Bioremediation of Atrazine Herbicide Contaminated Soil Using Different Bioremediation Strategies

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ABSTRACT: This study evaluated the bioremediation of atrazine herbicide contaminated agricultural soil under different bioremediation strategies using indigenous *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Aspergillus niger* as bioaugmentation agents and poultry droppings as biostimulation agent. The results showed that bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger*, bioaugmentation with bacterial-fungal consortium (*Pseudomonas aeruginosa*, *Bacillus subtilis* and *Aspergillus niger*), biostimulation with poultry droppings, and combined biougmentation and biostimulation (*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger* and poultry droppings) resulted in maximum atrazine biodegradation of about 97%, 95%, 84%, 99%, 100% and 100%, respectively. The kinetics of atrazine biodegradation in the soil were modelled using first-order kinetic model and the biodegradation half-life estimated. The first order kinetic model adequately described the kinetics of atrazine biodegradation in soil under the different bioremediation strategies. The rate constants (k_1) of atrazine biodegradation in soil subjected to bioaugmentations with *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger*, and bacterial-fungal consortium ranges between 0.059 day^{-1} and 0.191 day^{-1} while for that subjected to natural bioattenuation, biostimulation and combined bioaugmentation and biostimulation are 0.026 day^{-1}, 0.164 day^{-1} and 0.279 day^{-1}, respectively. The half-life ($t_{1/2}$) of atrazine biodegradation in soil under natural bioattenuation was obtained to be 26.7 days. This was reduced to between 2.5 and 11.7 days under the application of bioaugmentation, biostimulation and combined bioaugmentation and biostimulation strategies. The bioremediation efficiencies of the different bioremediation strategies in influencing atrazine biodegradation or removal is of the following order: Combined bioaugmentation and biostimulation > Bioaugmentation with bacterial-fungal consortium > Biostimulation with poultry droppings > Bioaugmentation with *Pseudomonas aeruginosa* > Bioaugmentation with *Bacillus subtilis* > Bioaugmentation with *Aspergillus niger* > Natural bioattenuation.

World-wide, herbicides have been widely utilized for controlling noxious or undesirable plant growth generally referred to as weeds in crop and non-crop areas and this has contributed greatly to modern agriculture (Wang *et al*., 2018). One of the most widely used herbicides in agriculture is atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) (Ackerman, 2007; El-Bestawy *et al*., 2014; Cao *et al*., 2018). It is a non-polar, non-volatile and low soluble compound (El-Bestawy *et al*., 2014). It is primarily applied as a selective herbicide for controlling broad-leaf and some grassy weeds in crop production such as corn, maize, pineapple, sorghum and sugar cane. (El-Bestawy *et al*., 2014; Sopid, 2016; Zhao *et al*., 2018; Cao *et al*., 2018) and as a non-selective herbicide for non-cropped fallow lands and industrial lands (Ghosh and Phillip, 2006; Solomon *et al*., 2013). Nevertheless, the long-term and excessive

application of atrazine could lead to its high concentrations presence and persistence in the soil which could be dissipated through leaching into ground water and drinking water as well as washed (as run-off) into surface water, if applied prior to irrigation or heavy rainfall (Nasseri *et al*., 2009; Dehghani *et al*., 2010). The maximum atrazine contaminant level in drinking water is 3.0 μgL^{-1} (USEPA, 1990). However, the type of soil determines the mobility and environmental fate of Atrazine through sorption to soil particles. Atrazine is more readily adsorbed on muck or clay soils than on soils of low clay and organic matter content; therefore, adsorption to certain soil constituents significantly limits the downward movement or leaching (Dehghani *et al*., 2005; Correia *et al*., 2007; Danrong *et al*., 2009).

Atrazine has been classified as a priority pollutant

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(Cao *et al*., 2018) that is toxic (Muñoz-Leoz *et al*., 2011; Imfeld and Vuilleumier, 2012; Zhao *et al*., 2018) and carcinogenic (VanLeeuwen *et al*., 1999; Zhao *et al*., 2017). It is harmful by inhalation and swallowing as well as by contact with the skin (Extension Toxicology Network, 1996; El-Bestawy *et al*., 2014). Additionally, atrazine has been reported to possess long-term reproductive and endocrine-disrupting effects, epidemiological link to low sperm counts in men and a probable human carcinogen (VanLeeuwen *et al*., 1999; Lasserre *et al*., 2009; Zhao *et al*., 2017) that causes breast and ovarian cancer (Wang *et al*., 2018). Therefore, the toxicity of atrazine has raised serious concerns and the need to remediate the soil contaminated with it. There are conventional physical and chemical technologies for soil remediation, however, they have the disadvantages of high economic costs, formation of secondary contaminants and damage to soil organisms (Cao *et al*., 2018). Innovative strategies for remediating atrazine-contaminated soils are critically needed (Lima *et al*., 2009; Pandey *et al*., 2009; Cao *et al*., 2018).

