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# INFLUENCE OF SOME SELECTED ENVIRONMENTAL FACTORS ON THE NATURAL ATTENUATION OF ATRAZINE IN TROPICAL AGRICULTURAL SOILS.

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## ABSTRACT

Understanding environmental factors that help to optimize the natural attenuation of atrazine in agricultural soils is necessary to minimize contamination risks and human health risks, as well as support a healthy soil ecosystem. This study, therefore, investigated the influence of pH, temperature, soil organic matter content, often measured as total organic carbon (TOC), and moisture content on atrazine attenuation by soil microbes. Agricultural soil with a history of atrazine application was spiked with the herbicide. The effects of pH (3, 7, 10), moisture content (10%, 15%, 25%), total organic carbon (TOC, 2%, 3%, 4%), and temperature (15°C, 25°C, 45°C) on atrazine attenuation and bacterial count were evaluated at the interval of 30 days each for 60 days of incubation in nutrient agar. The residual concentrations of atrazine attenuation were determined using GC-MS analysis at the interval of 30 days each for 60 days of incubation. The results indicate that higher bacterial counts (*Arthrobacter* sp.) were observed at neutral pH (7.0) and moderate moisture (10%). At a neutral pH (7), only 52.1% of the initial atrazine concentration remained after 60 days due to attenuation processes. Also, Atrazine natural attenuation increased with increasing TOC where only 25.8% attenuation residual concentration was left at 4% TOC and reached a maximum of 15% moisture content with a residual concentration of 20.13% after 60 days. Similarly, the concentration of atrazine decreased as the temperature increased, with only 29.01% of the residual concentration remaining at 45ºC treatment after 60 days. Statistical analysis revealed significant differences in natural attenuation between 30 and 60 days, but not after 30 days. These findings suggest that natural attenuation was favoured by higher organic matter content and moderate moisture levels, highlighting the importance of soil properties for atrazine fate in tropical soils and emphasizing the importance of studying natural attenuation processes as a viable strategy, particularly in tropical agricultural settings to minimize unnecessary contamination of soil to ensure a healthy and safe agricultural environment.

**Keywords:** *Atrazine, Bioremediation, Attenuation, Soil, Microbes.*

## INTRODUCTION

Atrazine is a widely used herbicide that has been effective in controlling broadleaf weeds in crops like corn and sugarcane. However, its persistence in the environment and potential for contamination of soil and water resources raise significant concerns while responsible application remains a challenge in many developing countries, particularly those with vast tropical landscapes (Tudi *et al.,* 2021). Unlike developed nations with well-established regulations and practices, these regions often lack the infrastructure and resources for proper pesticide management (van Den Berg *et al.,* 2021; Ojo, 2016). Research has documented the potential environmental impacts of atrazine, including its toxicity to non-target organisms, potential human health effects, and disruptions to soil ecosystem health (de Albuquerque *et al.,* 2020; Singh *et al.,* 2018; Pathak and Dikshit, 2011). Consequently, concerns regarding environmental contamination from pesticides have become paramount, especially in tropical areas.

Atrazine, a widely used herbicide, exemplifies this challenge. While it effectively controls weeds, its fate in soil is influenced by various factors, including: i) Sorption to soil components: Atrazine can bind to soil particles, impacting its mobility and potential for leaching (Swann & Eschenroeder, 1983); ii) Bio-attenuation: Soil microbes can break down atrazine, but this process can be influenced by factors like soil organic matter content (Swann & Eschenroeder, 1983); iii) Leaching: Herbicide mobility and leaching through soil are gradual processes, but factors like irrigation and soil texture can significantly impact the rate (Swann & Eschenroeder, 1983). A researcher investigated atrazine leaching in humid tropical soils and found that while leaching was moderate in acidic clay soils with high organic matter, sandy soils showed rapid transport regardless of sorption properties (Chai, 2009). This suggests preferential flow mechanisms may be at play in some soil types; iv Adsorption-desorption: Atrazine exhibits rapid initial sorption to soil followed by a slower process. Desorption, however, occurs at a much slower rate (Dehghani *et al.,* 2013). Understanding these dynamics is crucial for predicting atrazine movement in soil.

