

RESEARCH ARTICLE

ECOTOXICOLOGICAL RISK EVALUATION OF HERBICIDES ON NON-TARGET ENVIRONMENTAL RECEPTORS

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ABSTRACT

Globally, with the application of over 4 billion tons of herbicides yearly to suppress or kill weeds, the uncontrollable / unregulated use and disposal of these sometimes-recalcitrant chemicals could result in deleterious effects on non-target ecological receptors. This evaluation recorded varying degree of toxic effects on the environmental receptors (onions - *Allium cepa* L) and (snails - *Archachatina marginata*). The highest effective concentration (EC₅₀) for onions was recorded in Dragon® (0.042 ± 0.008 mg L⁻¹) while the lowest effective concentration was in Uproot® (2.185 ± 0.52 mg L⁻¹). Similarly, the highest EC₅₀ in the snail test was obtained in Cotrazine® (0.41 ± 0.012 mg kg⁻¹) and the lowest in Striker® (9.51 ± 0.94 mg kg⁻¹). The risk level estimated using the Ecotoxicological Risk Assessment Matrix (ERAM) revealed that the least toxic herbicide were rated E 3 (P;A;E;C) or 15 (P;A;E;C) while the most toxic herbicides were rated E 5 (P;A;E;C) or 25 (P;A;E;C), which was considered medium to high risk to plants (P), animal (A), the environment (E) and community (C). The use, handling and disposal of these lethal herbicides should be prudently managed so as to safe guide non-target species in the environment of application.

KEYWORDS

Allium cepa L, *Archachatina marginata*, chemical stressors, ecological species, risk matrix

1. INTRODUCTION

In Africa and most regions of the world, farming is a major occupation and means of livelihood especially among rural dwellers. However, the recent fluctuation experienced by farmers in plant yield due to invasion of weeds in some of these regions has led farmers to resort to chemical weed control. Globally, the class of pesticides most universally used are herbicides, which have extensive applications to suppress or kill unwanted vegetation (weeds). They have acquired prominence over the last few decades as the main strategy weed control for both agricultural practices and non-agricultural purposes (Sandier, 2010; Ogeleka et al., 2016).

Herbicides are versatile materials that provide a myriad of benefits including food, material, crop protection and disease control amongst others. There are two types of herbicides - selective and non-selective. Selective herbicides suppress or kill specific types of plants (i.e. they impact certain targets and leave the preferred crop undamaged). On the other hand, non-selective herbicides suppress or inhibit all plants they come in contact with (i.e. they destroy a very wide range of plant) (Baker et al., 2002). Herbicide usage in developing countries is often spurious and prodigious with little or no consideration for environmental and public health safety. Herbicides are essential in agriculture, however, under certain situations they could act as toxicants that can affect non-targeted organisms (plants, animals, human) and environment (air, water, soil) due to indiscriminate use and disposal (Beckie et al., 2006). The inherent toxicity herbicides pose have been evaluated by some researchers who Prominent herbicides commonly used in Nigeria include: Dragon®

found that herbicides could actually build up in soil to harmful levels and affect organisms and humans (Hayes et al., 2010, 2011).

The negative effects generated by herbicides on human health and the environment due to lack of knowledge with respect to safety limitations on the part of end users have made herbicides use in farming, one of the most controversial issues today (Hayes, 2006). The first warning signal of the unintended environmental impact of pesticides was given (Carson, 1962). Silent spring remained one of the most effective denunciations of industrial malpractices ever written and is widely attributed to activating popular environmental awareness in the United States and Europe (Mufflin, 2012). However, pesticides usage has more than doubled since silent spring. It is on record that more than 4 million kilograms of herbicides are applied each year (USEPA, 2015). Some major constraint resulting from the use and subsequent disposal of herbicides include:

- The unrestricted access to herbicides by farmers and non-farmers with little or no background knowledge of the chemicals is a problem in developing countries.
 - The lack of regulatory / implementation policy on the use, handling and disposal of herbicides.
 - The lack of adequate knowledge by the users concerning safety measures.
 - The indiscriminate combination of different herbicides and repeated prodigious applications of herbicides.
 - The potential of persistence of herbicides in the environment and consequent impact on biodiversity.
- (Paraquat Dichloride), Dazzle® (Glyphosate), Cotrazine® (Altrazine) and

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Force Uron® (Diuron) amongst other classes of herbicides. The major choice of *Allium Cepa L* in this study include the fact that it is a very important edible crop highly consumed by humans globally, used as seasoning and is relatively abundant with very high sensitivity to toxicants and can be cultivated all the year round. In addition, onions are weak against weed due to their nature of poor competitiveness, initial slow growth, their inability to have appropriate foliage and their cylindrical upright leaves (Wick et al., 1973; Ghosheh, 2004; Carlson and Kirby, 2005; Qasem, 2006). Similarly, *Archachatina marginata* was chosen because the species are easy to collect and identify. They are abundant all the year round and can be cultured in controlled environment. Most importantly, like onions, they are consumed by humans and other higher organisms, getting a high percentage of protein from them.