Bioremediation which is the use of microorganisms or plants has been considered a cost-effective, environmentally friendly strategy to solve soil contamination (Gregoire *et al*., 2008; Agnello *et al*., 2016). Thus in recent years, bioremediation has aroused increasing concern in the field of contaminated soil remediation (Agarry *et al*., 2013; Agnello *et al*., 2016; Agarry, 2018). It is currently being used to clean a wide variety of chemicals. In soil, many microorganisms and plants play the major role in the biodegradation and elimination of chemicals converting them into simpler non-toxic compounds (Marecik *et al*., 2008). Some reports have demonstrated the ability of some soil microorganisms to degrade atrazine partially or totally directing it to carbon dioxide and ammonia formation (Rosseaux *et al.,* 2001).

Therefore, this study aimed to experimentally investigate and evaluate the bioremediation of atrazine contaminated agricultural soil under different bioremediation strategies of bioaugmentation, biostimulation, bioaugmentation coupled with biostimulation (i.e. combined bioaugmentation and biostimulation), and natural bioattenuation.

MATERIALS AND METHODS
Material: The apparatus and equipment used includes the following: 500 ml measuring cylinder, 500 ml conical flasks, test tubes, glass slides, staining rack, filter paper, sterile earthen pots, needles and syringes,

pipette, cotton wool, inoculating loop, aluminum foil, spatula, vortex mixer, laboratory incubator 37 ºC (DNP-9052), compound microscope, autoclave (Yx-280A), and electrical weighing balance (Ohaus PA512), measuring tape. The reagents and chemicals used are:atrazine, ethanol, distilled water, safranin, n-hexane, acetone, crystal violet, Grams iodine, nutrient agar (NA), nutrient broth (NB), Eosin methylene blue (EMB), potato dextrose agar (PDA), potato dextrose broth (PDB) and Mac Conkey.

Collection and Preparation of Sample: Agricultural soil was collected from Landmark University Teaching and Research Farm, Omu-Aran, Kwara state, Nigeria. The farm had been planted with maize crop and leguminous crop on an annual rotation since 2014. The soil samples were obtained from the top 0 – 30 cm using a shovel. The soil samples were dried, homogenized, passed through a 2 mm sieve and then stored in a polyethylene bag. The poultry droppings used as nutrients for biostimulation were collected from Landmark University Commercial Farm. The poultry droppings were sun dried, grinded and sieved to obtain uniform size particles. The particles were mixed with water and the mixture was allowed to compost for two weeks with regular mixing. The atrazine [6-chloro-N2-ethyl-N4-isopropyl-1, 3, 5-triazine-2, 4-diamine] (Figure 1) used as the model herbicide was purchased from an Agro- chemicals store in Omu-Aran, Kwara State, Nigeria.
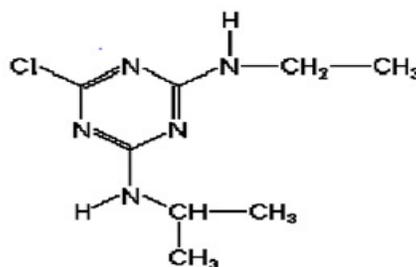
Fig 1. Chemical structure of *s*-triazine herbicide

Characterization and Artificial Contamination of Soil Sample: The collected soil and poultry dropping samples were characterized for physical and chemical properties using standard methods (APHA, 2005). The characterized soil (1 kg) was distributed into two different aluminum cores. Different volumes of the atrazine herbicide (10 and 20 ml) was added to the soil in each of the two aluminum cores, respectively. The cores and its contents were placed under a green vegetation area within the university environment and left for 30 days to allow for aging and development of microorganisms that are capable of utilizing atrazine as source of carbon and energy. Within this period, small volume of water was added to the contents in the

aluminum cores and stirred at regular intervals to ensure proper aeration and even distribution of temperature needed for microbial species development.