Studies have documented the persistence of atrazine in soil, with half-life estimates ranging from several weeks to months depending on various factors. Research has also explored the microbial degradation pathways for atrazine and identified the environmental factors influencing these processes (Liu *et al.,* 2023; Mili *et al.,* 2022; Jia *et al.,* 2021; Jablonowski *et al.,* 2010). Previous work has established the influence of soil properties like organic matter content, pH, and moisture levels on atrazine adsorption, movement, and degradation rates (Bhatti *et al.,* 2022; James *et al.*, 2021; Weber *et al.,* 2006). Studies have shown that higher organic matter content and moderate moisture levels generally promote atrazine degradation by soil microbes (Oyeyiola *et al.,* 2024; Luo *et al.,* 2021).

While previous research provides valuable insights, there are still gaps in our understanding of atrazine in tropical agricultural soils. Much of the existing research focuses on temperate climates. Tropical soils often have unique characteristics, such as higher temperatures, distinct microbial communities, and different clay mineralogy. Prior studies often focus on individual environmental factors. Understanding the combined effects of various factors like temperature, moisture, and organic matter on atrazine degradation in tropical soils is crucial. In another context, some studies explore bioremediation techniques for atrazine removal, but the focus on natural attenuation processes specifically in tropical regions remains limited.

Therefore, this current study aims to address these knowledge gaps by investigating the influence of selected environmental factors on the natural attenuation of atrazine in tropical agricultural soils. The study was conducted by using soil samples collected from representative tropical agricultural regions, ensuring the findings are relevant to these specific environments. The experimental design experiments involved consideration of the combined effects of multiple environmental factors (e.g., pH, temperature, moisture, organic matter) on atrazine degradation rates. By understanding the natural attenuation processes within tropical soils, this research can inform the development of management strategies that promote the breakdown of atrazine without relying solely on expensive or impractical deliberate removal techniques.

Despite its effectiveness in weed control, atrazine raises concern due to its potential for persistence and off-site mobility. Ideally, herbicides should be highly targeted and readily degrade, minimizing environmental and human health risks. Unfortunately, atrazine often falls short of these ideals. Thus, a deeper understanding of atrazine natural attenuation patterns and residues in tropical soil ecosystems is necessary to ensure public health and environmental safety. On the other hand, while deliberate bio-physicochemical techniques exist for atrazine removal, they can be expensive, require specialized equipment, and may not be readily available or practical for all situations, particularly in resource-limited settings like small-scale tropical agriculture. Therefore, understanding the natural attenuation processes that occur within the soil itself is crucial. Hence, this research aims to address this knowledge gap by investigating the environmental fate of atrazine in tropical soils. We explored how soil physicochemical characteristics (pH, TOC, and moisture content) and varying temperature conditions influence the atrazine natural attenuation process, ultimately contributing to more sustainable and responsible agricultural practices in tropical regions, as well as providing insights for developing management strategies, such as bioremediation, to minimize unnecessary contamination of soil and water resources.

## MATERIALS AND METHODS

### Collection of soil samples

Soil samples were collected with a hand-driven soil auger at a depth of 10cm. The samples collected were sieved through 2.0mm dish mesh to remove stones, plant debris, and grassroots. For the spiking procedure, the soil sample was spiked with 0.01 µg/mL of atrazine commercial grade in 10mL of ethanol in a container containing 100g of oven-dried soil sample. The ethanol was evaporated in a fume hood for 24 hours. Several of these were repeated in the determination of the influence of the selected environmental factors.

### Determination of total colony count (TCC)

The microbial load of the enzyme solutions and the soil samples were monitored weekly.

The determination of total colony count was done using the spread plate technique using nutrient agar (Oxide). Incubation of the plate was done at 35°C for 18–24 hours (Agarry & Oghenejoboh, 2015; Agarry & Latinwo, 2015). Serially diluted samples (0.1 $cm^3$) of appropriate dilution ($10^{-6}$, $10^{-7}$, $10^{-8}$) of the suspension of the contaminated soil in distilled water (prepared by dissolving 1.0 g of soil in 9 $cm^3$ of distilled water) were spread on nutrient agar plates

using the spread plate technique. Colonies were counted after incubation and the number of colonies in colony-forming units per gram of soil (cfu/g) was calculated using equation (1).