The habitat which supports organisms and natural resources could be adversely contaminated following the indiscriminate application of herbicides and wrong agricultural practices, and this could impact or threaten vulnerable environmental species, vegetation and human lives.

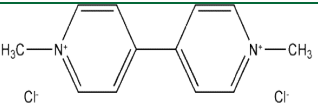
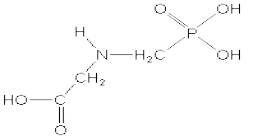
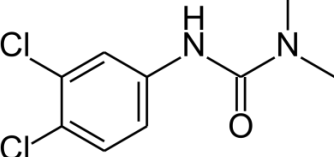
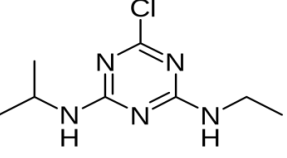
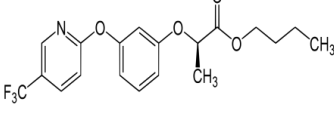
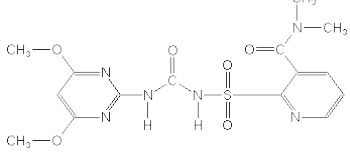
Thus the study focused on evaluating the deleterious effects of some commonly used selective and non-selective herbicides on non-target environmental receptors - *Allium cepa L* and *Archachatina marginata* using the Ecotoxicological Risk Assessment Matrix (ERAM).

2. MATERIALS AND METHODS

2.1 Test Chemicals (Toxicants)

Ten (10) herbicides including selective and non-selective were used as test chemicals for the bioassay. The active components, formulation and class of the herbicides are displayed in Table 1. Stock concentrations (1000 mg L⁻¹) of the different herbicides were prepared and serially diluted to obtain the concentrations used in the definitive bioassessment. As weed killer, these chemicals are widely and currently applied by farmers in the Niger Delta and most parts of the world.

Table 1: Common herbicides and their active ingredients used for the study

S/N	Trade name	Active components	Class	Formulation	Structure
1	ParaEforce®	Paraquat Dichloride	Non-selective, contact, post emergent herbicide	276 g of paraquat dichloride (200g Paraquat)/L SL	
2	Dragon®	Paraquat Dichloride	Non-selective, contact, post emergent herbicide	Paraquat dichloride 276 g/L	Ditto
3	Uproot®	Glyphosate	Non-selective, systemic, post emergent herbicide	360 g/L of Glyphosate in the form of 480g/Lt Isopropylamine salt	
4	Dazzle®	Glyphosate	Non-selective, systemic, post emergent herbicide	360 g Glyphosate (Glyphosate-Isopropylamine) 41%SL	Ditto
5	BushKlear®	Glyphosate	Non-selective, systemic, post emergent herbicide	Isopropylamine salt of Glyphosate – 41% Inert materials 59% = 100%	Ditto
6	ForceUron®	Diuron	Selective systemic post emergent herbicides	Diuron 80% W.P	
7	Rooter®	Diuron	Selective, systemic, Pre-emergent and post-emergent herbicides	Diuron 80% W.P	Ditto
8	Cotrazine®	Atrazine	Selective, systemic, pre-emergent and post-emergent herbicides	Atrazine 80% W.P/Atrazine 860g 1kg as W.P	
9	StarForce®	Fluazifop-p-butyl	Selective, systemic, post emergent herbicides	Fluazifop-p-butyl 150 g/L EC	
10	Striker®	Nicosulfuron	Selective , systemic: post emergent herbicide	Nicosulfuron 40g/L	

2.2 Test species

The test species used for the assessment are onions (*Allium cepa* L) and snails (*Archachatina marginata*) conditioned in the laboratory. *Allium cepa* L is an important edible vegetable crops used globally for seasoning and as an antioxidant while *Archachatina marginata* are used as biological indicators for estimating the ecological damage to soil and other environmental media since a major aspect of their cycle occurs in soils, including metabolic activities and reproduction.