Table 1: Physicochemical Characteristics of Uncontaminated Soil

Parameters	Un-impacted Soil	Poultry Droppings
Total nitrogen (%)	1.32 ± 0.01	1.62 ± 0.02
Total organic carbon (%)	0.66 ± 0.03	35.6 ± 1.12
Available phosphorus (%)	0.28 ± 0.02	1.56 ± 0.01
Potassium (%)	0.16 ± 0.03	0.34 ± 0.02
pH	6.8 ± 0.02	6.5 ± 0.1
Moisture content (%)	7.3 ± 0.3	11.8 ± 0.2

Isolation, Characterization and Identification of Microbial Species: The serial dilution and spread plate techniques (APHA, 2005) using NA, EMB and PDA were employed for the isolation of microorganisms in soil and poultry droppings. One gram each of the soil contaminated with 20 ml and 30 ml of atrazine herbicide was introduced into two (2) sterilized conical flask containing 50 ml of de-ionized water. Vortex mixer was used to mix it and then labelled as stock solutions. 1 ml was taken from each of the stock solutions and dispensed into two different sterilized test tubes containing 9 ml each of de-ionized water and mixed using a vortex mixer and labelled as 10^{-1}. 1 ml was then drawn from each of the test tubes labelled 10^{-1} and dispensed into another two separate sterilized test tubes and mixed with vortex mixer and labelled 10^{-2}. This same procedure was repeated for the rest of the test tubes and labelled 10^{-3} to 10^{-10}, respectively. This process is called serial dilution. After the serial dilution, 1 ml was taken from each of the test tubes labelled 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}, and 10^{-10} and introduced into 10 different sterilized Petri dishes containing EMB agar and then allowed to solidify. The same procedure was done for PDA and NA. The Petri dishes with EMB and NA were inverted and kept in an incubator at 37 °C for 18 - 24 h, while the Petri dishes with PDA were inverted and kept at ambient temperature (27 °C) for 72 h to allow for microbial growth. The bacterial and fungal colonies isolated from soil contaminated with 20 ml and 30 ml of atrazine herbicide were further selected for colony purification by sub-culturing and for further evaluation of their capacity to utilize atrazine herbicide. The colonies considered to be visually different were streaked separately on NA, EMB, MacConkey and PDA agar plates containing 30 ml of atrazine herbicide and incubated at 37 °C. This procedure was repeated severally to ascertain the purity of the isolated colonies. To be able to characterize the isolated bacterial and fungal isolates, several biochemical tests (citrate test, indole test, catalase test, oxidase test, urease test, mannitol test,

methyl red/Voges-Proskauer (MR/VP) test) were carried out on the samples. The shape and morphology of the bacterial and fungal cells were determined using light microscopy (Granados and Villaverde, 1996), while identification of the isolates was made using Bergey's Manual of Determinative Bacteriology (Krieg *et al*., 1994). Bacteria and fungi isolated from the atrazine contaminated soil include *Pseudomonas aeruginosa*, *Acetobacter* sp, *Bacillus subtilis*, *Flavobacterium* sp., and *Aspergillus niger*. The indigenous microorganisms present in the un-impacted soil were identified to be made up of *Pseudomonas aureofaciens*, *Curvularia* sp, *Alternaria* sp, *Candida famata*, *Phialoplora* sp., and *Penicillum* sp., respectively. Meanwhile, the microorganisms present in the poultry droppings were identified to be made up of mainly *Escherichia coli*, *Flavobacterium*, *Pseudomonas* sp., *Staphylococcus sp.,* and *Mucor*. The isolated pure colonies of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger* and their consortium were used as agents of bioaugmentation as well as poultry droppings as agent of biostimulation.

Bioremediation Experimental Design: Twenty (20) grams of un-impacted soil sample having no history of atrazine contamination was dispensed into seven different earthen pots (diameter, 15 cm; depth, 24 cm) and labeled A, B, C, D, E, F and G. Isolates of *Pseudomonas aeruginosa* and *Bacillus subtilis* were cultured in nutrient broth (NB) liquid while *Aspergillus niger* was cultured in potato dextrose broth (PDB) liquid medium until they all reached the stationary phase. Cell pellets of the three different isolates were collected and washed twice with sterile phosphate buffered saline (PBS). Thereafter, the cell pellets of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Aspergillus niger* were suspended in PBS and adjusted to 1.1×10^6, 1.3×10^6 and 0.8×10^6 cfu g^{-1}, respectively. The ratio of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Aspergillus niger* in the mixture (bacterial-fungal consortium) was 1:1:1 (v:v). Two (2) ml of de-ionized water was added to each of the pots so as to moisten the soil and then contaminated with 5% (w/w) of atrazine herbicide. The contaminated soils in pots B, C and D were correspondingly augmented with *Pseudomonas aeruginosa* (bioaugmentation), *Bacillus subtilis* (bioaugmentation) and *Aspergillus niger* (bioaugmentation); poultry droppings were added to the contaminated soil in pot E (biostimulation); the contaminated soil in pot F was augmented with mixed bacterial-fungal consortium (consists of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Aspergillus niger*) (bioaugmentation) and the combined mixture of *Pseudomonas aeruginosa*, *Bacillus subtilis*,