$$Number\ of\ \left(\frac{cfu}{g}\right)\ of\ soil = \frac{Average\ number\ of\ colonies\ \times dilution\ factor}{Initial\ weght\ of\ soil}$$

**Experiment on the influence of pH on the natural attenuation of Atrazine**

The soil sample was brought to the desired pH content by the addition of buffered solvents. Soil pH was adjusted after preparation and maintained at pH 3, 7, and 10 respectively. Continuous monitoring and buffer replenishment was adopted where the desired pH was monitored regularly and replenished periodically to maintain the desired pH throughout the experiment. These corrective actions were taken if it deviated significantly from the target value. Contents were maintained constant throughout the incubation period at 25°C, and the atrazine residue was determined in triplicate by GC-MS analysis (GC 7890B, MSD 5977A, Agilent Tech)

**Experiment on the influence of moisture on the natural attenuation of Atrazine**

The soil sample was brought to the desired moisture content by the addition of sterile deionized water and maintained constant throughout the research period by weighing and correcting for any weight loss by adding sterile deionized water. The soil samples were placed in a constant temperature incubator to investigate the attenuation within 30 and 60 days under treatment at 25°C and varied moisture content of 10, 15, and 25%, respectively. The moisture content was monitored and adjusted periodically (e.g., by adding sterile water) to ensure consistency across treatments (Chowdhury *et al.,* 2021). The content of atrazine residue was determined in triplicate using GC-MS Analysis.

**Experiment on the influence of temperature on the natural attenuation of Atrazine**

The test soil samples were prepared to contain 10% water with the addition of sterile deionized water. After stirring to mix well, the soil samples were placed in a bacteriological incubator (Meditech, MTBI) under treatment at 15°C, 25°C, and 45°C, respectively to investigate the attenuation within 30 and 60 days. The residue of atrazine was determined in triplicate by GC-MS analysis at different treatment times.

*Experiment on the influence of TOC on natural attenuation of Atrazine*

The soil sample was prepared to a desired organic matter by the addition of C (in the form of glucose) for its influence on the natural attenuation of atrazine. The soil organic matter was prepared and maintained at 0.08 g/L, 0.09 g/L, 0.10 g /L of C equivalent to 2%, 3%, and 4% glucose levels respectively (Qi *et al.*, 2022). The content was maintained constant throughout the incubation period at 25°C and the atrazine residue was determined in triplicate by GC-MS analysis.

## RESULTS AND DISCUSSION

**Total bacterial load in the soil sample after 60 days of incubation**

The result of bacterial count is presented in Table 1. The bacterial count was determined in triplicate for soil samples with different moisture content under treatment at 25°C. There were differences in bacterial count among the three

soil samples with different moisture content and treatments. After 30 days, the soil with 25% moisture content had the highest bacterial count of $4.7x10^4$ cfu/g, while the soil with 10% moisture content had the lowest count. After 60 days, the soil with the highest moisture content had the highest count of $1.5x10^5$ cfu/g. This difference is attributed to the fact that bacterial cells thrive and metabolize more at a certain moisture level over time. Statistical analysis revealed a significant difference ($p>0.05$) in bacterial load between moisture contents of 10%, 15%, and 25%. Furthermore, the TOC in the soil influenced bacterial metabolism and growth, as shown in Table 1. After 30 days, the bacterial load tended to increase with increasing TOC percentages in the soil samples. For example, TOC percentages of 2%, 3%, and 4% resulted in bacterial counts of $6.1x10^4$ cfu/g, $7.8x10^4$ cfu/g, and $8.3x10^4$ cfu/g, respectively.