2.3 *Allium cepa* root tip assay

The Organization for Economic Co-operation and Development, (OECD) protocol #208 was applied for root growth inhibition bioassay (OECD 2003a; Olorunfemi et al., 2012). *Allium cepa* L., (2n = 16) of average weight and length of 73.10 ± 0.24 g and 6.30 ± 0.07 cm respectively previously air-dried for six weeks was prepared by exposing the fresh meristematic tissues after removing the dead root ends (Plate 1). The bio-assessment for root growth inhibition commenced with a range-finding test to establish the amount to be used for the actual (definitive) test.

The exposure concentrations were arrived at after the range-finding experiment for each test herbicide. The acute toxicity biotest was carried out in the dark for 96 hours. The roots of each onion bulb were removed with a forcep and the root growth measured and used to determine the EC₅₀ (the effective concentration is the concentration that inhibits 50% root growth on exposure to the toxicant) after subjecting the evaluation to a Probit analysis (Finney, 1971).



Plate 1: *Allium cepa* Linn (Source: sakata.co.za)

2.4 *Archachatina marginata* bioassay

Archachatina marginata of length 1.23 ± 0.5 cm and weight 0.81 ± 0.05 g were collected from Songhai farms (cultured) in Delta State at a coordinate of N05° 51' 0.29" and E005° 44' 36.71" (Plate 2). Songhai, Delta is located along the Amukpe-Eku road and is approximately 7 km from the center of Sapele town, in the Delta State and this pristine site is very strategically positioned. The bioassay was performed using the guidelines for snail toxicity test as recommended by International Organization for Standardization (ISO), # 15952 (ISO 2006).

The test organisms - juveniles of seven days old were acclimated for one week in an unspiked soil. During the period of acclimation, the species were fed with cellulose in order to prevent starvation. The bioassay began with a preliminary test to determine the working concentration done by obtaining the least concentration that had no effect and the maximum concentration that would result in 100% death. This formed the basis for the concentrations used in the definitive (actual) bioassay.

For each of the test concentrations, 1000 g (1 kg) of natural soil from the organism's habitat was placed in the test vessel. Five (5 g) of cellulose was mixed into the soil as food for the organisms. Thereafter, 100 mL of each test herbicide was introduced into the vessel containing the soil substrate to make the corresponding concentrations. After attenuation of the soils and the test toxicants, ten (10) juvenile snails were placed in each test vessel. For the five exposure concentrations, three replicates per treatment was prepared and control setup (water) without the test toxicants was prepared along with the other treatment. Assessment for mortality count was evaluated on the 7th and 14th day. The exposed species were regarded

dead when organisms are prodded at the foot (pedal) region with a pointed metal rod and there was no movement of the snail or if after 5 minutes there was no activity of the organisms on placement on moist filter paper.



Plate 2: The giant African Snail (*Archachatina marginata*) - (Source: en.wikipedia.org)

Table 2: Toxicity rating		
Ratings		EC50 (mg/kg)
5	Very highly toxic	< 0.1
4	Highly toxic	0.1-1
3	Moderately toxic	1-10
2	Slightly toxic	10-100
1	Practically non-toxic	100-1000
0	Non-hazardous	> 1000

2.5 Growth rate evaluation

The growth rate, percentage growth rate relative to control and the percentage growth inhibition efficiency of the metals were calculated using the equations (1) – (3) (Owodeinde et al., 2017).

$$\text{Growth rate (cm/hr)} = \frac{\text{mean length}}{\text{time}} \quad (1)$$

$$\text{Percent growth rate (\%)} = \left(\frac{GR_s}{GR_c} \right) \times 100 \quad (2)$$

$$\text{Percent growth rate relative to control (\%)} = \frac{GR_c - GR_s}{GR_c} \times 100 \quad (3)$$

Where:

GR_s = growth rate for sample

GR_c = growth rate for control

2.6 Ecotoxicological risk assessment (ERA) of herbicides

On the Ecotoxicological Risk Assessment Matrix (ERAM), risk levels can be classified as low, medium, or high (Table 3). If an environment is contaminated with toxicants (pollutants), animals (A), plants (P), environment (E) and community (C) may be affected. Factors considered for classification include: exposure concentration, exposure duration and potency of the toxicant amongst others. The risk levels are categorized in a numbered format (USEPA 2015; Ogeleka et al., 2017). Each hazard is given a rating, and this was multiplied by the probability that these hazards would occur using the relationship:

Risk level = Hazard severity x likelihood of exposure (Table 6).

Hazard severity are rated as 1 (slight effect), 2 (minor effect), 3 (localized effect or damage), 4 [major effect (deaths)] and 5 [extensive effect (death of population)]. Similarly, the likelihood of occurrence or exposure are rated as 1 (seldom – A - yearly), 2 (frequent – B - quarterly), 3 (very likely – C - monthly), 4 (near certain – D - weekly) and 5 (certain – E - daily) (SETAC 1997; USEPA 2015).

