

Effect of Atrazine on Microbial and Physicochemical Parametrs of Atrazine Contaminated Soil

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ABSTRACT

Rates of biodegradation are affected by atrazine's low solubility; surfactants may increase the degradation rate. Though the two alkyl moieties readily support growth of certain microorganisms, the atrazine ring is a poor energy source due to the oxidized state of ring carbon. The physiochemical parameters and microbial loads of the soil were determined using standard microbiological standards where tenfold serial dilution was carried out on the various soil samples which was inoculated using pour plate technique while the colorimetric method was employed in the determination of the nitrogen, phosphorous and Potassium concentration. The pH meter was used to determine the soil pH. The experiment was carried out for a period of four weeks with the soil microbial load declining from 7.33×10^8 Cfu/ml to 1.11×10^1 Cfu/ml across the various concentrations of atrazine from 0% to 120%. The pH increased from 5.17 to 8.96, the soil nutrients decreased as the days passes by were, the soil nitrogen content decreased from 6.543 nm to 1.341nm while Phosphorus decreased from 6.772nm to 0.877nm while the Potassium content also decreased from 6.456nm to 0.566nm. Three bacterial isolates were obtained from this study which includes *Clostridium* sp., *Pseudomonas* sp. and *Bacillus* sp. The *Clostridium* sp. has the highest inhibition zone diameter at 500mg/ml of atrazine with 22.4 ± 0.00 mm followed by *Pseudomonas* sp. with 20.5 ± 0.05 mm and *Bacillus* sp. with the least inhibition diameter of 17.5 ± 0.01 mm. These antimicrobial activities at various concentrations of atrazine reveals that atrazine was actually the major cause of the decline in the soil microbial load.

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INTRODUCTION

Soil is a dynamic, living system and a chemically intricate medium that supports life on land. It functions as a storage system for nutrients and water, provides habitat for a wide range of microorganisms, and sustains primary production, carbon cycling, and overall ecological balance Rohr *et al.*, (2005). Sustainable agricultural productivity relies on the maintenance of soil physicochemical characteristics (including pH and availability of key nutrients) as well as the biochemical activities of native microorganisms, which break down organic materials, recycle vital elements, and preserve soil fertility Pathak *et al.*, (2012). However, prolonged intensive farming practices, rapid urbanization, population growth, and the increasing demand for higher crop yields have resulted in the widespread and often uncontrolled use of

synthetic agrochemicals, particularly pesticides and herbicides Nwani *et al.*, (2010). Among these, atrazine (ATZ) is one of the most frequently detected herbicides in aquatic environments in the United States Cheremisinoff *et al.*, (2010). It is classified as a restricted-use pesticide by the United States Environmental Protection Agency (EPA), with a maximum contamination level (MCL) of 3 ppb or 0.003 µg/L Cheremisinoff *et al.*, (2010). In contrast, the European Union adopted stricter pesticide regulations in 1991, restricting substances considered harmful to the environment, groundwater, or human health Cheremisinoff *et al.*, (2010). Atrazine's high-water solubility enables it to easily infiltrate and spread within aquatic ecosystems. Repeated and careless applications have led to its accumulation and eventual transport into water bodies De Campos *et al.*, (2008). Studies

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conducted in China have reported the presence of ATZ in 97% of soil samples and 89% of groundwater samples Geng *et al.*, (2013). Although initially believed to selectively inhibit photosynthesis in target plants without affecting other organisms, it has since been established that ATZ can exert both direct and indirect harmful effects on aquatic life and human health. These effects may include genetic alterations and physiological disruptions, and in severe cases, death of exposed organisms. At lower concentrations, the effects may not be immediately apparent but can gradually reduce the lifespan of affected organisms De Campos *et al.*, (2008). Fish serve as important bioindicators for evaluating the risks associated with ATZ exposure, as they can be affected directly through contaminated water or indirectly through the food chain Lakra *et al.*, (2009). Additionally, certain herbicides are known to induce oxidative stress by promoting excessive production of reactive oxygen species (ROS) Achuba *et al.*, (2003). These reactive molecules can, however, be neutralized by antioxidant compounds and the