After 60 days, the bacterial load decreased and then increased with the increase in TOC value. TOC 2%, 3%, and 4% have $2.5x10^4$, $1.5x10^{4,}$ and $3.1x10^4$ cfu/g, respectively. This is because a microbial population increases in the soil rich in organic matter environment, they tend to deplete the nutrients in the soil, thereby creating competition which leads to a decrease in their population as observed in bacterial growth (Iven *et al.,* 2023; Adomako *et al.,* 2022; Rashid *et al.,* 2016). All life form exists under a certain pH value as most survive at a neutral pH value of 7. The bacterial load was $9.4x10^4$ cfu/g at neutral pH, which was the highest count in all the experiments after 30 days, followed by $2.1 \times 10^4$ cfu/g at pH 10 and $1.1 \times 10^4$ cfu/g at pH 3. At 60 days, $1.4 \times 10^4$ cfu/g at pH 3, $8.2 \times 10^4$ cfu/g at neutral, and $1.9 \times 10^4$ cfu/g when the pH was 10. The highest bacterial load ($9.4 \times 10^4$ cfu/g) at neutral pH (around 7) after 30 days suggests that these conditions were most favourable for bacterial growth in this experiment. Most soil bacteria prefer a neutral or slightly acidic environment (pH 6-7.5) for optimal growth and reproduction (Xia *et al.,* 2024). This could explain the higher bacterial count at neutral pH compared to the acidic (pH 3) or alkaline (pH 10) conditions. The decrease in bacterial load at neutral pH (from $9.4 \times 10^4$ cfu/g at day 30 to $8.2 \times 10^4$ cfu/g at day 60) might indicate a shift in the bacterial community. Some bacteria that thrived initially at neutral pH might have depleted available resources or faced increased competition over time (Ng *et al.,* 2023). Conversely, the increase in bacterial load at pH 3 (from $1.1 \times 10^4$ cfu/g at day 30 to $1.4 \times 10^4$ cfu/g at day 60) suggests that some acid-tolerant bacteria adapted and grew better in the acidic environment over time (Whitman *et al.,* 2014; Samelis *et al.,* 2001).

**Table 1**. Total bacterial load of the soil sample after 60 days

| SAMPLE MOISTURE | MEAN cfu/g 30 DAYS | MEAN cfu/g 60 DAYS |
|---|---|---|
| 10% | $1.2 \times 10^4$ | $1.4 \times 10^4$ |
| 15% | $2.5 \times 10^4$ | $2.4 \times 10^4$ |
| 25% | $4.7 \times 10^4$ | $1.5 \times 10^5$ |
| ORGANIC MATTER | | |
| 2% | $6.1 \times 10^4$ | $2.5 \times 10^4$ |
| 3% | $7.8 \times 10^4$ | $1.5 \times 10^4$ |
| 4% | $8.3 \times 10^4$ | $3.1 \times 10^4$ |
| pH | | |
| 3 | $1.1 \times 10^4$ | $1.4 \times 10^4$ |
| 7 | $9.4 \times 10^4$ | $8.2 \times 10^4$ |
| 10 | $2.1 \times 10^4$ | $1.9 \times 10^4$ |
| TEMPERATURE | | |
| 15ºC | $5.2 \times 10^4$ | $1.2 \times 10^4$ |
| 25ºC | $5.4 \times 10^4$ | $1.5 \times 10^4$ |
| 45ºC | $9.9 \times 10^4$ | $2.9 \times 10^4$ |

In the case of temperature, temperature of 45ºC had a high bacterial count of $9.9 \times 10^4$ cfu, $5.4 \times 10^{4,}$ and $5.2 \times 10^4$ cfu/g at 25ºC and 15ºC, respectively, all after 30 days. Conversely, at 60 days, $1.2 \times 10^4$, $1.5 \times 10^4$ and $2.9 \times 10^4$ cfu/g at 15 ºC, 25 ºC, and 45ºC, respectively. This implies that the highest bacterial load ($9.9 \times 10^4$ cfu/g) at 45°C after 30 days suggests that some bacteria in the experiment thrived at this higher temperature. Certain bacteria, known as thermophiles, prefer warmer environments and grow optimally at temperatures above 40°C (Burkhardt *et al.,* 2024). These thermophilic bacteria might have dominated the population at day 30, leading to the highest count at 45°C. The decrease in bacterial load at 45°C (from $9.9 \times 10^4$ cfu/g on day 30 to $1.2 \times 10^4$ cfu/g on day 60) could indicate several possibilities: i) Nutrient Depletion: The initial surge in thermophilic bacteria at 45°C might have depleted available nutrients in the environment. This could have limited their growth by day 60; ii) Heat Stress: While some bacteria are thermophilic, prolonged exposure to high temperatures can be stressful, even for heat-loving microbes. This could have slowed their growth or reproduction over time. Conversely, the increase in bacterial load at lower temperatures (15°C and 25°C) between day 30 and 60 suggests: i) Mesophilic Growth: These temperatures might favor the growth of mesophilic bacteria, which thrive in moderate temperature ranges (20-40°C). They might have taken more time to establish but could have grown steadily by day 60; ii) Community Adaptation: The overall bacterial community might have adapted to the experimental conditions over time. Some thermophiles might have died off due to resource limitations or heat stress, allowing mesophilic bacteria to take hold at the lower temperatures. Statistical analysis shows a significant difference ($p>0.05$) among the temperatures of 15 ºC, 25 ºC, and 45ºC. This implies that the significant difference ($p > 0.05$) among the bacterial loads at 15°C, 25°C, and 45°Cat day 30 highlights the clear impact of temperature on bacterial growth in this experiment.