Aspergillus niger and poultry droppings was added to the content in pot G (i.e. combination of bioaugmentation and biostimulation). No microbial isolates and poultry droppings were added to the content in pot A (natural bioattenuation). The contents in each of the Petri dishes A, B, C, D, E, F, and G were then incubated for 30 days at laboratory ambient temperature. All the experiments were carried out in triplicate. Samples were withdrawn at 5 days interval for residual atrazine determination.

Extraction and Determination of Residual Atrazine Herbicide: Ten (10) millilitre of hexane was added to 1 g of soil sample and the mixtures were thoroughly shaken for 20 min in a mechanical shaker to allow for hexane to mix properly and extract all the residual atrazine in the soil sample. The mixtures were left for 48 h in a separating funnel at the end of which it separated into two layers: the organic upper layer made up of water, hexane and the extracted residual atrazine and the bottom solid layer (residue) made up of soil particles. The upper organic layer was decanted and centrifuged at 4000×g for 5 min after which it was filtered through a clean pad of cotton. The filtrate was evaporated to dryness at 40 °C in a rotary evaporator to remove the hexane and the residual atrazine was analyzed using gas-liquid chromatography-mass spectrometry. The gas-liquid chromatography conditions were as follows: N2 was used as carrier gas at a flow rate of 3 ml min^{-1}. The capillary column is of 30 m length × 0.32 mm internal diameter × 0.52 μm film thickness with 5% phenyl methyl polysiloxane. The initial temperature of column was set at 180 °C for 2 min and then raised at 3 °C per minute, and finally held at 200 °C for 15 min. The injector and detector temperatures were set at 230 °C and 280 °C, respectively.

Kinetics and Estimation of Biodegradation Half-Life: The kinetics of biodegradation is of great significance as it provides at any time the contaminant concentration that will be left or remain and consequently allow for the prediction of the amount likely to be present at some future time (Agarry *et al.*, 2013). The rate of organic compound biodegradation by microbes in soil is often described by a first-order kinetics as given in the Eq. (1) (Agarry *et al.*, 2013):

$$\ln C_A = a + k_1 t \qquad (1)$$

Where C_A, k_1 and t are the concentration of the contaminant at time t, biodegradation rate constant and time, respectively.

Biodegradation half-life ($t_{1/2}$) is the time taken for half the amount of a substance to be lost or degraded or removed. Biodegradation half-life time can be obtained from the rate constant k_1 using Eq. (2) (Zahed *et al.*, 2011; Agarry *et al.*, 2013):

$$t_{1/2} = \frac{\ln 2}{k_1} \qquad (2)$$

Data Analysis: The data were subjected to one-way analysis of variance (ANOVA) at 5% probability. The data analysis was performed using statistical package for social sciences, version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION
Atrazine Biodegradation: The atrazine biodegradation profile in soil subjected to different bioremediation strategies of natural bioattenuation, bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger*, biostimulation with poultry droppings, bioaugmentation with bacterial-fungal consortium and combined bioaugmentation and biostimulation is shown in Figure 1. It is observed that the percentage atrazine biodegradation was relatively rapid within the first 15 days of remediation in all the soil subjected to bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger*, biostimulation with poultry droppings, bioaugmentation with bacterial-fungal consortium and combined bioaugmentation and biostimulation and rapidly continued up to day 30 when compared to that of the soil under natural bioattenuation. At the end of day 30, the atrazine concentration reduced from 50,000 mg/kg to 1,451±240, 2,450±350, 8,000±470, 480±40, 0 and 0 mg/kg corresponding to 97.1%, 95.1%, 84%, 100%, 99% and 100% reduction in atrazine contaminated soil subjected to bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger*, biostimulation with poultry droppings, bioaugmentation with bacterial-fungal consortium, and combined bioaugmentation and biostimulation, respectively.

