IMAGE: A MAP OF THE STARS OF THE ORION CONSTELLATION

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ABSTRACT

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Keywords: farm sizes, groundwater, seasons; contamination risk; sanitary survey.

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Lydiah L. Mbula Kitonga[α], Wilkister Nyaora Moturi[σ], Samuel Mutuku Mwonga[ρ] & Isaiah Taabu (Deceased)[Ↄ]

ABSTRACT

Sanitary survey refers to an evaluation and on-site inspection of the physical environment of the water source to identify possible sources of environmental contamination (USEPA, 1999). The information generated by a sanitary survey helps identify existing and potential sanitary risks to the water quality. Groundwater contamination can be as a result of poor sanitation and subsequent leaching from site especially in the vicinity of the well (Rahman, 1996 and Olsen et al., 2002). The aim of the project was to identify and assess the Sanitary Risk Factors (SRF) associated with the wells and subsequently determine the Contamination Risk Score (CRS) as predictors of water quality in different farm sizes. Onsite sanitary survey of the wells and the homesteads were carried out within farms of different sizes through, visual inspection, observations and interviews whereby a score was allocated for a positive answer and no score for a negative answer. There were 11 Sanitary Risk Factors (SRF) adapted to assess the susceptibility of the well water to contamination. The CRS were categorized as Very High Risk (VHR) = 9-11; High Risk (HR) = 6-8; Intermediate Risk (IR) = 3-5; Low Risk (LR) = 0-2. There were highly significant differences in well CRS within the different farm sizes. Wells within the large and medium mixed farm sizes had an Intermediate CRS because most wells are protected and the well vicinity was relatively clean. Wells within the small farm sizes and which were communal shallow water sources, did not have a wall protection and were located down slope. Rain water flowed into these wells damping collected debris and waste into the wells. Although NO_3-N concentrations in the wells did not exceed the statutory guiding limits of 10mg/l, well attributes increase the susceptibility of wells to pollution. The CRS is a predictive factor of well contamination and the most important risk factors to the wells are the well protection constructions and the activities within the well vicinity. There is therefore ,need for local county initiatives to construct protective raised walls at the communal wells and educate communities on aspects of water quality.

Keywords: farm sizes, groundwater, seasons; contamination risk; sanitary survey.

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I. INTRODUCTION

Groundwater is the main source of domestic water supply for the community of Ainabkoi Sub-County and is exploited through shallow wells (Uasin Gishu Integrated Development Plan (UGCIDP), 2013). Groundwater is considered to be more stable in quality, is conveniently available and accessible for the family and wells can be developed at comparatively low costs. However drinking water quality is of major concern in developing countries with regard to microbiological, inorganic contaminants and physico-chemical properties which deteriorate water quality (Sorlini et al., 2003). Communities should have access to safe drinking water as a basic need for health and sustainable development as outlined in the sustainable development goals (SDGs) which focus on ensuring universal and equitable accessibility of

safe water for all by 2030 (6th SDG) (Osborn et al., 2015).

Under natural conditions, fresh water in shallow aquifers has a relatively short residence time, and its chemistry remains practically unchanged under the effect of a set of natural influences such as physical, geographical, geological and hydro-geological factors. However human economic activities can distort this natural balance (Rutkoviene, Kusta, and Èesoniene, 2005). Well characteristics such as well depth, well age, type of well and its structural features, distance from vegetable gardens, and slope of the land, have been found to contribute to the level of pollution in the groundwater (Bruggeman *et al.*, 1995).

Groundwater may become contaminated naturally or from numerous types of anthropogenic activities within the vicinity of the well, as well as inappropriate well construction (Fawcett, 1992). Thus, a poorly organized environment around the homestead, with poultry and livestock kept near the well will have an impact on the pollution of the water of shallow wells. According to Kutra, Kusta, & Rutkoviene, (2002) the distance at which the household premises, the cowsheds, greenhouses, vegetable gardens, pit latrines, dumps and other aggressive sources of pollution can be located and still have an impact on well water quality is 145 meters (Kutra *et al.* 2002). Animal wastes from active or abandoned feedlots may be a significant source of nitrates to groundwater (Kirder, 1987). When manure is stored in open lots for eight months, 7% nitrogen, 14% phosphorus and potassium enter the environment in the form of leachate, resulting in groundwater pollution from the leachate greatly exceeding the maximum allowable concentrations for the area (Kirder, 1987). The direction of groundwater flow also has an important influence on the probability of contamination. A widely-held tenet of groundwater hydrology states that water flows downslope along the gradient of the groundwater surface or water table (Rutkoviene, *et al.*, 2005). This gradient generally conforms to the surface contours. Thus, water quality in wells is highly influenced by pollutants moving from up-slope in the vicinity of the well (Rutkoviene, *et al.*, 2005). Therefore, an insufficiently dimensioned sanitary zone or a surface incline towards the well can lead to seeping of the surface water down into the well.

Groundwater contamination can be as a result of poor sanitation and subsequent leaching from the site especially in the vicinity of the well (Abdulsalam and Zubairu, 2013). In view of this a sanitary surveillance method was developed by Lloyd and Helmer (1991) to assess the drinking water quality and the associated risks or hazards in the water supplies in rural areas. A sanitary survey refers to an evaluation and on-site inspection of the physical environment of the water source to identify possible sources of environmental contamination (USEPA, 1999). Sanitary survey can be a complex technical task which involves inspection and the use of questions to assess the key elements of a water source itself, sources of contaminants and water handling (Lloyd and Helmer 1991 and USEPA, 1999). An inspection format developed by Lloyd and Helmer, (1991) consists of a set of questions that have 'yes' or 'no' answers. The questions are structured such that a 'yes' response indicates that there is reasonable risk of contamination and a 'no' indicates that the risk is negligible. A 'yes' response scores one point and a no scores zero points. Upon completion of the inspection, the points are summed up to give a sanitary risk inspection risk score which is referred to as the Contamination Risk Score (CRS) in this study. A higher CRS indicated a greater risk of well contamination by faecal pollution from the immediate surroundings of the well. The information generated by a sanitary survey helps identify existing and potential sanitary risks to the water quality. A sanitary survey systematically lists every fault in the system as a sanitary risk factor (Lloyd and Bartram, 1991).