**Influence of pH on natural attenuation of Atrazine**

The attenuation of atrazine varies significantly depending on the pH level, which affects the effectiveness of many chemical and biological activities (Dehghani *et al.,* 2013). The Influence of various pH on atrazine attenuation is

shown in Fig. 1. It was observed that atrazine attenuation at pH 3, 10, and 7 had 91.5%, 83.9%, and 91.1% respectively. This indicates little percentage attenuation after 30 days. While at 60 days, pH 10, 3, and 7 had 97.1%, 65.7% and 52.1% respectively. This implies that atrazine attenuation was higher at pH 7. This rate is consistent with findings from similar studies (Korpraditskul, 1993). In addition, several studies emphasized that an increase in soil pH (between 6-7.5) results in an increase in soil microbial biomass and enzymatic activities, which in turn helps the microbial community to adapt and develop gene-enzyme systems for the enhanced attenuation of pesticides (Xia *et al.,* 2024).

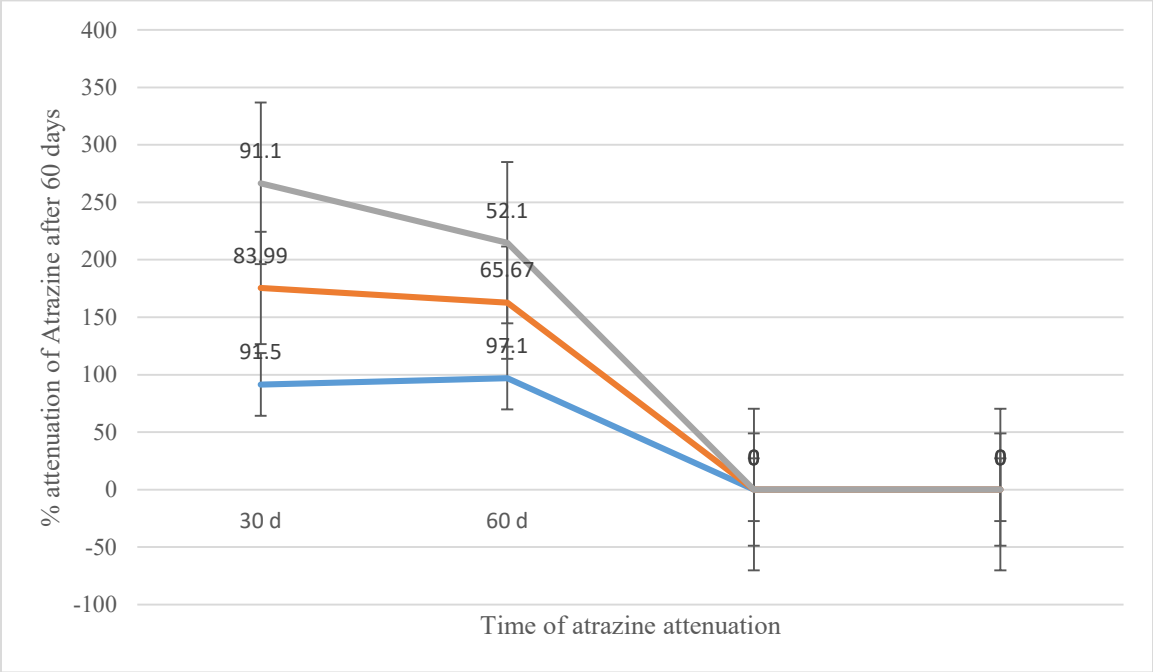


**Figure 1**: % attenuation of Atrazine under the influence of pH 3, 7, and 10 after 30 and 60 days.

**Influence of moisture content on natural attenuation of Atrazine**

In wet soil, herbicides break down differently than in dry soil. Atrazine breaks down faster without oxygen. The more water and time, the more atrazine breaks down. Wet soil makes atrazine break down 3-4 times more (Piccolo *et al.,* 1998). The attenuation curves are shown in Fig. 2. The attenuation of atrazine in soil was studied at different moisture levels. At 30 days, the moisture content of 10%, 15%, and 25% led to atrazine attenuation percentages of 97.7%, 55.6%, and 56.6% respectively. After 60 days, the attenuation percentages were 70.7%, 20.13%, and 73% for the same moisture content. It was observed that as the soil moisture increased from 5% to 20%, the attenuation rate increased by 3-4 fold. This was attributed to the increased microbial activity, atrazine diffusion, and chemical availability due to higher moisture content.