For the atrazine contaminated soil subjected to natural bioattenuation, the atrazine concentration of 50,000 mg/kg reduced to 22,500±1340 mg/kg corresponding to 55% reduction at the end of day 30.
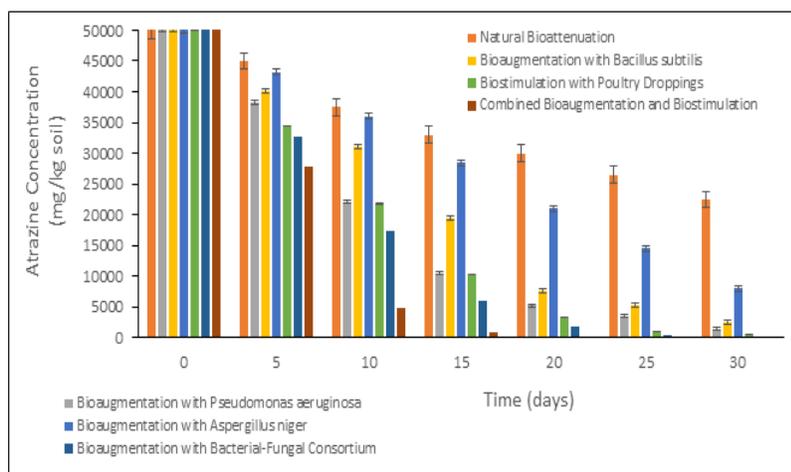
Fig 1.Time course for atrazine biodegradation in soil under natural bioattenuation, bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger*, biostimulation with poultry droppings, bioaugmentation with bacterial-fungal consortium and combined bioaugmentation and biostimulation. Bars indicate the average of triplicate samples while the error bars show the standard deviation.

This observation showed that during the atrazine biodegradation in soil, bioaugmentation, biostimulation and combination of bioaugmentation and biostimulation individually provided a more effective and enhanced bioremediation response than the natural bioattenuation. The reason(s) for this observation may be due to the fact that biostimulation with poultry droppings provided more nutrients (Table 1) required by the autochthonous microorganisms and more microbial load in the soil (i.e. acting as biostimulation-bioaugmentation agent) (Agarry *et al*., 2013; Agarry and Jimoda, 2013), while the bioaugmentations with *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Aspergillus niger* either as pure culture or as mixed bacterial-fungal culture increased the level of organic compound degrading microbial species in the soil. Similar observation of enhanced bioremediation or biodegradation of atrazine in soil has been reported for the use of *Pseudomonas* sp. ADP, mixed bacterial consortium (*Pseudomonas* sp., *Providencia* sp., and *Bacillus* sp.) mixed bacterial consortium (*Chenggangzhangella methanolivorans* CHL1 and *Arthrobacter* sp. ATR1) as bioaugmentation agents (i.e. bioaugmentation) (Lima *et al*., 2009; El-Bestawy *et al*., 2014; Wang *et al*., 2018); dairy manure, farmyard manure, citrate and molasses as biostimulation agents (i.e. biostimulation) (Topp *et al*., 1996; Mukherjee, 2009; Qui *et al*., 2009; Nousiainen *et al*., 2015); and mixture of *Pseudomonas citronellolis* ADP and citrate (i.e. combined bioaugmentation and biostimulation) (Nousiainen *et al*., 2015). Poultry droppings used as biostimulation