2.7 Statistical analysis

The effective concentration (EC₅₀) and ecological risk assessment was used to ascertain the proneness of *Allium cepa* L and *Archachatina marginata*

exposed to the test herbicides for the specific duration. Statistical analysis to establish the variation between the controls and the treatment groups at significance level of $P = .05$ was performed using the one-way analysis of variance (ANOVA).

3. RESULTS

The results of the toxic impact of herbicides exposed to *Allium cepa L* and *Archachatina marginata* are presented in Tables 4 – 6. The Figures 1-2 showed the most toxic herbicide exposed to the two test species.

Table 3: Ecotoxicological Risk Assessment Matrix (ERAM)

Table 3: Ecotoxicological Risk Assessment Matrix (ERAM)											
SEVERITY	CONSEQUENCE						INCREASING PROBABILITY				
			P	A	E	C	A	B	C	D	E
							Never experience the chemical in the area	Had been exposed / used in the area	Had been exposed / used in the area and other locations	Had been exposed / used several times in the area	Had been exposed / used several times in the area and other locations
0	Practically non-toxic	>1000	No injury	No effect	No effect	No impact	Area 1	Area 2	Area 3	Area 4	Area 5
1	Practically non-toxic	>1000	Slight injury	Slight effect	Slight effect	Slight impact					
2	Slightly toxic	100-1000	Minor injury	Minor effect	Minor effect	Limited impact					
3	Very toxic	10-100	Major injury	Localized effect	Localized effect	Considerable impact					
4	Extremely toxic	1.0-10	Single fatality	Major effect (deaths)	Major effect	National impact					
5	Super toxic	<1.0	Multiple fatality	Extensive effect (kills)	Massive effect	International impact					

Abbreviations: LC50 median lethal concentration in ppm. Data from (OECD, 2003b; GESAMP, (1997), Ogeleka *et al.* (2017)

Table 4: Effective concentration (EC₅₀) values of herbicides exposed to *Allium cepa L*

Test Chemical	Line equation (Y – value)	EC ₅₀	Rating
BushKlear®	y = -37.193x + 53.99	1.279 ± 0.16	Moderately toxic
Dazzle®	y = -43.834x + 61.83	1.861 ± 0.21	Moderately toxic
Dragon®	y = -43.854x - 10.4	0.042 ± 0.008	Very highly toxic
ParaEforce®	y = -30.233x + 7.3	0.197 ± 0.06	Highly toxic
Uproot®	y = -39.517x + 63.414	2.185 ± 0.52	Moderately toxic
Cotrazine®	y = -47.664x + 41.223	0.636 ± 0.042	Highly toxic
ForceUron®	y = -42.527x + 46.716	0.169 ± 0.024	Very highly toxic
Rooter®	y = -44.841x + 49.437	0.971 ± 0.11	Highly toxic
StarForce®	y = -71.096x + 5.6	0.237 ± 0.024	Highly toxic
Striker®	y = -43.522x + 32.4	0.394 ± 0.092	Highly toxic

Data were processed and expressed as mean ± SD based on three replicates

Data were processed and expressed as mean ± SD based on three replicates. The phytotoxicity of the herbicides exposed to *Allium cepa L* varied from moderately toxic to very highly toxic while effects on *Archachatina marginata* was moderately toxic to highly toxic based on the OECD rating (OECD 2003b). The effective concentration EC₅₀ derived from the *Allium cepa L* bioassay varied from 0.042 ± 0.008 mg L⁻¹ (Dragon®) to 2.185 ± 0.52 mg L⁻¹ (Uproot®) while exposure to *Archachatina marginata* resulted in the highest (0.41 ± 0.012 mg kg⁻¹) (Cotrazine®) to the lowest (9.51 ± 0.94 mg kg⁻¹) (Striker®) influence on the snail species (Table 4 and 5).

For the ecotoxicology risk assessment for the study, the application of herbicides vary according to the type of plant or the weed intended to be eliminated. In addition, the frequency of application by farmers and agriculturists was determined based on the daily uptake by the species in the environment upon application. Thus, the release of a herbicide (for

instance Dragon®) (hazard) into the environment could result in major damage or death (a severity with a rating of 5. Following the exposure, the frequency of application will then be considered. For a risk level following a hazard severity of 5 and a daily uptake for 4 days and 14 days (*Allium cepa L* and *Archachatina marginata* respectively), a frequency (daily) is assigned E. The risk level of Dragon® by the plant species would be 5 x E and represented as 5 E (P; E; C) or 25 (P; E; C) (very highly toxic - EC₅₀ = 0.042 ± 0.008 mg L⁻¹) while the risk level for animal (for Cotrazine®) could be given as 4 E (A; E; C) or 20 (A; E; C) (highly toxic - EC₅₀ = 0.41 ± 0.012 mg kg⁻¹ (Figure 1-2). The risk levels, hazard rating, ecological influences for the other herbicides are listed in Tables 6 - 9.