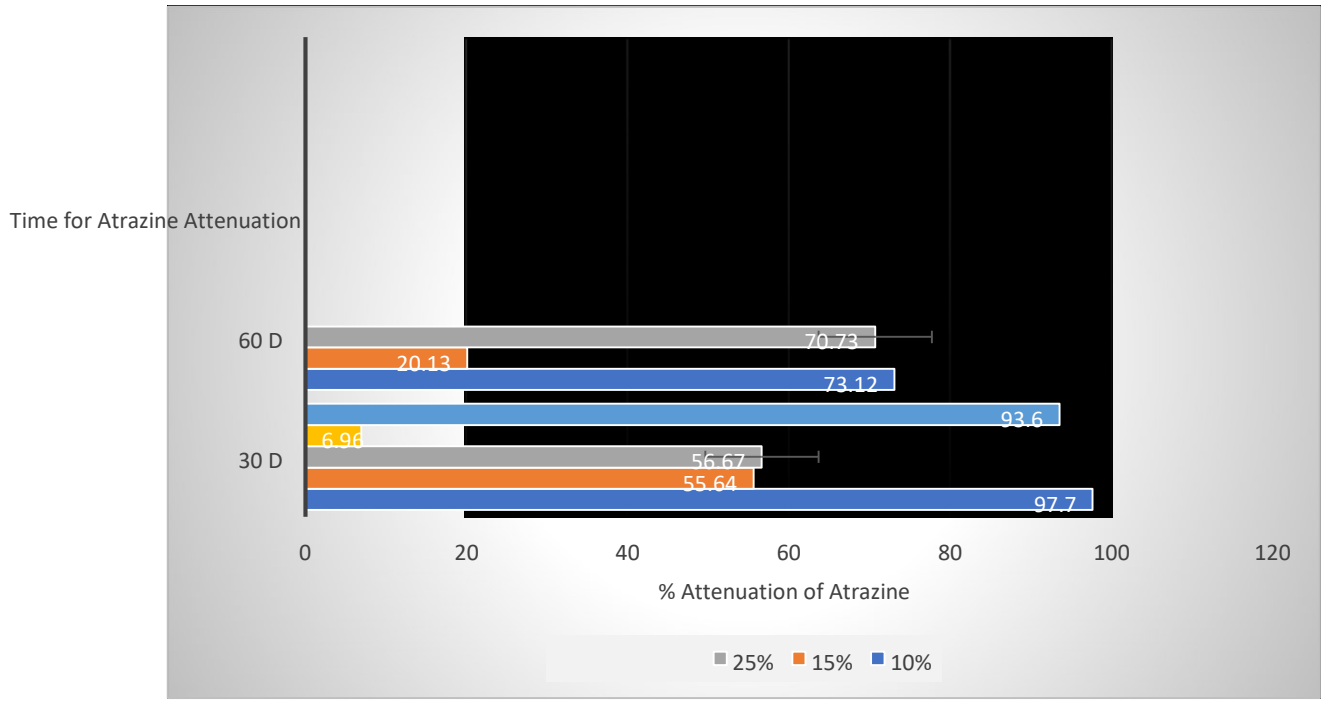


**Figure 2**: % attenuation of Atrazine after 30 and 60 days under the moisture content influence of 10%, 15% and 25%.

**Influence of temperature on natural attenuation of Atrazine**

The % attenuation of atrazine was determined at different influences of 25ºC and 45ºC, respectively after 30 and 60 days, as shown in Fig. 3. In general, the quantity of atrazine decreases with the increase in temperature. At treatment temperatures of 25°C and 45°C, the attenuation rate was 67.9% and 29.01% after 60 days, at 30 days the gap in attenuation rate was not much. This implies that volatilization and the rate of chemical and microbial attenuation are favoured by high temperatures, and the dissipation of pesticides is significantly faster under tropical conditions. Warmer temperatures, within a moderate range, typically accelerate microbial activity and atrazine attenuation. However, excessively high temperatures can be detrimental to some microbes (Arbeli & Fuentes, 2007).