agents in this study contained organic nitrogen as part of the nutrients supplied to the soil and consequently played a role in the enhancement of atrazine biodegradation. This is in agreement with the observation of Topp *et al*. (1996) that used dairy manure as biostimulation agent in the biodegradation of atrazine. However, it is reported that the catabolism of atrazine is repressed in the presence of another nitrogen source and activated when nitrogen is limiting (Dehghani *et al*., 2013). Thus many investigators have reported the negative effect of nitrogen supplementation on atrazine biodegradation by indigenous microorganisms in soils (Alvey and Crowley, 1995; Abdelhafid *et al*., 2000; Dehghani *et al*., 2013). From Figure 1, it is observed that at day 20 and day 30, complete atrazine biodegradation was attained in contaminated soil subjected to combined bioaugmentation and biostimulation, and bioaugmentation with bacterial-fungal consortium, respectively. This observation indicates that combined bioaugmentation and biostimulation as well as bioaugmentation with bacterial-fungal consortium elicited complete atrazine biodegradation in soil than the individual bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger* and biostimulation with poultry droppings, respectively. A similar observation has been reported for the use of combined bioaugmentation and biostimulation in eliciting a higher atrazine biodegradation than the use of individual bioaugmentation or biostimulation (Lima *et al*., 2009; El-Bestawy *et al*., 2014).
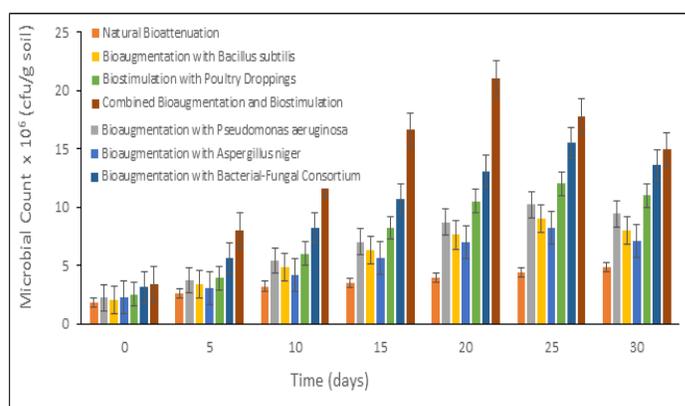
Fig 2 shows the growth profiles of the organic compound degrading organisms in atrazine contaminated soil subjected to different bioremediation strategies of natural bioattenuation, bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger*, biostimulation with poultry droppings, bioaugmentation with bacterial-fungal consortium and combined bioaugmentation and biostimulation, respectively.

Figure 2. Time course for the growth of organic compound degrading organisms in atrazine contaminated soil under natural bioattenuation, bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger*, biostimulation with poultry droppings, bioaugmentation with bacterial-fungal consortium and combined bioaugmentation and biostimulation. Bars indicate the average of triplicate samples while the error bars show the standard deviation.

As shown in Figure 2, it is observed that the microbial counts maximally increased from day 0 to day 25 and slightly decreased on day 30 in each of the atrazine contaminated soil subjected to different bioremediation strategies. For the contaminated soil under natural bioattenuation, the microbial count maximally increased from 1.8 to $4.8 \pm 0.4 \times 10^6$ cfu-g^{-1}, while it increased from 2.2 to $10.2 \pm 1.1 \times 10^6$ cfu-g^{-1}, 2.0 to $9.0 \pm 1.2 \times 10^6$ cfu-g^{-1}, 2.3 to $8 \pm 1.4 \times 10^6$ cfu-g^{-1}, 2.4 to $12.0 \pm 1.0 \times 10^6$ cfu-g^{-1}, 3.1 to $15.5 \pm 1.3 \times 10^6$ cfu-g^{-1}, and 3.4 to $17.8 \pm 1.5 \times 10^6$ cfu-g^{-1} corresponding to a growth increase of 364%, 350%, 257%, 380%, 400%, and 424% for atrazine contaminated soil subjected to bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger*, biostimulation with poultry droppings, bioaugmentation with bacterial-fungal consortium and combined bioaugmentation and biostimulation,

respectively. The microbial growth increase in contaminated soil under natural bioattenuation is 167%. These observations revealed that bioaugmentation, biostimulation and the combination of bioaugmentation and biostimulation enhanced the rate of microbial growth which accounted for the higher counts of the microorganisms observed in all the soil subjected to bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger*, biostimulation with poultry droppings, bioaugmentation with bacterial-fungal consortium and combined bioaugmentation and biostimulation than the microbial counts observed in soil under natural bioattenuation. The higher count in microbial populations observed in atrazine contaminated soil subjected to bioaugmentation, biostimulation and the combination of both may be due to increased level of nutrient which stimulated increase in microbial activities and microbial population as well as increased level of organic compound degrading organisms and activities thus resulted to high carbon demand by the degrading microbes (Agarry *et al*., 2013).

Evaluation of Atrazine Biodegradation Kinetics and Half-Life: First-order kinetics model equation (Eq. (1)) fitted to the biodegradation data (Figure 1) was used to determine the rate of atrazine biodegradation in the various bioremediation treatments.
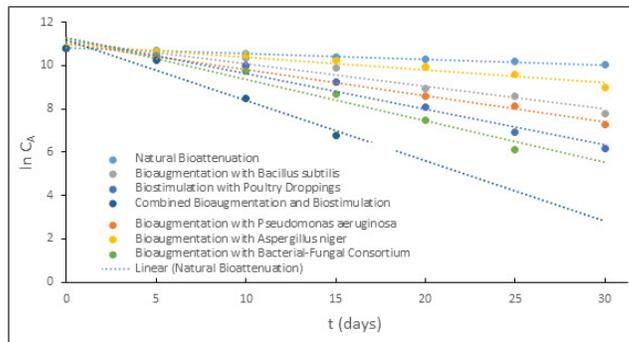
Fig 3. First-order kinetic model fitted to the atrazine biodegradation data under the influence of natural bioattenuation, bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger*, biostimulation with poultry droppings, bioaugmentation with bacterial-fungal consortium and combined bioaugmentation and biostimulation.