Table 5: Effective concentration (EC₅₀) values of herbicides exposed to *Archachatina marginata*

Test Chemical	Line equation (Y – value)	EC ₅₀	Rating
BushKlear®	y = 14.137x + 41.722	3.85 ± 0.84	Moderately toxic
Dazzle®	y = 23.44x + 30.78	4.74 ± 0.69	Moderately toxic
Dragon®	y = 25.911x + 54.80	0.66 ± 0.05	Highly toxic
ParaEforce®	y = 34.216x + 60	0.51 ± 0.011	Highly toxic
Uproot®	y = 35.907x + 40.512	1.84 ± 0.82	Moderately toxic
Cotrazine®	y = 37.87x + 64.8	0.41 ± 0.012	Highly toxic
ForceUron®	y = 28.569x + 59.2	0.48 ± 0.027	Highly toxic
Rooter®	y = 31.226x + 58.8	0.52 ± 0.026	Highly toxic
StarForce®	y = 47.277x + 29.218	2.75 ± 0.48	Moderately toxic
Striker®	y = 22.298x + 21.81	9.51 ± 0.94	Moderately toxic

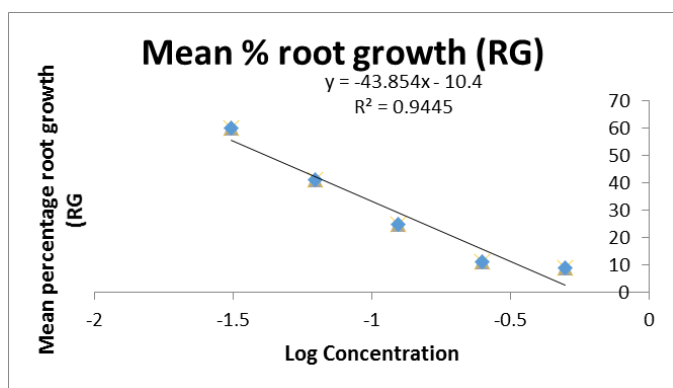
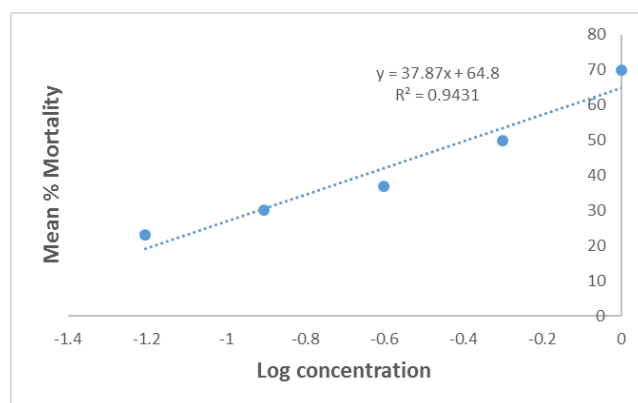

 Figure 1: Mean % root growth (RG) for *Allium cepa* L in Dragon®

 Figure 2: Mean % mortality for *Archachatina marginata* in Cotrazine®

 Table 6: Ecotoxicology Risk Assessment for the *Allium cepa* L bioassessment

Types of herbicide	EC50 rating	Frequency of exposure (daily for 4 days) (a)	Hazard severity (b)	Risk level (a X b)	Hazard rating
BushKlear®	Moderately toxic	E	3	E3	E3 or 15 (P,E,C)
Dazzle®	Moderately toxic	E	3	E3	E3 or 15 (P,E,C)
Dragon®	Very highly toxic	E	5	E5	E5 or 25 (P,E,C)
ParaEforce®	Highly toxic	E	4	E4	E4 or 20 (P,E,C)
Uproot®	Moderately toxic	E	3	E3	E3 or 15 (P,E,C)
Cotrazine®	Highly toxic	E	4	E4	E4 or 20 (P,E,C)
ForceUron®	Very highly toxic	E	5	E5	E5 or 25 (P,E,C)
Rooter®	Highly toxic	E	4	E4	E4 or 20 (P,E,C)
StarForce®	Highly toxic	E	4	E4	E4 or 20 (P,E,C)
Striker®	Highly toxic	E	4	E4	E4 or 20 (P,E,C)