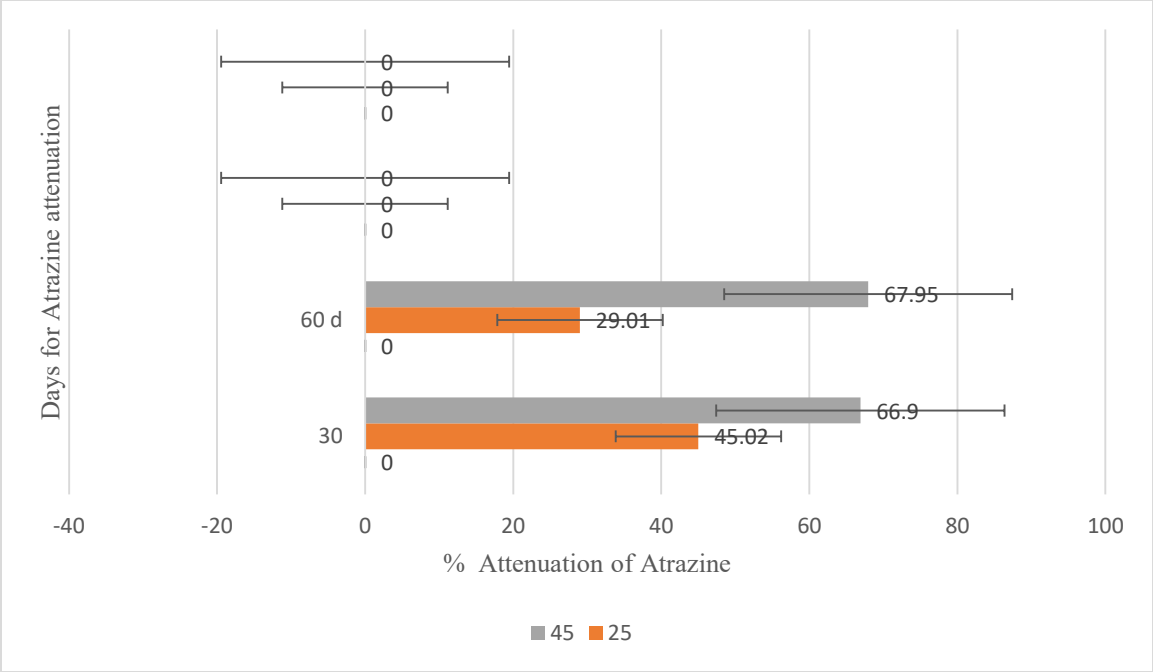


**Figure 3:** % Attenuation of Atrazine under the influence of temperature 25ºC, and 45 ºC after 30 and 60 days.

**Influence of TOC on natural attenuation of Atrazine**

The influence of the three different soil samples containing 2%, 3% & 4% TOC on the attenuation of Atrazine after 30 and 60 days at temperature 25ºC is shown in Fig. 4. The attenuation of atrazine was observed to increase with higher levels of TOC. At 30 days, the % attenuations with TOC of 2%, 3% and 4% were 80.2%, 66.6%, 25.8%. At 60 days; TOC of 2%, 3% & 4% were 68.1%, 73.1% & 83.9%, respectively. The results suggest that the attenuation process seems to be faster with readily available carbon resources that support a higher population of atrazine-degrading microbes.

