated earlier. The soil samples were for atrazine residue using spectrophotometric analysis with Atomic Absorption Spectrophotometer (AAS) MILTON ROY 21D. Soil samples for microbial population estimates were taken at two weekly intervals from the day of herbicide application three times. Standard methods were used to prepare nutrient agar (NA) and potato dextrose agar (PDA) for estimation of microbial population. One gramme each of the soil samples were measured into the test tube containing 9 ml sterile distilled water and serially diluted to dilution factor (10^{-5}) and 1ml of the last dilution was pipette into sterile plate which were incubated at 37^oC for NA and PDA incubated at $28\text{-}30^oC$. All the plates were incubated inverted wise. Microbial counts were done at 48 hr. for NA and 72 hr. for PDA in the Petri plates.

Extraction of Herbicide Residues

5g of soil sample was weighed using a Mettler top weighing balance and 25 g of ethyl acetate and methanol in the ratio 4:1 was added in the presence of anhydrous sodium sulphate and sodium chloride using 10 g each.

They were homogenized on shaker at a high speed for 3 minutes. The homogenate was filtered through a Whatman no. 1 filter paper. The filtrate was left to pass through activated charcoal, (i.e. the activated charcoal was put on the filter paper when the solution was then poured).

Atrazine standard was prepared by taken 100 ppm of atrazine solution. Volumes of 10, 2.0, 1.5, 1 and 0.5 ml to give 10, 2, 1.5, 1, and 0.5 ppm concentrations. 100 ml volumetric flask was used and these five levels (10, 2.0, 1.5, 1 and 0.5 ml) atrazine samples were made up to 100 ml capacity volumetric flask with the extraction solution (ethyl acetate and methanol) at the ratio 1:1). The clear filtrate was then read on the Spectronic 21D at 420nm wavelength. Calculation was done as follows:

Absorbance x Slope x Dilution Factor Miller *et al*. (1981); (AOAC (2005)}

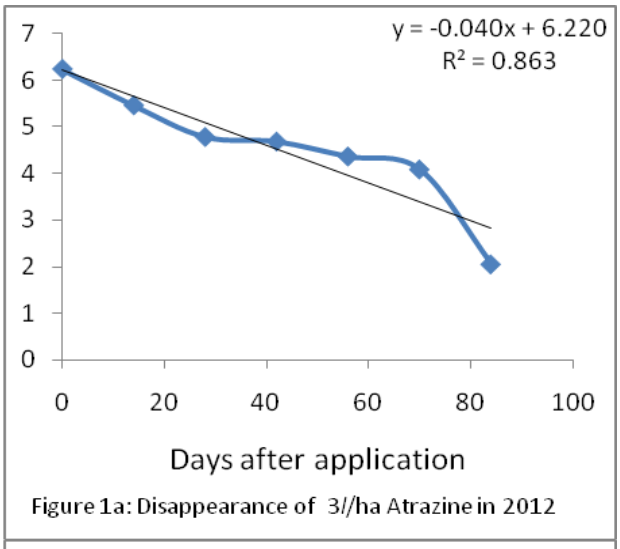

Figure 1a: Disappearance of 3l/ha Atrazine in 2012

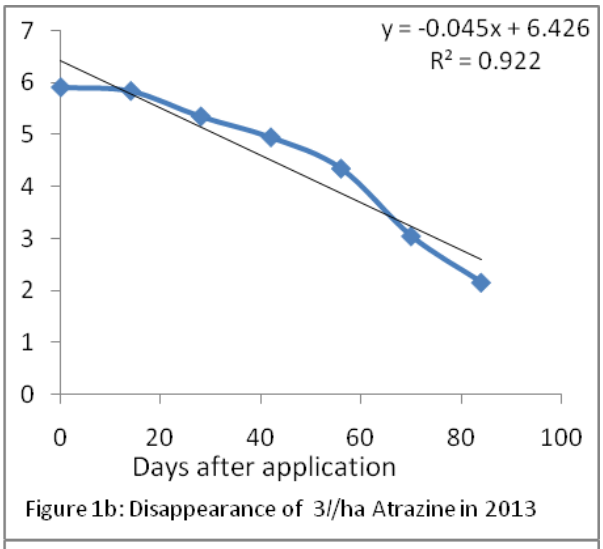

Figure 1b: Disappearance of 3l/ha Atrazine in 2013

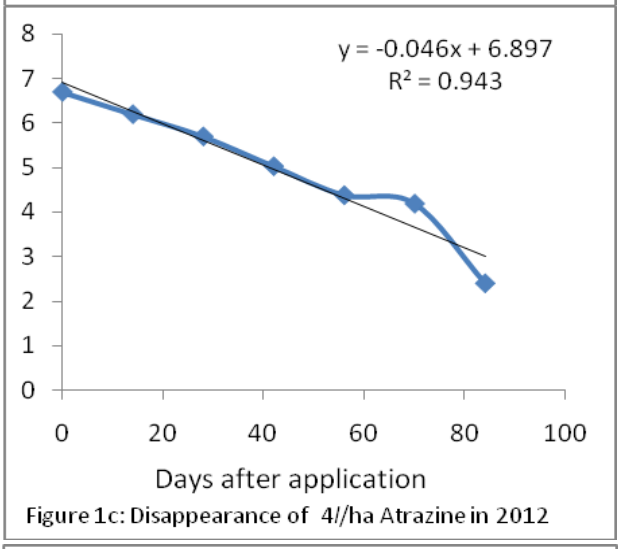

Figure 1c: Disappearance of 4l/ha Atrazine in 2012

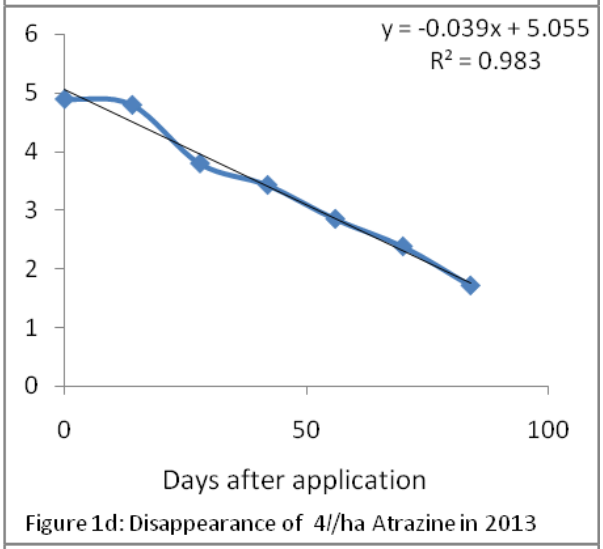

Figure 1d: Disappearance of 4l/ha Atrazine in 2013

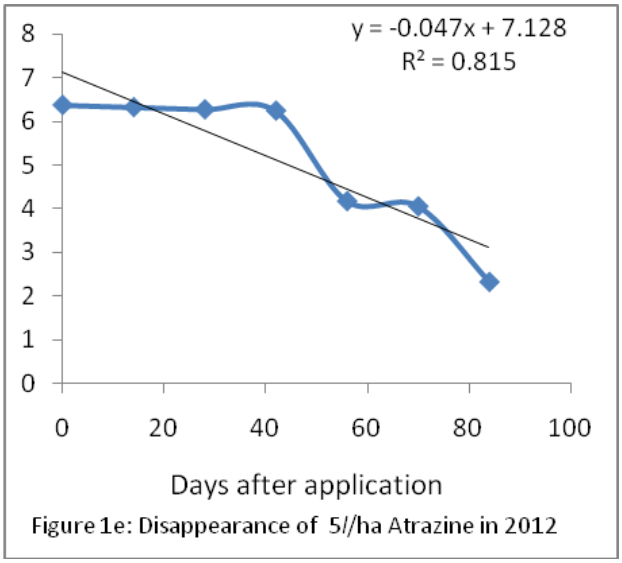

Figure 1e: Disappearance of 5l/ha Atrazine in 2012

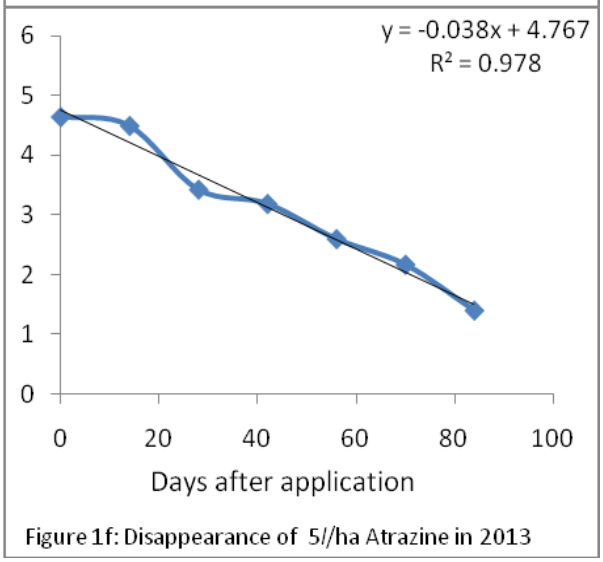

Figure 1f: Disappearance of 5l/ha Atrazine in 2013

Fig. 1a-f. Disappearance time (DT_{50}) of atrazine at three dosage rates under southern guinea savanna ecology in 2012 and 2013 planting season.

Table 3. Disappearance time (DT)(days) for 10, 50, 75% and 90% atrazine from Southern Guinea Savannah soil.

Rate (L/ha) ↓	DT_{10}		DT_{50}		DT_{75}		DT_{90}	
	2012	2013	2012	2013	2012	2013	2012	2013
2.2 L/ha	4	4.2	24.5	20.6	39.7	31.3	46.2	32.7
3 L/ha	7.5	4.2	24.5	19.6	52.5	28.9	63	34.8
3.75L/ha	8.4	9.8	41.5	46.9	58.8	71.6	69.3	85.9

Table 4. Physical and chemical properties of the soil in 2012 and 2013 seasons.

	pH(water) 1:1	%OC	%N	P(µg/g)	K(c mol.+/kg)	ECEC	%Sand	%Silt	%Clay
2012	6.3	0.66	0.05	3.29	0.13	17.83	79.0	8.0	13.0
2013	6.7	0.80	0.06	11.11	0.41	6.96	64.0	8.0	13.0

RESULTS

Bacteria