The values of the biodegradation rate constants (k_1) obtained from fitting of the model are presented in Table 2.

Table 2: First-order kinetic equation with correlation determination (R^2) results of atrazine herbicide biodegradation under the influence of bioaugmentation and biostimulation

Bioremediation Strategy	First-Order Kinetic Equation	k_1 day^{-1})	R^2	$t_{1/2}$ (days)
Natural Bioattenuation	$C_A = -0.026\,t + 10.82$	0.026	0.9948	26.7
Bioaugmentation with *Pseudomonas aeruginosa*	$C_A = -0.120\,t + 11.04$	0.120	0.9874	5.8
Bioaugmentation with *Bacillus subtilis*	$C_A = -0.104\,t + 11.12$	0.104	0.9605	6.7
Bioaugmentation with *Aspergillus niger*	$C_A = -0.059\,t + 10.99$	0.059	0.9457	11.7
Bioaugmentation with Bacterial-Fungal Consortium	$C_A = -0.191\,t + 11.26$	0.191	0.9642	3.6
Biostimulation with Poultry Droppings	$C_A = -0.164\,t + 11.27$	0.164	0.9680	4.2
Combined Bioaugmentation and Biostimulation	$C_A = -0.279t + 11.17$	*0.279*	*0.9605*	*2.5*

The results in Table 2 as shown by the relatively high coefficient of determination (R^2) values that ranges between 0.94 and 0.99 revealed that atrazine biodegradation adequately fitted well to the first-order kinetic model. The half-life time ($t_{1/2}$) of atrazine biodegradation was calculated using Eq. (2). The values of k and $t_{1/2}$ for the different bioremediation treatments are presented in Table 1. Higher k value indicates higher or faster rate of biodegradation and consequently lower half-life time. It is seen from Table 1 that for soil augmented with the mixture of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger* and poultry droppings (i.e. combined bioaugmentation and biostimulation) had a higher k (0.279 day^{-1}) and lower $t_{1/2}$ (2.5 days) and this is followed by the soil augmented with bacterial-fungal consortium which consists of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Aspergillus niger* (k = 0.191 day^{-1} and $t_{1/2}$ = 3.6 days), poultry droppings (i.e. Biostimulation) (k = 0.164 day^{-1} and $t_{1/2}$ = 4.2

days), *Pseudomonas aeruginosa* (k = 0.120 day^{-1} and $t_{1/2}$ = 5.8 days), *Bacillus subtilis* (k = 0.104 day^{-1} and $t_{1/2}$ = 6.7 days), and *Aspergillus niger* (k = 0.059 day^{-1} and $t_{1/2}$ = 11.7 days), respectively. A k value of 0.026 day^{-1} and $t_{1/2}$ of 26.7 days was obtained for atrazine biodegradation in the non-augmented soil (natural bioattenuation). The k value is lower and the $t_{1/2}$ value is higher than the values obtained for the atrazine contaminated soil subjected to either bioaugmentation, biostimulation or both. This observation indicates that atrazine biodegradation in soil undergoing natural bioattenuation (bioremediation) is slower and with a higher biodegradation half-life time.

Effectiveness of the Different Bioremediation Strategies: A one-way Analysis of Variance (ANOVA) was carried out to compare the atrazine biodegradation efficiency of the different bioremediation strategies adopted in this study and the result is presented in Table 3.