Point sources of pollution are those where the origin of contamination can be identified such as localized agricultural practices that affect aquifers directly below the site (feedlots), septic tanks and landfills (Bolger and Stevens, 1999). Sources of pollution in groundwater may include runoff or seepage from fertilized agricultural lands, municipal and industrial waste water, refuse

dumps, animal feedlots, septic tanks and private sewage disposal systems, urban drainage and decaying plant debris (Hudak, 1999 and Nas and Berktay, 2006). Non-point source pollution from agricultural activities such as animal farming and pit latrines have been reported to degrade groundwater quality and thereby threaten people's health. The use of poorly protected groundwater sources has been linked to acute diarrhoea in developing countries (Nasinyama, 2000).

The study was carried out within Ainabkoi Sub-County of Uasin Gishu County in Kenya, where shallow hand dug wells are the main source of water for domestic use. These hand-dug wells are constructed manually as irregular holes in the ground that intersect the water table and are prone to pollution from several sources (Todd, 1980). The wells may be exposed to pollution from surface run-off, poor sanitation in the vicinity of the well, effluent discharge from agricultural production activities and by the specific physical attributes of well construction. Mixed farming agriculture (food/commercial crops and livestock-dairy) characterized by different farm sizes is the predominant economic activity for the rural community of Ainabkoi Sub-County with farmers gradually shifting to intensive horticultural farming (UGCIDP, 2013). According to Goswami et al., (2014), the selection of factors that define farm typology varies greatly from study to study and may be governed by the purpose of research. For purposes of this study different farm sizes were determined as a working farm typology because it captured common characteristics within farms in each ward in Ainabkoi Sub-County. Therefore, farms in Ainabkoi, Kipsinende and Olare wards were classified as large, medium and small farm sizes respectively. Groundwater is the main source of water for drinking and other domestic needs in Ainabkoi Sub-County. Therefore, the aim of this project was to identify and assess the sanitary risk factors associated with the wells and subsequently determine the contamination risk score (CRS) as predictors of water quality.

II. METHODOLOGY

The study was conducted in 2012 and 2013 in three wards within Ainabkoi sub-county namely Ainabkoi, Olare and Kaptagat (Kipsinende) which have extensive agricultural activities. A baseline reconnaissance farm survey found that farmers predominantly practiced mixed farming whereby they grew maize, kept some farm animals, and had a variety of vegetables and fruit crops in small gardens beside their homes. However, each farm had its own unique characteristics with regard to the farm sizes, number and types of domestic animals kept, the maize acreage, variety of vegetable and fruit crops grown, and the homestead/property development such as landscaping, housing, toilet construction, well ownership and construction.

A conceptualized working typology therefore identified farms in Ainabkoi ward as mainly large, family-generations-owned mixed farming size and ranged more than 40 acres in size (>40 acres) with privately owned wells. In Kipsinende ward, farms were medium sized (10-40 acres) mixed farming size with privately owned wells. The farms in Olare ward were small mixed farm size which ranged 2-10 acres in size and with communally owned wells. Purposive random sampling technique was applied in selection of the representative farms within each ward whereby only accessible farms that had access to a well for evaluation of the groundwater sources were selected. For each farm size, Large mixed, Medium mixed or Small mixed farm systems, five farms were purposively selected such that each had access to a well within the farm or a centrally communal well.

2.1 Survey and Assessment of wells in relation to Sanitary risk factors.

Onsite sanitary survey of the wells and the homesteads was carried out in each farm in order to identify significant potential deficiencies, which could explain possible trends in the water quality with regard to the integrity of the whole system (USEPA, 1999). Observations and interviews were used to collect information on the sanitary aspects of the wells. Visual inspection and observations of

the wells and the immediate environments were conducted on each farm in the different farm sizes. For the purpose of the study, the sanitary survey encompassed the essential components of water source as described in the sanitary survey assessment form adapted from Lloyd and Helmer (1991) and modified in the context of the observations specific to the study area. Visual examination of each well at the time of groundwater sampling was done along with interviews with the landowner. Interviews were used to determine land ownership, well ownership and age, ward of septic tanks, toilets and cowshed. During the survey, farm owners ascertained their land/farm management practices which included the stock of animal farms and water use.

The field and well inspections were carried out to find out the proximity of the wells to latrines and other sources of pollution, nature of well surrounding, well construction such as lining of the well (parapet), and mode of water withdrawal. A positive response indicated the presence of a risk and a score was allocated for a positive answer and no score for a negative answer. The positive answer scores were added up to give an overall sanitary contamination risk score.

The Contamination Risk Score (CRS) was as follows:

i. Very High Risk (VHR) = 9-11
ii. High Risk (HR) = 6-8
iii. Intermediate Risk (IR) = 3-5
iv. Low Risk (LR) = 0-2

The average CRS was determined for the wells within each farm system. The average percentage of wells within each farm size that were exposed to each of the sanitary risk factors was determined.