Pre-treatment soil sample was observed to have higher and significantly different bacterial population (21.30 and 21.63 $Cfug^{-1}$) than soil from all the rates of atrazine for the two years. There were generally no significant different in bacterial populations among all the atrazine rates used although the population increased from the fourth to the sixth week of sampling. Though not significantly different from the other rates, the highest rate of atrazine had the highest bacterial colony for the three consecutive samples (Table 1).

Fungi

Fungal populations were highest (3.7 $Cfug^{-1}$) in the initial soil samples compared with the rates of atrazine and throughout the duration of the sampling for both seasons. There was steady increase in fungal populations from two to six weeks after atrazine application during the two years. The three rates of atrazine were not significantly different among themselves when comparing the fungal colonies throughout the experiment (Table 2).

Persistence of atrazine

The general trend in the rate of disappearance of atrazine during the two seasons was that the rate within the first 28 days was higher (1.33, 1.05 and 0.66 mg/kg for 2.2, 3.0, and 3.75 L/ha respectively) than the second 28 days (1.16, 0.91, and 1.47 mg/kg for 2.2, 3.0, and 3.75 L/ha respectively). The last 28 days recorded the highest rates 2.26, 1.81 and 1.53 mg/kg for 2.2, 3.0, and 3.75 L/ha respectively from the regression plots (Figs. 1 a-f).