 Table 7: Ecotoxicology Risk Assessment for the *Archachatina marginata* bioassessment

Types of herbicide	EC50 rating	Frequency of exposure (daily for 14 days) (a)	Hazard severity (b)	Risk level (a X b)	Hazard rating
BushKlear®	Moderately toxic	E	3	E3	E3 or 15 (A,E,C)
Dazzle®	Moderately toxic	E	3	E3	E3 or 15 (A,E,C)
Dragon®	Highly toxic	E	4	E4	E4 or 20 (A,E,C)
ParaEforce®	Highly toxic	E	4	E4	E4 or 20 (A,E,C)
Uproot®	Moderately toxic	E	3	E3	E3 or 15 (A,E,C)
Cotrazine®	Highly toxic	E	4	E4	E4 or 20 (A,E,C)
ForceUron®	Highly toxic	E	4	E4	E4 or 20 (A,E,C)
Rooter®	Highly toxic	E	4	E4	E4 or 20 (A,E,C)
StarForce®	Moderately toxic	E	3	E3	E3 or 15 (A,E,C)
Striker®	Moderately toxic	E	3	E3	E3 or 15 (A,E,C)

 Table 8: Consequences of the effects of herbicides using ecological risk assessment matrix (ERAM) for *Allium cepa* L bioassessment

Types of herbicide	Consequences	Environment (E)	Community (C)	Inference
	Plant (P)			
BushKlear®	Major injury	Localized effect	Considerable impact	Moderately toxic
Dazzle®	Major injury	Localized effect	Considerable impact	Moderately toxic
Dragon®	Multiple fatality	Massive effect	International impact	Very highly toxic
ParaEforce®	Single fatality	Major effect	National impact	Highly toxic
Uproot®	Major injury	Localized effect	Considerable impact	Moderately toxic
Cotrazine®	Single fatality	Major effect	National impact	Highly toxic
ForceUron®	Multiple fatality	Massive effect	International impact	Very highly toxic
Rooter®	Single fatality	Major effect	National impact	Highly toxic
StarForce®	Single fatality	Major effect	National impact	Highly toxic
Striker®	Single fatality	Major effect	National impact	Highly toxic

Table 9: Consequences of the effects of herbicides using ecological risk assessment matrix (ERAM) for *Archachatina marginata* bioassessment

Types of herbicide	Consequences			Inference
	Animal (A)	Environment (E)	Community (C)	
BushKlear®	Localized effect	Localized effect	Considerable impact	Moderately toxic
Dazzle®	Localized effect	Localized effect	Considerable impact	Moderately toxic
Dragon®	Major effect (deaths)	Major effect	National impact	Highly toxic
ParaEforce®	Major effect (deaths)	Major effect	National impact	Highly toxic
Uproot®	Localized effect	Localized effect	Considerable impact	Moderately toxic
Cotrazine®	Major effect (deaths)	Major effect	National impact	Highly toxic
ForceUron®	Major effect (deaths)	Major effect	National impact	Highly toxic
Rooter®	Major effect (deaths)	Major effect	National impact	Highly toxic
StarForce®	Localized effect	Localized effect	Considerable impact	Moderately toxic
Striker®	Localized effect	Localized effect	Considerable impact	Moderately toxic

4. DISCUSSION

The application and disposal of herbicides commonly used on soils and agricultural premise cannot be underestimated. The benefits are important and enormous, however, deleterious consequences resulting from inappropriate dose or application of these chemicals could lead to loss of environmental non-target biodiversity. It has been reported that 70% of paraquat poisoning resulted in inhibition and death of exposed species (Zain et al., 2013). Paraquat dichloride, an organochlorine based herbicide (1,1-dimethyl-4,4-bpyridinium dichloride) represented in this assessment as Dragon® and ParaEforce® acts by inhibiting photosynthesis. These herbicides accept electrons and generate extremely reactive hydroxyl radicals, which destroy cell membrane. The cells leaks and result to sudden leaf leaflessness and desiccation (dryness or wilting), and eventually plant death (Summers, 1980; WSSA, 1994; Roberts et al., 2002; Sebiomo et al., 2011; Oyeyiola et al., 2017). The data from this assessment for the two paraquat-based herbicides (Dragon® and ParaEforce®) are in conformance with that reported by Summers, 1980, Song, 2014 and more recently (Oyeyiola et al., 2017).