2.2 Description of the Risk Assessment Factors

In the context of the study area there were 11 sanitary risk factors (SRF) used to assess the quality of the well water and were modified and described as follows:

1. Distance of Pit latrine from well. The question aimed at determining if the well was located at a safe distance from contamination by the pit-latrine. In this case a 10 m distance was used as a general guideline value. It was common for the homesteads to have a pit latrine near the main house and may therefore be near the well.
2. Position of Pit latrine on higher ground in relation to the well.
   The observation question was based on the assumption that water flows downwards and hence the potential to contaminate wells downhill because the land was generally undulating.
3. Is there any source(s) of possible pollution (man-made attributes, animal excreta, rubbish, Septic tanks, constructions, feedlot runoffs, cowshed runoffs) within 10m of the well?
   The aim of this question was to check for any sources of pollution that may wash into the well. It was common for animals to be tethered and graze within the well vicinity where green grass was common. Some cowsheds/barnyards were not far from the well. Disposal of rubbish is done within the homestead.
4. Well Ownership: Wells were either privately or communally owned. This question focused on the assumption that communally owned wells may not be as well managed and protected like the privately owned one.
5. Was the well depth less than 15ft? This question was adapted because of the varied well depth in the different farm sizes. Deeper wells may indicate a lower water table and hence less likely to be polluted through leaching.
6. Does the general land terrain slope towards the well? This was an observation question of the land terrain to determine if it slopes towards the well. This was deemed important in sanitary risk determination because undulating land enhanced the likelihood of storm runoff into the well.
7. Do animals graze and water in the well vicinity? Livestock such as sheep were tethered and watered within a 10 m radius of

the well vicinity. The excreta from these animals can be a source of nitrogen pollution of the wells.

8. Is the water extracted by use of a bucket and rope?
   This question was based on the probability of well water pollution when buckets and ropes left in unsanitary positions such as lying on the well surface or grounds around the well. This question was aimed at determining if the water abstraction means were left in such conditions that they contaminated or polluted the water source. Water extraction from wells was done manually by use of a metal or plastic container which was tied to a rope for deep wells. However some wells had a windmill, and hand pumps which were used for water extraction.
9. Is the well open (not constructed)? Wells either had a wall (parapet) constructed around them or not. Wells that were at the same level as the ground were deemed susceptible to pollution from runoff and other sources of pollution.
10. Is there a likelihood of runoff entering the well? Runoff possibility into the well could be due to a wall (parapet) around the well that was not adequately high (more than 1m high) and other preferential pathways for the runoff to enter the well such as cracks on the wall. This observation question was aimed at determining if there was a wall (parapet) around the well that was adequately high (more than 1m high) to prevent surface water flow from entering the well?
11. Is the maize garden less than 5m from the well? The question assumed the likelihood of groundwater pollution through leaching of fertilizer N into groundwater.

III. DATA ANALYSIS

The data collected was subjected to the analysis of variance using SAS statistical package Version 6.12, (1997). ANOVA was done to determine if there were any significant differences between the farm sizes in the overall CRS and mean values were compared by least significant difference (LSD) at the 5% level.

IV. RESULTS

The results of the sanitary risk conditions of the wells in the different farm sizes in Ainabkoi ward are presented in Table 1. The sanitary survey revealed that there were highly significant differences between the farm sizes in the sanitary contamination risk scores. The homesteads within the large and medium farm sizes were well organized and landscaped whereby farm areas were subdivided into functional areas. These functional areas included grazing paddocks, the main house and homestead area, kitchen garden area, recreation/relaxing areas and utility areas. The wells within the large farm sizes were privately owned, 30-40ft in depth and were either protected from runoff by a raised construction (parapet) or semi-protected with a concrete wall that was close to the ground surface and covered with iron sheets (Figs. 1 to 3).

The medium-sized farm sizes were well planned, organised and landscaped with modern houses. Functional areas, grazing paddocks, cow sheds and utility areas such as the toilets were located in the backhouse and screened with live fences. The wells within the medium farm sizes had both protected and semi-protected constructions around the well (Figs. 4 to 7). Kaptagat ward, where the medium sized farms were located was generally flat with gentle slopes in some parts hence pollution from runoff may not be a common occurrence.

Most of the farms and homesteads within the small farm size, were not well planned. The houses were mostly semi-permanent and ranged from one house to about six houses within the homesteads. Wells within the small farm sizes were communally owned, shallow and unprotected making them vulnerable to pollution from runoff (Fig. 8-12). Water extraction from the wells was done by use of hand buckets and cans because the water wells were very shallow and the water level was always high. Within the small farm sizes, the general terrain sloped towards the wells and livestock were tethered to graze and were also watered in the well vicinity. This consequently littered the area around the well with animal excreta (Fig. 9).

It was apparent that the water table in the region of the small mixed farm sizes of Olare ward was mostly high and therefore the wells were shallow and remained full throughout the wet and dry season (Fig. 6). These wells were located at the bottom of the terrains or slopes which facilitated drainage and runoff down slope into the wells. Observation of the maize crop around the area around the well showed significant N fertilizer deficiency as shown in Fig. 7.

Table 1: Sanitary Risk Factors (SRF) observed in wells in the different Farm sizes in Ainabkoi Sub-County

Percentage of wells exposed to the sanitary Contamination Risk Factors				
Sanitary Risk Factors (SRF)		Farm sizes		
		Large	Medium	Small
		Percentage observed		
1	Latrine within 10m of well	33	20	60
2	Latrine on higher ground than well	0	100	80
3	Any other source of possible pollution (animal excreta, rubbish, fertilizer)?	67	40	100
4	Is the well communally owned?	0	20	100
5	Is the well less than 15ft?	0	40	80
6	Does the general land terrain slope towards the well?	100	40	100
7	Is the well vicinity livestock grazing ground?	33	20	100
8	Is the water extracted by bucket and rope?	66	60	100
9	Is the well open (Not constructed)?	0	40	80
10	Is there likelihood of runoff entering the well	66	60	100
11	Is the garden less than 5m from the well?	33	80	80
	Average of Sanitary Risk Factors(out of 11)	4.0	4.2	9.65
	*Contamination Risk Score (CRS) Range	IR (36%)	IR (38%)	VHR (87%)
	Significance (p=0.05))	***		
	Least Significant Difference (LSD)	0.148		

Adapted and modified from Lloyd and Helmer (1991)
*Contamination Risk Score Range: 9-11 = Very High Risk (VHR); 6-8 = High Risk (HR); 3-5 = Intermediate Risk (IR); 0-2 = Low Risk (LR).
*** Highly significant at p≤0.05.