antioxidant enzyme system (AOS). In humans, exposure to ATZ is particularly common among agricultural workers and herbicide applicators due to frequent contact. The herbicide can enter the body through inhalation or ingestion of contaminated food, water, or soil, although it is not readily absorbed through the skin. Once in systemic circulation, ATZ is metabolized and distributed to various organs, including the liver, ovaries, kidneys, red blood cells, and adipose tissue. It does not persist in the body for long and is typically excreted primarily through urine and, to a lesser extent, through feces within one to two days Dorsey (2003). Epidemiological studies have reported mixed findings regarding its health effects. A prospective cohort study found a positive association between ATZ exposure and cancers such as multiple myeloma, non-Hodgkin lymphoma, as well as bladder and lung cancers Rusiecki *et al.*, (2004). In contrast, another study found no significant relationship between ATZ use and most cancer types Freeman *et al.*, (2011).

METHODS

Study Area: This study was conducted in Clifford University, Ihie in Isialangwa North L.G.A of Abia State, Nigeria, with GPS of latitude 5.2955° N and longitude 7.3150° E.



Fig.1.0 Map of Study Area in Clifford University, Ihie.

Study Site and Soil Sample Collection

The soil sample used for this research was collected from Clifford University Garden in Ihie campus, Abia State. The topsoil of the depth of about 15cm was taken using a spade and 3kg was weighed and transferred into different labelled containers.

Pollution of Soil Samples with Atrazine

Different concentrations of atrazine (0%, 20%, 40%, 60%, 80%, 100% and 120%) were applied to the different containers with the soil sample and mixed properly and

allowed for 48 hours with constant sprinkling of water for acclimatization.

Microbiological Analysis

The method of Chigbu *et al.*, (2025) was adopted where tenfold serial dilution was carried out by weighing one gram of each of the different mixtures of soil into 9ml of sterile distilled water in a test tube to have 10^{-1} , 1ml was transferred from the first tube to the second to have 10^{-2} it was continued till the last fifth test tube which was 10^{-5} , 1ml aliquot was discarded. Pour plate technique was used to inoculate the inoculum by transferring 0.1ml from tube 10^{-3} , 10^{-4} and 10^{-5}

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into an empty sterile petri dish followed by pouring sterile nutrient agar. The plate was swirled to mix properly and was finally incubated at 28– 30 °C for 24 hours in a bacteriological incubator. Digital colony counter was used to count the resultant colonies after incubation.

NPK Determination

Total nitrogen in the soil was measured using the Kjeldahl method. In this process, the soil sample was chemically digested to convert organic and ammonium nitrogen into ammonia. The ammonia released was then distilled and titrated to determine the total nitrogen present in the sample. Phosphorus was analyzed using a color-based method. After extracting phosphorus from the soil, a 1% ammonium molybdate solution was added, which produced a blue color; the deeper the blue, the higher the phosphorus content. Potassium was measured using a spectrophotometric method. Potassium permanganate was added to the soil extract, producing a purple color. The intensity of the purple color indicated the level of potassium in the soil, with a stronger color showing a higher potassium concentration.

pH Determination

The soil pH was determined using a digital pH meter (Lansny model), following proper calibration before measurement.

The acid 0.1N HCl and 0.1N NaOH was used to standardize the pH meter. The pH meter's prob. was inserted into the respective soils to determine their pH.

Characterization of Bacterial Isolates

The purified isolates which were obtained through constant sub culturing were characterized by Gram staining and different biochemical tests like catalase, coagulase, oxidase, indole, urea, methyl red, Voges Prokuer and sugar fermentation tests.