The highest rate of atrazine applied took the longest time to disappear from the soil followed by the recommended rate while the lowest rate, 2.2 L/ha was fastest. The disappearance time for 50% atrazine (DT_{50} or half-life) irrespective of the rate applied was less than 50 days. The highest rate had 69.3 and 85.9 days as DT_{90} for 2012 and 2013 respectively. The DT_{50} for this rate, 3.75 l/ha were, respectively 41.5 and 46.9 days for 2012 and 2013 (Table 3).

Table 4 shows the physical and chemical properties of the soil in which the pH showed it to be slightly acidic which may be favourable to fungal activities and textured as sandy loam meaning no substantial atrazine adsorption.

DISCUSSION

The higher rates of disappearance of atrazine within the first month could be attributed more to other factors of degradation rather than microbial because the microbial population dropped within the month in question. Silva *et al.* (2004) demonstrated the occurrence of fast atrazine mineralization after an acclimatization period of approximately 28 days. The structural activity of atrazine is enormously affected by several potent environmental factors including pH, humidity, environmental temperature and microbial activity (Feria-Reyes *et al*., 2011). It had been reported that biodegradation of atrazine consists of complex physical and biological processes that mainly depend upon the nature and total amount of atrazine available in soil and water, and that some important factors may limit the biodegradation of atrazine in the environment including soil and water and its limited availability to the microorganisms (Abigail and Nilanjana, 2012). As most atrazine-degrading bacteria use this herbicide as a nitrogen source, the presence of preferential nitrogen sources in the environment is detrimental to atrazine degradation. Nitrogen amendments were in fact shown to decrease atrazine degradation rates by *Pseudomonas* sp. ADP (Clausen *et al.*, 2002; García González *et al.*, 2003). In fungi, nitrogen suppressed mineralization of atrazine but stimulated the primary growth, thereby suggesting that N could alter the microbial processes and C uptake and thus influence the

rates of herbicide degradation (Entry *et al.*, 1993). The decreased loss rate of disappearance in the following 28 days could be due to the microorganisms trying to adapt to the herbicide as it was reported that the halogen, methylthioether, and N-alkyl substituents on the s-triazine ring of this group of herbicides hinder the microbial metabolism (Wackett *et al.*, 2002). The highest loss rate recorded at last could result from microbial activities as their population had started appreciating substantially then. The slightly acidic nature of the soil may be favourable to fungal activities and the sandy loamy texture may reduce adsorption making the herbicide available to microbes. Studies of Canadian and French soils (Houot *et al*., 2000) indicated that accelerated mineralization of atrazine was only observed in soils with a pH greater than 6.5. The bioavailability of most herbicides for microbial biodegradation is limited by the sorption to organic matter or clay minerals (Alexander, 1994; Skow and Johnson, 1997). In addition, organic carbon substrates may affect microbial community structure and the potential for degradation of herbicides such as atrazine (Rhine *et al*., 2003). Using an antibiotic-inhibition assay, Levanon (1993) demonstrated that the mineralization of the alkyl side-chains of atrazine was attributed to fungal metabolism, whereas the mineralization of the triazine ring was due to bacterial metabolism. These loss rates were higher than those reported by Akinyemiju *et al*. (1986) for humid tropical Soil in the rain forest area of Nigeria.

All the rates of atrazine applied were moderately persistent with DT_{50} less than 60 days (Roberts, 1996). The average DT_{50} (22.05 days) recorded for 3 *l*/ha was lower than that (36.4 days) reported by Akinyemiju *et al*. (1986) in their research. Relatively high populations of atrazine degraders (>1,000 degraders g^{-1} soil) were associated with soils that exhibited enhanced atrazine degradation. Half-lives of atrazine in soils exhibiting accelerated mineralization were typically less than 10 d. Accelerated degradation of atrazine may reduce the weed control efficiency of this herbicide and may also reduce potential for off-site movement (Robert *et al*., 2006). Alabama field trials have indicated an average half-life of atrazine of less than 20 d in warm Southern US soils (Buchanan and Hiltbold, 1973). Based on these results, it took atrazine less than 100 days to be reduced appreciably in the soil meaning that the follow-crop problem that is usually associated with the persistence of the herbicide would have been reduced to some extent in the Southern Guinea Savannah of Nigeria.

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