Figure 1: A protected well (parapet) with a hand-manipulated water extractor

Figure 2: Semi-protected well showing the laundry activities and vegetable garden within the well vicinity a Large Farm Size

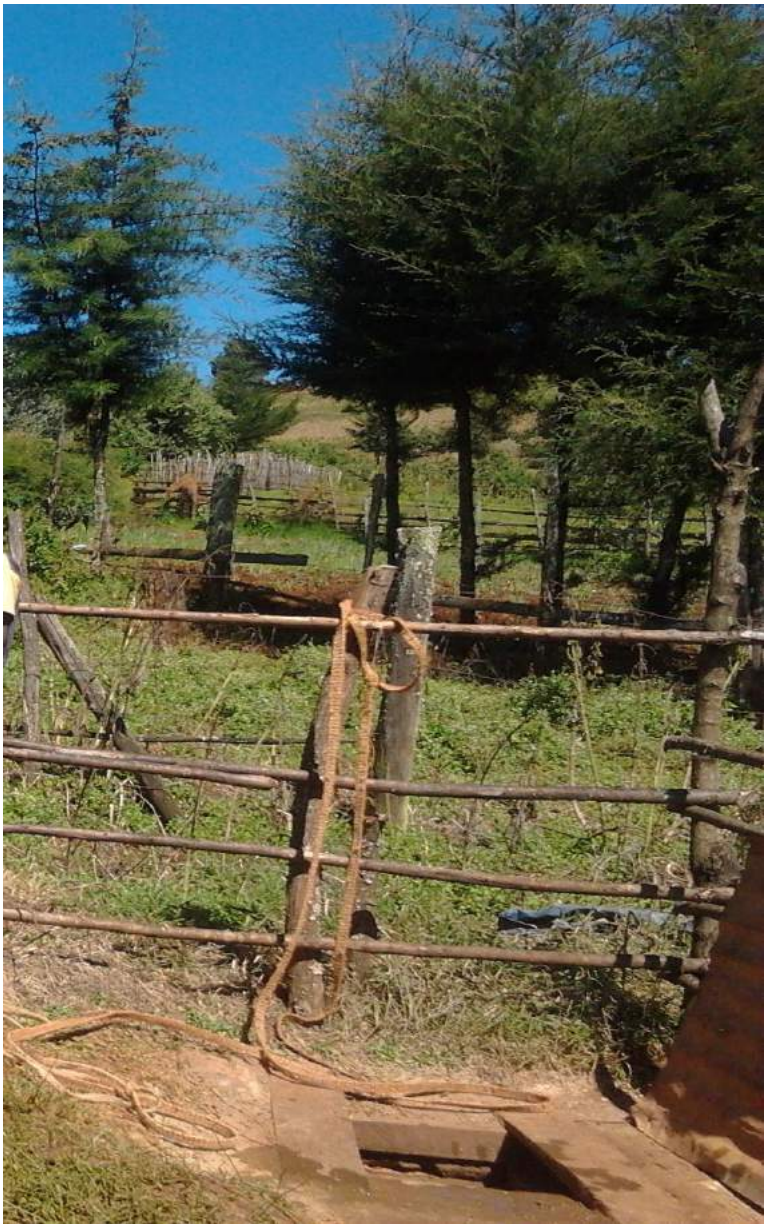

Figure 3: Close up of the semi-protected well in Fig. 2

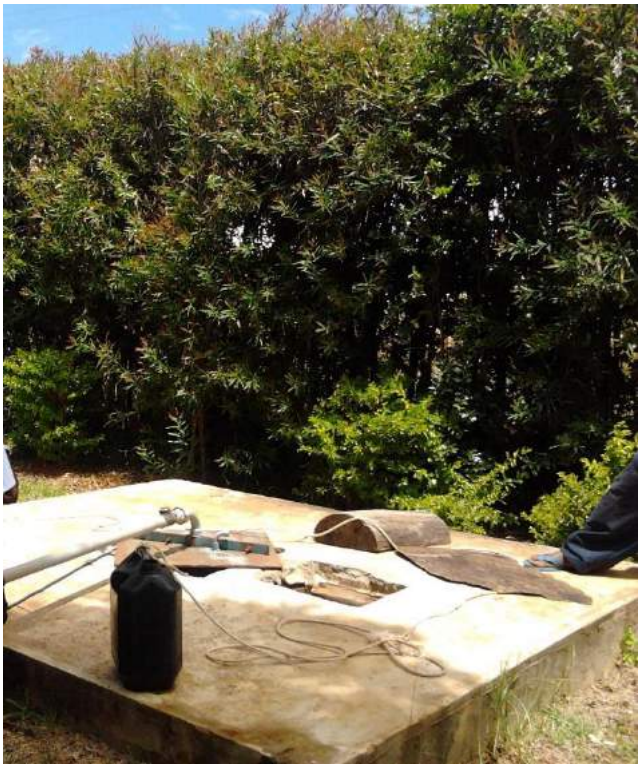

Figure 4: Protected well within a medium farm size showing modes of water extraction using a bucket and rope

Figure 5: Protected wells within the medium farm sizes showing windmill mode of water extraction

Figure 6: Semi-protected wells within the medium farm system surrounded by a vegetable garden

Figure 7: Semi-protected wells within the medium farm system surrounded by maize production in the vicinity of the well

Figure 8: A shallow unprotected communal wells used for both home water consumption and also for watering cattle in the small mixed farm size

Figure 9: A communal shallow well within the small farm system showing livestock grazing (left) and cow dung (right) within the vicinity of the well

Figure 10: A communal shallow well within the small farm system showing livestock waiting for water and cow dung within the vicinity of the well