Antimicrobial Activities of Atrazine

Atrazine stock solutions were prepared by dissolving the herbicide in distilled water to obtain concentrations of 500 mg, 250 mg, 125 mg, 62.5 mg, and 31.25 mg, respectively as described by Chigbu *et al.*, (2025). Mueller Hinton agar was prepared according to the manufacturer's instruction while soil-isolated fresh bacterial cultures including *Pseudomonas sp.*, *Clostridium sp.* and *Bacillus sp.* were spread on the surface of the sterile Mueller Hinton agar. The different concentrations of the atrazine were applied using agar well diffusion method. Incubation of the plates at 28– 30 °C was for 24 hours. The different zones of inhibition were measured using a transparent ruler.

RESULTS

Table 1: Baseline Physicochemical and Microbial Quality of Test Soil Sample

Measured Parameters	Concentration Absorbance
Microbial Load	5.5x 10 ⁸ (Cfu/g)
Nitrogen (N)	6.891 (nm)
Phosphorus (P)	6.773 (nm)
Potassium (K)	6.566 (nm)
pH	5.04

Table 2: Mean Viable Count of Bacteria Isolated in Atrazine contaminated soil at Week 2

Mean Viable Count of Bacteria Isolated in Atrazine Contaminated Soil at Week 2								
Conc.	Of	0	20	40	60	80	100	120
Atrazine (%)								
Mean		7.33x 10 ⁸	2.0 x 10 ⁸	4.41x 10 ⁷	5.2 x 10 ⁶	3.20x 10 ⁵	4.10x 10 ⁴	3.41x 10 ⁴
Viable								
Count								
(cfu/g)								
pH		5.17	5.22	5.23	5.30	5.33	5.36	6.35
Nitrogen		6.543	6.021	5.306	5.121	4.880	4.592	3.076
Phosphorus		6.772	6.211	5.554	4.001	3.992	3.311	2.155
Potassium		6.456	5.900	4.857	4.122	3.597	3.274	2.782

Table 3: Mean Viable Count of Bacteria Isolated in Atrazine Contaminated Soil at Week 4

3. Mean Viable Count of Bacteria Isolated in Atrazine Contaminated Soil at Week 4								
Conc. Of	0	20	40	60	80	100	120	
Atrazine (%)								
Mean Viable Count (cfu/g)	1.10x 10 ⁶	5.4 x 10 ⁵	4.11x 10 ⁴	3.7 x 10 ³	2.50x 10 ²	7.0 x 10 ¹	1.11x 10 ¹	

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pH	5.72	5.88	6.02	6.45	7.71	8.32	8.96
Nitrogen	6.441	4.210	3.330	2.567	2.110	1.762	1.341
Phosphorus	6.300	4.702	3.258	2.157	1.632	1.121	0.877
Potassium	5.135	3.102	2.810	2.332	1.190	0.786	0.566

Table 4: Morphological and Biochemical Characteristics of Bacteria Isolates

Isolate codes	Gram stain	Shape	Cat	Oxi	Coa	Ind	Cit	H ₂ S	Gas	Probable Identity
GSR1	G-ve	Rod	+	+	-	-	+	-	-	<i>Pseudomonas</i> sp.
GSR2	G+ve	Rod	+	-	-	-	-	-	-	<i>Bacillus</i> sp.
GSR3	G+ve	Rod	+	-	+	-	+	-	-	<i>Clostridium</i> sp.

Key: positive (+), negative (-), gram positive (G+), gram negative (G-), catalase (cat), oxidase (oxi), coagulase (coa), Indole (ind), citrate (cit) and hydrogen sulphide (H₂S).