Glyphosate is been regarded by producers as a herbicide with low toxicity and is environmentally friendly, which can be viewed as a silver bullet when handling undesired plants (WHO/UNEP/ILO 1994; Cox 1998; 2001). The United State Environmental Protection Agency (USEPA), termed glyphosate as extremely persistent with a half-life of over 100 days (USEPA 1986). Glyphosate has shown from many studies to pose a variety of health and environmental hazards. As a non-selective herbicide, it has lethal toxic effects on lots of plant species and induces serious effects such as reduced plant / seed quality, reduced metabolic activities, inhibit growth and causes death of target / non-target biodiversity (Zabaloy et al., 2008; Ogeleka et al., 2016; Ani et al., 2017). Considering the mode of its reactions in plants, glyphosate (phosphonomethyl) glycine organophosphorus based-herbicide shikimate pathway yields the amino acids - tyrosine, phenylalanine and tryptophan. The herbicide inhibits by disrupting the enzymatic pathway thus, preventing energy and other metabolic processes from taking place and eventually leading to the death of the plant (Qasem 2006; Cavusoglu et al., 2011; Jawale et al., 2017). The data for the glyphosate test herbicides - Bushklear®, Dazzle® and Uproot® indicated toxicity and harm to plants and animals assessed in this appraisal.

In the same way, atrazine-based selective herbicides (1-chloro-3-ethylamino-5-isopropylamine-2,4,6-triazine) have been found to cause hermaphroditism and chemoreceptor alteration in exposed organisms. Atrazine inhibits photosynthesis in vulnerable plants, which die as a result of starvation and oxidative impairment (stress) generated in the electron reaction process (Jooste et al., 2005a; Hayes et al., 2010; 2011). Effective concentrations reported for this study showed that the atrazine-based herbicide (Cotrazine®) recorded toxic effect in plant and animal, in line with studies reported (Hayes et al., 2006; 2010; 2011; Cox, 2001; Jooste et al., 2005a; 2005b).

It has long been reported that diuron-based herbicides have been found to cause methemoglobin, oxidative stress and endocrine damage in organisms (Wang, 2004; Ezemonye and Tongo, 2010). In its reactive mode like other herbicides, the diuron - phenylurea herbicide (3-(3,4-dichlorophenyl)-1,1- dimethylurea) can be regarded as an inhibitor of photosynthesis. In this way, it disturbs the photosynthetic pathway and this reduces the capability of the plant to convert light energy into chemical energy - adenosine triphosphate (ATP). Force Uron® and Rooter® which are diuron-based herbicides assessed in this research showed very highly toxic to highly toxic rating in plant and moderate toxicity in animals (Metz et al., 1986; Singh and Singh, 2015).

The nicosulphuron - sulphonylurea herbicides [2-(4,6-dimethoxypyrimidin-2-ylcarbamoyl sulphamoyl)-N, N-

dimethylnicotinamide] control weeds by inhibiting acetolactate synthase (ALS). Acetolactase synthase is a key enzyme in the production of three amino acids namely isoleucine, valine and leucine. Once weeds are treated with the herbicide - nicosulphuron, they first begin to develop chlorosis and gradually, as the weed continues to be starved of the essential amino acids, the chlorosis symptomology (yellowing of plant due to a lack of chlorophyll development) will be more pronounced and this will result into necrosis (plant death) (Brown, 2000). This type of herbicide inhibited the growth of plants in studies reported by some authors was in agreement with the data presented in this study for Striker®, together with remarkable effects noted in the snail bioassay (Brown, 2000; Beckie et al., 2006).

Similarly, for the fluzafop-butyl ester - phenoxy herbicides - [Butyl 2-(4-[[5-(trifluoromethyl) pyridin-2-yl]oxyphenoxy]propanoate] (Starforce® in this study), mimic the natural auxin (indole-3-acetic acid - IAA) at the cellular and molecular level. Natural auxins are important phytohormones consisting of IAA, which plays a vital role in the growth of plant. These herbicides attacks the plant and as a result, the leaves and stems twist, thicken and elongate, resulting to plant death. Starforce® was highly toxic to *Allium cepa* L and moderately toxic to *Archachatina marginata* (Song, 2014).

Previous studies have reported conflicting results and information of some commonly used herbicides on non-target species leading to controversies in these harmful but useful chemicals. For instance, in their study noted that frog exposed to levels of atrazine 30 times below the recommend concentrations by the United State ($0.1 \mu\text{g L}^{-1}$ to $30 \mu\text{g L}^{-1}$) in water either showed hermaphroditism, were incapable of reproducing or died (Hayes et al., 2011). Similarly, in the study, they observed that periwinkles (*Tympanotonous fuscatus var radula*) in environment having significant amount of Bis-Tributyltin Oxide (TBTO) resulted in imposex (a disorder in which the toxicant causes the female organs to develop male sex organs such as penis and vas deferens) thus preventing reproduction (Ogbomida and Ezemonye, 2013).