Figure 11: In a small mixed farm in Olare ward showing the visibly high water table (right)

Figure 12: The road to a communal well in the small mixed farm system shows common N deficiency symptoms on the maize crop on the right

V. DISCUSSION

The results showed that farm characteristics can influence the SRF associated with individual wells and consequently the CRS. The differences in well contamination risk in the different farm sizes could have been due to individual farm endowments and ownership of the wells. In the large and medium farm sizes wells were privately owned and therefore it was apparent that efforts were made to maintain the sanitary standards of the well. However, wells within the small farm sizes were 100% communally owned and this may have contributed to the degradation of the area within the vicinity of the wells because the well was communally accessed by more people. This indicated that the people were either ignorant of the dangers associated with SRF or that the people/community could not control the use of communal property. The high percentage of wells located in positions where they are prone to pollution from the vicinity signifies that the well sanitary risk was not of importance in the choice of its ward. Similar results were reported by Abdulsalam and Zubairu, (2013) who reported that 80% of the wells were within 10m of the latrines and 70% were very close to the source of pollution indicating the indiscriminate positioning of wells in relation to sanitary risk.

The raised construction on the wells reduced the likelihood of contamination from pollutants in the well vicinity however the semi-protected wells were subject to runoff such as during the rainy season even though they had lid covers that helped reduce entry of surface flow of water into the well. The fact that the wells were not protected and that the terrain slopes towards the wells were major predisposing factors to the sanitary risks of the wells. The Large and Medium mixed farm size wells have an Intermediate Contamination Risk Score because most wells are protected and homesteads were moderately organized such that the well vicinity was relatively clean. This concurs with results by Llopis-Gonzalez, Sanchez, Marti-Requena & Suarez-Varela, (2014) who reported significant differences between percentages of protected and unprotected wells with regard to risk factors.

The wells within the small farm sizes were shallow due to the high-water table within the area of Olare unlike the low water table found in the large and medium farm system areas of Ainabkoi and Kaptagat. Llopis-Gonzallez *et al.*, (2014), also reported that the depths of wells at high risk of

contamination ranged from 0 to 300m and therefore making deeper wells have an increased ability to filter contaminants through different soil layers. Kibona, Mkoma, & Mjemah, (2011) observed a decrease in nitrates with increase in well depth, with high nitrate concentrations occurring mainly in wells with depths less than 41m. They attributed it to anoxic conditions in the deeper wells where the oxygen levels are depleted and reduction of other electron acceptors such as NO_3^- become energetically favorable. According to Hallberg (1989), groundwater nitrate contamination is often detected in aquifers less than 30m deep because the major nitrate sources occur at the surface and there is a delay in the migration of nitrates

It was observed that wells within the small mixed farm sizes were very shallow, communal water sources, lacked a well protection construction and were located down slope. The water levels in these wells did not recede like in the other wells ever during the dry season. Rain water flowed into these wells collecting any debris and waste into the wells. A widely held precept in groundwater hydrology is that water flows downslope along the gradient of the groundwater surface or water table and this gradient generally conforms to the surface contours (Rutkoviene, *et al.*, 2005). Therefore, this affects well water because pollutants are carried down slope by runoff or general water flow. This tenet explains the high sanitary risk of wells found within the large and small farm sizes whereby the land slopes towards the well vicinity unlike in the medium farm sizes of Kaptagat ward where the farm lands are generally flat. Runoff down slope may introduce pollutants such as nitrates from fertilizers applied in the farms, animal excreta, organic waste, inorganic wastes.

These wells in the small farm sizes have a very high sanitary risk because they are found downslope and are not protected by raised construction. Livestock are often tethered and watered within the well vicinity; hence any animal wastes are washed into the wells from runoff down slope. Water quality in wells is highly influenced by pollutants moving from upslope in the vicinity of the well. The deeper wells of the large farm sizes tended to have relatively lower than expected nitrate concentration which may be attributed to the below surface groundwater flow. It was observed that the water level in these wells frequently fluctuated with rainfall amount received unlike in the shallow wells whose water level remained noticeably visible.

VI. CONCLUSION

From this study it was apparent that there are multiple pollution point sources and risk factors that may determine the potential for environmental degradation on well water quality. The source of groundwater pollution comes from a variety of factors including the fertilizer application rates, well protection, well depth, groundwater level fluctuations and recharge conditions of the groundwater. The most important risk factors to the wells are the well protection and the activities within the well vicinity.

Farm characteristics can influence the SRF associated with individual wells and consequently the CRS. Efforts can be made to maintain the sanitary standards of the well as evidenced in the large and medium size farms. People may not be able to control the use of communal wells. Well sanitary risk was not of importance in choice of well location. There seemed to be lack of knowledge on the risks associated with well location. The raised construction on the wells reduced the likelihood of contamination from pollutants in the well vicinity. Well covers may not protect wells from rain runoff though they reduce the entry of surface flow of water. A widely held precept in groundwater hydrology is that water flows downslope along the gradient of the groundwater surface or water table and this gradient generally conforms to the surface contours. The small farm sizes had a very high sanitary risk because they were downslope and are not protected by raised construction.

There is therefore need for a local county initiative to construct protective raised walls at the communal wells and educate the community on aspects of water quality. It will be necessary to evaluate the microbial load and thereby determine

the level of contamination associated with each well. Examination of the microbial levels alongside the sanitary risk scores will make it possible for local remedial actions. In addition it will be important in realising the national policy on Kenya's groundwater resources of providing a common framework to protect its quality by minimising the risks posed by pollution (Republic of Kenya (ROK). 2013).