Table 3: Analysis of variance (ANOVA) for the different bioremediation strategies

Source	Sum of squares	Degree of freedom	Mean of squares	*F*-value	*P*-value
Treatment	0.1307	6	0.0218	6929.27	1.11E-16
Error	0.000	14	0.000		
Total	0.1307	20			

The result suggests that all the bioremediation strategies (natural bioatenuation, bioaugmentation, biostimulation, and combined bioaugmentation and biostimulation) had a statistically significant influence on the atrazine biodegradation in soil at the 5% probability level (p = 0.05). Therefore, the effectiveness of each strategies was tested. Through evaluation of the rate constant of atrazine biodegradation in non-augmented soil (natural bioattenuation) and augmented soil (bioaugmentation and/or biostimulation), the bioremediation efficiency

(E (%)) was calculated at the end of 30-day bioremediation period using Eq. (3):

$$E\ (\%) = \frac{k_{(Aug)} - k_{(Non-A)}}{k_{(Aug)}} \times 100 \quad (3)$$

Where, $k_{(Aug)}$ is the rate constant of atrazine biodegradation in the augmented soil, and $k_{(Non-A)}$, the rate constant of atrazine biodegradation in the non-augmented soil. The E (%) results are shown in Table 4.

Table 4: Percentage biodegradation of atrazine and bioremediation efficiency of the different bioremediation strategies at the end of 30 days

Bioremediation Strategy	k (day⁻¹)	E (%)
Natural Bioattenuation	0.026	-
Bioaugmentation with *Pseudomonas aeruginosa*	0.120	78.3
Bioaugmentation with *Bacillus subtilis*	0.104	75
Bioaugmentation with *Aspergillus niger*	0.059	56
Biostimulation with Poultry Droppings	0.164	84.1
Bioaugmentation with Bacterial-Fungal Consortium	0.191	86.4
Combined Bioaugmentation and Biostimulation	0.279	90.7

The results in Table 4 generally revealed that there are relative differences in the bioremediation efficiencies (E (%)) of the different strategies (bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, biostimulation with poultry droppings, bioaugmentation with bacterial-fungal consortium and combined bioaugmentation and biostimulation) utilized in the soil bioremediation of atrazine.

Nevertheless, to actually determine the significant difference in the bioremediation efficiencies, post hoc comparisons using Tukey's (HSD) test at 5% probability level were out between the different strategies.

The difference in mean k (rate constant) between pairs of strategies was greater than the HSD value, hence, the grouping of mean k using the Tukey's test for the different bioremediation strategies as presented in Table 5 reveals that there are significant differences in the biodegradation rate and thus in the bioremediation efficiencies of natural bioattenuation, bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger*,

biostimulation with poultry droppings, bioaugmentation with bacterial-fungal consortium and combined bioaugmentation and biostimulation.

The Tukey's test showed that there are significant differences in the bioremediation efficiencies between each of the different bioremediation strategies (natural bioattenuation, bioaugmentation with *Pseudomonas aeruginosa*, bioaugmentation with *Bacillus subtilis*, bioaugmentation with *Aspergillus niger,* biostimulation with poultry droppings, bioaugmentation with bacterial-fungal consortium and combined bioaugmentation and biostimulation).

Therefore, in comparison between the different bioremediation strategies, combined bioaugmentation and biostimulation suggests to be relatively more effective with higher E (%) (90.7%).

This is followed by bioaugmentation with bacterial-fungal consortium (86.4%), biostimulation with poultry droppings (84.1%), bioaugmentation with *Pseudomonas aeruginosa* (78.3%), bioaugmentation with *Bacillus subtilis* (75%) and bioaugmentation with *Aspergillus niger* (56%), respectively.

Table 5: Grouping of k mean for the different bioremediation strategies computed by Tukey's method

Bioremediation Strategy	k_1 Mean[*]	Standard Deviation	P-Value
Natural Bioattenuation	0.026[A]	0.0006	$p < 0.01$
Bioaugmentation with *Pseudomonas aeruginosa*	0.120[B]	0.0012	$p < 0.01$
Bioaugmentation with *Bacillus subtilis*	0.104[C]	0.0006	$p < 0.01$
Bioaugmentation with *Aspergillus niger*	0.059[D]	0.0012	$p < 0.01$
Bioaugmentation with Bacterial-Fungal Consortium	0.191[E]	0.0012	$p < 0.01$
Biostimulation with Poultry Droppings	0.164[F]	0.0012	$p < 0.01$
Combined Bioaugmentation and Biostimulation	0.279[G]	0.0012	$p < 0.01$

[*]*Means that do not share the same letter are significantly different*

Conclusions: From this study, it can be concluded that the use of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger* and poultry droppings as bioaugmentation and biostimulation agents accelerates or enhances the rate of atrazine biodegradation in soil and thus its removal from contaminated soil. Complete atrazine biodegradation or removal with higher biodegradation rate and lower biodegradation half-life can be attained at reduced remediation time using combined bioaugmentation and biostimulation strategy as compared to bioaugmentation or biostimulation alone.

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