Table 5: Inhibition Zone Diameters of Atrazine on Soil Bacterial Isolates

Conc. (Mg/ml)	<i>Clostridium</i> sp.	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp.
500	22.4 ± 0.00	20.5 ± 0.05	17.5 ± 0.01
250	18.5 ± 0.04	14.07 ± 0.00	14.5 ± 1.12
125	13.4 ± 0.20	10.00 ± 1.00	12.5 ± 1.21
62.5	8.0 ± 1.03	0.00 ± 0.00	10.00 ± 0.05
31.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

DISCUSSION

Atrazine may be catabolized as a carbon and nitrogen source in reducing environments, and some aerobic atrazine degraders have been shown to use the compound for growth under anoxia in the presence of nitrate as an electron acceptor, Bichat *et al.*, (1999) a process referred to as a denitrification. When atrazine is used as a nitrogen source for bacterial growth, degradation may be regulated by the presence of alternative sources of nitrogen. In pure cultures of atrazine-degrading bacteria, as well as active soil communities, atrazine ring nitrogen, but not carbon are assimilated into microbial biomass (Ralebitso *et al.*, 2002). Low concentrations of glucose can decrease the bioavailability, whereas higher concentrations promote the catabolism of atrazine (Cai *et al.*, 2003). The result of this research shows that atrazine though an herbicide which destroys weeds and help in the development of crops and agricultural products has an adverse effect on the soil microbial community hence it leads to the reduction in microbial population of the soil and other physiochemical parameters of the soil as shown on table 2 above. After two weeks of application the microbial load declined from 7.33×10^8 Cfu at 0% of atrazine to 3.41×10^4 Cfu at 120% these results opposes the previous report of (Nazia *et al.*, 2000) who worked with different concentration of atrazine on the growth of millet and the previous research of Ana *et al.*, (2020) who reported on the soil samples collected in the 2nd and 8th weeks after the application of atrazine to soil had significantly lower alpha-diversity and high increase in the number of soil microorganisms after treatment. The mean growth ranges from 6.82 ± 0.14 , 6.88 ± 0.12 to 7.21 ± 0.06 . This results also agrees with the previous report of Abdullahi *et al.*, (2022) who reported on the reduced microbial diversity of atrazine and the negative impact on soil beneficial

microorganism like nitrogen fixing bacteria and Phosphate solubilizing microorganisms. Atrazine also showed a clear decline in the nutritional abundance of nitrogen, Phosphorus and Potassium. After the first two weeks application the nitrogen content of the soil reduced from 6.343 nm at 0% concentration to 3.076 nm at 120% atrazine concentration, Phosphorus declined from 6.772 to 2.155 while Potassium declined from 6.456 to 2.782. After four weeks of application the nitrogen content reduced from 6.441 to 1.341, Phosphorus declined from 6.300 to 0.877 while Potassium declined from 5.125 to 0.566 respectively. This report is in agreement with the previous report of Wang *et al.*, (2025) who also reported on the reduced nutrients in the soil after the application of atrazine in the soil. The Bacteria isolated from the soil samples contaminated with atrazine includes *Pseudomonas* sp, *Bacillus* sp and *Clostridium* sp. Fernandes *et al.*, (2020) reported the bacterial communities in soils after the application of atrazine to include members of the phyla *Proteobacteria*, *Actinobacteria* and *Enterobacteriaceae*, *Burkholderiaceae*, *Conexibacteraceae*, *Solirubrobacteraceae* and *Gaiellaceae*. The result of antimicrobial activities shows that atrazine has clear inhibition activity on the three characterized bacterial isolates which is a clear indication that the reduction in number of soil microbes were due to the lethal actions of atrazine as also reveal decline in bacteria viable count after week four of treatment. *Clostridium* sp had the highest inhibition zone diameter of 22.4 ± 0.00 mm at 500mg/ml followed by *Pseudomonas* sp which has 20.5 ± 0.05 mm and *Bacillus* sp with 17.5 ± 0.01 mm respectively. As concentration of the atrazine reduces the inhibition size reduces equally, however at 31.25mg/ml there was no inhibition activities on the isolates which shows that concentration is directly

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proportional the inhibition zones respectively. Herbicides may inhibit some microorganisms due to toxicity, but also stimulate specific groups because some herbicides, such as atrazine, are a nutrient source (Ribaudo and Bouzaher, 1994; Bonfleur *et al.*, 2015). Therefore, from the result, it can be deduced that *Pseudomonas* and *Baccillus* spp could be enhanced and used for the degradation of atrazine in highly contaminated soil.

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