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ABSTRACT

Many anthropogenic chemicals, especially from agricultural and environmental practices are generating global ecological concerns. The fact that many of these substances have been linked to endocrine system modulation, particularly the thyroid homeostasis is becoming a topical issue. Several studies support the hypothesis that the thyroid system represents major target of endocrine disruption. Using a Xenopus Metamorphosis Assay, this study assessed the comparative thyroidal impact of three glyphosate-based formulations including Roundup, Kilo Max and Enviro glyphosate. The result showed that Roundup at 0.6 mg/L significantly increased whole-body length (WBL), and reduced the whole-body mass (WBM) alongside a significant increase in the thyroid gland, but without impacting stage development. The Kilo Max significantly reduced both WBL and snout-vent length (SVL) at 190 and 280 mg/L, while it increased the full-limb length (FLL) at 90 mg/L only. Kilo Max also significantly reduced the stage development at 190 mg/L and 280 mg/L, without impacting the thyroid gland. Although, Enviro glyphosate, reduced WBL, SVL and WBM of the tadpoles at 28 mg/L, without impacts the stage development and thyroid histology. This result confirms that Roundup formulation is thyroid active as it affect the thyroid gland, while Kilo Max and Enviro glyphosate formulations only showed extra-thyroidal effects, since it has no effect on thyroid gland.

Keywords: thyroid, herbicide, amphibian, Roundup, Kilo Max, Enviro glyphosate.

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ABSTRACT

Many anthropogenic chemicals, especially from agricultural and environmental practices are generating global ecological concerns. The fact that many of these substances have been linked to endocrine system modulation, particularly the thyroid homeostasis is becoming a topical issue. Several studies support the hypothesis that the thyroid system represents a major target of endocrine disruption. Using a Xenopus Metamorphosis Assay, this study assessed the comparative thyroidal impact of three glyphosate-based formulations including Roundup, Kilo Max and Enviro glyphosate. The result showed that Roundup at 0.6 mg/L significantly increased whole-body length (WBL),and reduced the whole-body mass (WBM) alongside a significant increase in the thyroid gland, but without impacting stage development. The Kilo Max significantly reduced both WBL and snout-vent length (SVL) at 190 and 280 mg/L, while it increased the full-limb length (FLL) at 90 mg/L only. Kilo Max also significantly reduced the stage development at 190 mg/L and 280 mg/L, without impacting the thyroid gland. Although, Enviro glyphosate, reduced WBL, SVL and WBM of the tadpoles at 28 mg/L, without impacting the stage development and thyroid histology. This result confirms that Roundup formulation is thyroid active as it affects the thyroid gland, while Kilo Max and Enviro glyphosate formulations only showed extra-thyroidal effects, with no effect on thyroid gland. This result points to the underlying role of surfactants in the thyroid disruption and not the main glyphosate. Regulatory agencies must do the needful, to subject all aquatic formulations to relevant physiological endpoints to characterize their impacts particularly on amphibians, given their global declining status.

Keywords: thyroid, herbicide, amphibian, Roundup, Kilo Max, Enviro glyphosate.

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Highlights

- Roundup significantly increased thyroid gland at 0.6 mg L, increased the WBL and reduced the WBM, without impacting the developmental stage of the tadpoles.
- Kilo Max significantly reduced the developmental stage, WBL and SVL at 190 and 280 mg/L, while increasing the FLL at 90 mg/L, without impacting the thyroid glands.
- Enviro glyphosate reduced WBL, SVL and WBM at 28 mg/L without impacting the stage development.
- Roundup is thyroid active while Kilo Max and Enviro glyphosate only showed extra-thyroidal actions.

I. INTRODUCTION

There are no longer any pristine areas without environmental pollutants (Bergman *et al.,* 2013). The environment today harbors numerous anthropogenic substances emanating from

agricultural, industrial, and environmental management (WHO/UNEP, 1997; Bergman *et al.,* 2013; EPA, 2016). A particular concern is the intensive use of pesticides such as insecticides, herbicides, fungicides and acaricides among others, which has led to ubiquitous contamination in many environmental media (Oritz-Dalgado *et al*., 2019) that raises serious concern over their non-target impacts through contamination of soil and water matrices (Carvalho, 2017**;** Babalola *et al*., **2**019). These chemicals, apart from their toxic effects, also possess differential physiological effects that may include thyroidal, reproductive, genotoxicity and teratogenicity (Babalola *et al*., 2020). Even though there are about 800 chemicals known or suspected to interfere with the endocrine system in one way or the other (mostly *in-vitro*), only small fractions of these chemicals have been investigated in intact organisms (Bergman *et al.,* 2013).

Pesticides are regularly introduced into the environment, through a variety of anthropogenic practices to combat pests that mainly influences agricultural yield and sustainability. However, several of these pesticides alter the delicate balance between species that characterize a functioning ecosystem and produce many physiological and biochemical changes in freshwater organisms by influencing the activities of several enzymes (Khan and Law, 2005)**.** Many of these pesticides have also been shown with the capacity to decrease the total protein content in amphibians, suggesting changes in the biochemical system of the non-target organisms (Khan *et al*., 2003). Some of these pesticides have also been shown to increase the toxic nature of the non-target organisms, through the alteration of their biochemical system (Bokony *et al.,* 2017). There are increasing negative impacts on the ability of non-target organisms to develop and reproduce properly given the level of these harmful substances that are continually introduced into the environment (Kortenkamps *et al.* 2011). Today, despite the intensive ecotoxicological research, there are still gaps about the ecological impacts of many environmental contaminants on non-target organisms (Bokony *et al.,* 2020).