A group researcher in their study found out that newly hatched snails exposed to 36% of Round-up® - a glyphosate-based herbicide contaminated with soil for 168 days resulted in a decrease in albumen gland development with an inhibition of $43.5 \pm 32.8\%$ (Druart et al., 2011). Similarly, a group researcher concluded from their results that atrazine and glyphosate herbicides used in their study were regarded as highly genotoxic to *Biomphalaria glabrata* snails (Mona et al., 2013). A studied the effects of chlorophyllin on the reproduction and growth of *Lymnaea acuminata* and concluded that there was substantial decrease in prolificacy, hatching capability and survival of the juvenile snails (Singh and Singh, 2015). The data for the snail study in this assessment for the various test herbicides corroborates these researches and those described by other authors including (Snyman et al., 2005; de Vaufléury et al., 2006; Soso et al., 2007; Ogeleka et al., 2017).

In selective herbicides, the target weeds are suppressed with little or no harm to the plant. The crop absorbs and metabolizes the herbicide without experiencing the lethal effects. The selectivity to crop is due to the capacity of the plant to break down the herbicides into inactive metabolites. However, the weeds were not capable of such metabolism, thus, they are killed or controlled by the herbicide, while the non-selective herbicide kills both the crop and the weeds they come in contact with (Cheremisinoff and Rosenfeld, 2010). It should be noted that some herbicides are recalcitrant and do not degrade easily in the environment, these chemicals bioaccumulated in the lipid tissues of vulnerable species, thus leading to damage in the morphology of the organisms, chemoreceptor and endocrine impediment depending on the degree of exposure and the frequency of contact. The frequency and unregulated application of high concentrations of such herbicides can eventually lead to death and

subsequently extinct of a myriad of viable species of animals and plants (WSSA, 1994; Olorunfemi et al., 2012).

The intended target for selective and non-selective herbicides are weed (plants), however, in most exposure, the non-target organisms are the most susceptible when these herbicides are released into recipient environment. The mode of action / reaction with the exposed species have been documented and the data from this study aligned with those of many researchers including (Hayes *et al.*, 2010; 2011; Cavusoglu et al., 2011; Song, 2014; Jawale et al., 2017). Several researches have been conducted on the deleterious consequences of herbicides on non-target environmental receptors but the effects of herbicides on the sensitivity of the organisms are still quite limited despite the indiscriminate use of the herbicides to increase plant production (Cox, 2001; Jooste et al., 2005a; 2005b; Hayes et al., 2006; 2010; 2011; Ezemoye and Tongo, 2010; Ogbomida and Ezemoye, 2013; Ogeleka et al., 2016; Ogeleka et al., 2017; Prosser et al., 2017; Jawale et al., 2017; Ani et al., 2017).

5. CONCLUSION

In this article, the impact of selective and non-selective herbicides to *Allium cepa* L and *Archachatina marginata* was studied and major conclusions are drawn as follows:

In the two species and in all cases for the herbicides, there was high toxic effects, however, effects were more prominent in the selective than the non-selective herbicides. The phytotoxic effects / alterations in the plant species include: inhibited growth, twisting of roots, decolouration of the species, bulb deformation and root damage. The morphology of the animal specimens on exposure at high concentrations were intensely altered. Effects in the highly impacted concentrations include: slow burrowing, weakness, immobility and shell colour alteration. The effect of the herbicides on the test organisms (plant and animal) was considerably different from the control at levels of $P = 0.5$.

The study established data that would be useful in the determination of safe levels of herbicide application to minimize unintended harmful effects on non-target organisms. The data would assist farmers, agriculturists and non-farmers in the appropriate application and disposal of the test herbicides when applied on farms and soils. In addition, the assessment would enable users to safeguard plants, animals and protect humans who are end consumers of a wide variety of these edible species.

Similarly, for further evaluation, the authors recommend amongst others further researches on the synergic effects of a combination of herbicides, since most farmer and un-skilled users tend to combine different herbicides to achieve effective results on weeds inhibition. In addition, the risk associated with each evaluation should be assessed. There is also the need for comparative appraisal of field and laboratory studies.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Doris Fovwe Ogeleka and Felix Ebhodaghe Okieimen designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Beatrice Oghenetega Peretiemo-Clarke managed the analysis of the study and literature searches. All authors read and approved the final manuscript.

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