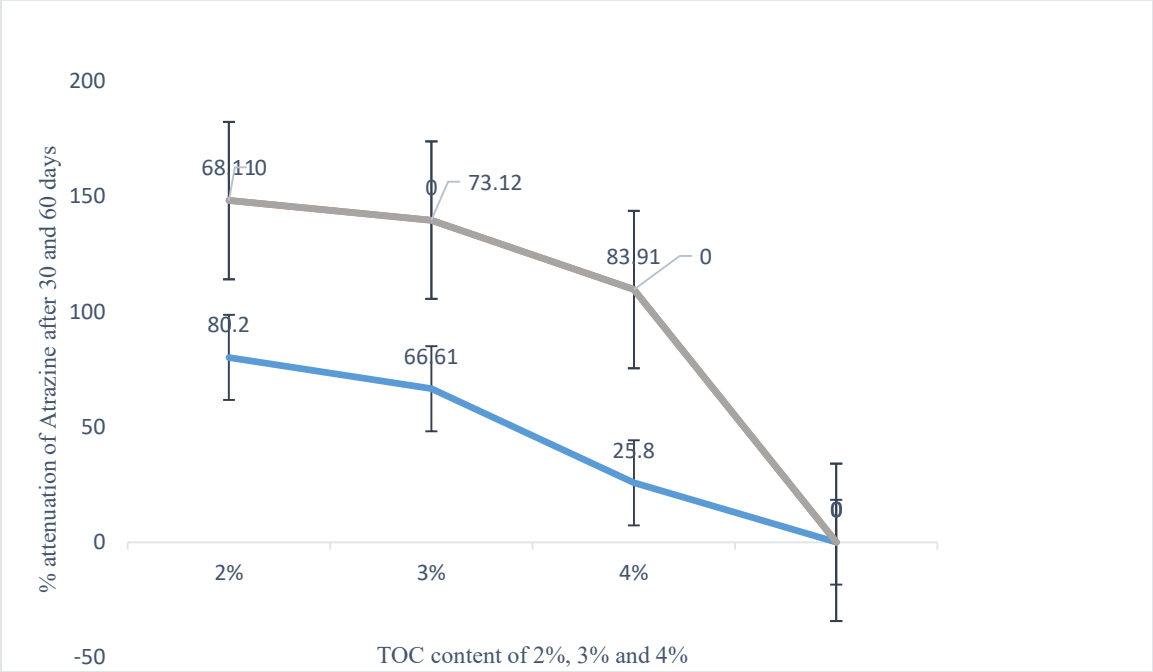


**Figure 4:** % Attenuation of Atrazine after 30 and 60 days under the influence of TOC content at 2%, 3% and 4%.

## CONCLUSION

This study investigated the influence of environmental factors on the natural attenuation of atrazine in tropical agricultural soils. The results demonstrate that natural attenuation processes can be a viable strategy for mitigating atrazine contamination, particularly in tropical regions. Higher soil organic matter content (TOC) and moderate moisture levels significantly enhanced atrazine degradation by soil microbes. The highest bacterial counts (*Arthrobacter sp*.) and the most efficient atrazine attenuation were observed at a neutral pH (7.0). Atrazine attenuation increased with higher temperatures, with the fastest rate observed at 45°C. These findings highlight the importance of considering soil health and properties when managing atrazine use in tropical agriculture. Practices that promote soil organic matter content and maintain moderate moisture levels can potentially enhance natural attenuation processes and reduce atrazine persistence in the environment. However, further research is needed to explore the specific mechanisms by which these environmental factors influence atrazine degradation pathways in tropical soils. Additionally, investigating the combined effects of these factors on different atrazine concentrations and diverse soil microbial communities can provide valuable insights for optimizing natural attenuation strategies in various tropical agricultural settings. Overall, this study emphasizes the potential of natural attenuation for minimizing atrazine contamination in tropical agricultural soils. Hence, by understanding the environmental factors that influence atrazine natural attenuation, we can explore strategies like bioremediation and targeted management practices to minimize potential contamination of soil and water resources. Ultimately, these efforts can contribute towards a healthier society and a more productive agricultural environment.

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