Amphibians are an important component of the aquatic habitat, especially in tropical regions of the world (McDarmid, 1992), they are decreasing at an alarming rate around the world (Stuarts *et al*., 2004; Khan and Law, 2005; Munoz *et al.,* 2014). Although several factors have been suggested as possible causes of this decline, chemical pollutants (such as pesticides) have been highlighted as a major contributing factor (Sparling *et al*, 2001; Egea Serano *et al*., 2012; Munoz *et al*., 2014). In most amphibian species, their active reproductive cycles and early onset of development coincide both in time and places with pesticides application, which may therefore impact a critical window of amphibian fecundity and survival (Carlsson, 2019)**.** This global extinction of species and population declines among the amphibians is considered a critical threat to the global biodiversity (Whittaker *et al*., 2013). Even though Egea-Seranno *et al.* (2012)have noted that there is lack of deep understanding of how different types of pollutants affect amphibians, but evidence have shown that low-dose effects and non-monotonic response in amphibian species suggests that many pathways might be affected differentially (Vanderbing *et al.,* 2012). This is particularly true for the thyroid system.

Due to the complex nature of the thyroid system, numerous possible mechanisms of agonistic and/or antagonistic actions may influence amphibian metamorphic processes at different molecular levels (Fort *et al*., 2011; Gilbert *et al.,* 2012)**.** Optiz *et al.* (2005) summarized various possible thyroid system-disrupting effects of chemicals that strongly support the hypothesis that thyroid system represents another important target of endocrine disruption activities**.** The central effects of many anthropogenic chemicals on the thyroid system can be seen in many metamorphic organisms like amphibians, where they disrupt, arrest or lead to abnormal growth. The fact that the rising thyroid hormones from the thyroid gland of a growing tadpoles orchestrate the sequential changes of metamorphosis in the tadpole organs (Schreiber *et al.* 2001), any insult on the thyroid gland by these anthropogenic chemicals results in disruption of metamorphic

processes. The alteration of the thyroid system is of serious concern because the thyroid hormones mediate major physiological processes, including growth regulation, general metabolism and metamorphosis (Denver, 1997; Hermelink *et al.* 2010). Today, because of the considerable number of chemicals that have been shown to alter the thyroid system in vertebrates, the disruption of the thyroid axis has been identified as an important consideration for the regulation of chemicals (Miyata and Ose, 2012).

The Xenopus metamorphosis assay (XEMA) is used globally as an *in vivo* assessment protocol to identify agonistic and/or antagonistic effects of chemical toxicants on the thyroid system of the African clawed frog, *Xenopus laevis*. This assay is based on the fact that the metamorphosis phase in amphibians development is generally dependent on proper synthesis and regulation of thyroid hormones, which when modulated can lead to measurable developmental effects in the test organisms (Grim *et al.*, 2009). These measurable endpoints of this assay include hind limb length, thyroid gland histopathology and assessment of metamorphic stage development compared to control exposure group, and the identification of growth abnormalities and morphological features of the exposed organisms (OECD, 2007). The uniqueness of this protocol is the fact that the entire process occurs in aquatic environments, the ecological niche of these anuran larva, where the effects of thyroid disruption are greater (Helbing *et al.* 2010).

Glyphosate, for example, is the current leading herbicide globally (Sihtmae *et al.,* 2013; Turhan *et al.*, 2020). They are used for plant control, in no-till farming practises, and in the agricultural production of "glyphosate-ready" genetically modified crops (Gomez-Oritz *et al.,* 2017). The herbicide is also one of the leading formulations approved for aquatic weeds management. For example, a Working for Water initiative by the Western Cape Government of South Africa project to eradicate invasive alien vegetation, aimed to promote freshwater security, relied on the use of glyphosate formulations as one of the herbicides of choice. There are several commercial formulations of glyphosate herbicide including Roundup, Kilo Max, Environ glyphosate, Touchdown, Glyfos Bio and Roundup Bioactive etc. These different glyphosate formulations contain various surfactants, some of which are already of global concern. Despite substantial advances in Glyphosate toxicological studies, several uncertainties and gaps still exist, particularly on amphibians (Munoz *et al.*, 2014; Turhan *et al.*, 2020). For example, studies have pointed at the polyethoxylated tallow amine (POEA) surfactant in Roundup for the adverse role, leading to the emergence and development of several new POEA-free formulations (Howe *et al.*, 2004; Turhan *et al.*, 2020).

For the purpose of extending the knowledge regarding the potential adverse health effects of glyphosate formulations on amphibian development, this study evaluated the thyroid-disrupting potential of three aquatic glyphosate-based formulations using a Xenopus Metamorphosis Assay protocol. The selected glyphosate formulations were selected due to their regular use in managing aquatic weeds in the South African Working for Water Project and hence, may be closely associated with the pollution of freshwater ecosystems and exposure to aquatic wildlife.

II. MATERIALS AND METHODS

2.1. Test chemicals

The three glyphosate formulations selected for the study include Roundup (360 g acid equivalent (a.e.)/L) by Monsanto Ltd, South Africa, Enviro glyphosate (360 g a.e /L) by Enviro Industries Ltd, South Africa and Kilo Max (700 g a.e /kg) Volcano Agro-science Ltd, South Africa. Roundup contains polyethoxylated tallow amine (POEA) surfactant. The Kilo Max formulation contains an undisclosed POEA-free surfactant. Enviro glyphosate contains polyethylene alkylamine surfactant.

2.2. Africa clawed frog (Xenopus laevis) husbandry and breeding of Tadpoles.

From the healthy in-house *X. laevis* breeding stock, two sexually mature males and females were selected. The males and females were

separately maintained in 15 L glass tanks, filled with charcoal filtered water buffered with 2.5 g iodated sea salt and 0.8 g $NaHCO_3$ /10 L (Kloas *et al.* 1999). The frogs were fed with fish pellets (Aqua-Nutro, South Africa) three times weekly, and their holding tanks were washed and refilled with fresh charcoal filtered water immediately after their feeding. The breeding induction was performed according to ASTM, (1998) protocol. In brief, the adult males and females were first primed with 100 IU human chorionic gonadotropin (hCG) (Merck Ltd, Germany), which was injected into their dorsal lymph sac, four days before the mating. The males and females were given a second round of 100 IU and 300 IU hCG respectively, just prior to the mating. Male and female breeding pairs were placed together in a 15 L glass tank that was lined with plastic netting to isolate and protect the eggs from the adults. After the eggs deposition, the breeding pair were removed from the chamber, and an oxygen pipe was dropped to the water tank to increase the oxygen volume available to the eggs. After the emergence of the tadpoles, the newly hatched tadpoles were spread into several new 15 L tanks at a density of 40 tadpoles per tank, to avoid the overcrowding effects. The resultant tadpoles were staged using developmental atlas by Nieuwkoop and Faber (1994). Starting from NF-stage 47-48, the tadpoles were fed with Sera Micron (Sera Heinsberg, Germany), at 30 mg/animal/day twice daily until they reached NF-stage 51. All the husbandry, breeding and maintenance of tadpoles and exposure protocols were approved by the Animal Research Ethical Committee of the Stellenbosch University (Approval no- SU-ACUM 12-00015).

2.3 Test procedure

2.3.1. Exposure set-up

At the attainment of NF-stage 51, a total of twenty (20) healthy tadpoles were selected from the holding tanks and allocated to new 15 L exposure tanks. The exposure tanks were replicated twice at each of the exposure concentrations. Following the XEMA experimental protocol, the exposures were done under controlled climate conditions including water temperature at 23 ± 1 ºC, pH range of 7.5 - 8.5, ensured dissolved oxygen of >6.5 mg/L and a 12 hour of light and dark photoperiod ($L_{12}D_{12}$) regime (Organization for Economic Co-operation and Development, 2008). The tadpoles food ration was increased from the initial 30 mg/animal/day, to 50 mg/animal/day to compensate for their increased growth. While exposure mortality was monitored twice daily, the exposure medium was totally renewed every third day. The whole experiment was repeated twice independently. Only mortality incidence below 10% in the control group was accepted as basis for the acceptance of the experiment.

2.3.2. Exposure Concentrations

Arising from the initial 96-hour LC_{50} value derived at NF-stage 48 of *X laevis tadpoles* (Babalola and Wyk, 2017), three exposure concentrations for each of the herbicide formulations were selected (Table I).

2.3.3. Analytical assessment of experimental concentration

Random samples from each of the exposure medium concentrations were taken and analyzed to confirm the experimental concentrations. In brief, 100 ml of water sample was taken from each of the selected exposure tanks into 150ml glass bottles. In the case of replicates, 100ml was taken from each and pooled together, from where a single 100ml was obtained. The water samples were then frozen in an iced pack and transported to the Synexa Life Sciences certified laboratory, (Cape Town, South Africa), for analysis within two hours after collection. The glyphosate analyses and quantification were performed using liquid chromatography coupled with mass spectrometry. The results of the exposure water sample analysis (Table I addendum) showed that there was no major difference to the predicted nominal concentrations during the exposure study.

2.4. Autopsy procedure and morphometric measurements.

At the termination of the 21-day exposure, all the survived tadpoles were collected and gently euthanized in 0.1 % benzocaine (Sigma). The tadpoles were then blotted dry, individually

weighed (to the nearest 0.01 g), and measured for their snout–vent length (SVL) (to the nearest 0.1 mm) prior to preservation in Davidson's solution **(**OECD, 2007**)** for 72 hours. The tadpoles were then transferred to 4 % neutral buffered formalin for preservation (OECD, 2007**;** Shi *et al.*, 2012). The fore-limb length (FLL) and hind-limb length (HLL) were measured using Leica EZ4D stereo microscope (Leica Microscope Ltd, Germany) (to nearest 0.1 mm). with metric trace ruler that has the capacity to measure both straight and curved lines using traced lines of the limbs. The tadpole's heads just posterior to the eye, (containing the thyroid gland) were carefully severed transversely using a sharp blade and subjected to routine (paraffin wax imbedding) for histological procedures (Bancroft and Steven, 1977), followed by sectioning, mounting and staining.

2.5. Developmental Stage (NF-stage) determination

To establish the NF developmental stages of the tadpoles (according to Nieuwkoop and Faber, 1994), five median developmental stage of the tadpoles per tank and per concentration were haphazardly selected and compared to the median developmental stage of the control group for histopathological studies of the thyroid gland.

2.6. Histological Procedures

The lower jaws housing the thyroid glands were removed from the formalin, and thoroughly washed in running tap water for 10 minutes before they were processed for routine paraffin wax-based histology. Following the routine paraffin wax embedding and staining (Bancroft and Steven, 1977), the jaw samples were first dehydrated in series of graded concentrations of alcohol before been embedded (in frontal plane to facilitate the caudal surface of the tissue first) in histowax (Histolab Product, Sweden). The embedded lower jaw tissues were sectioned at 8 µm using Reichert-Jung microtome (Cambridge Instrument, Germany). The sections were mounted on clean, albumin coated glass slides, before being oven-dried at 40 °C overnight. The sections were subsequently dewaxed, then stained with haematoxylin and eosin (Bancroft and Stevens, 1977). The stained slides were cleared in xylene and mounted with glass cover using resin-based mounting medium (DPX, Sigma Ltd)

2.6.1. Histological measurement of the thyroid

Using the image of the right-side of the thyroid from each of the tadpoles, Leica DMLB light microscope equipped with a digital camera (Leica Microscope Ltd, Germany) was used for all measurements of the thyroid follicle epithelium cell heights. This was done by determining the length of the base to the apical edge of the cell. For each tadpole specimen, 15 epithelial cell height measurements were taken for four thyroid gland follicles, resulting in 60 epithelial cell height measurements per individual. A mean value was then calculated with other individual group members for follicle cell height. Follicular cross sectional area (follicle lumen area), and the thyroid cross sectional area were also measured and calculated (using image analysis software (Sigmascan, Systat Software Inc.). This was done by measuring the cross sectional area of all the serial sections and then summed together. Ten follicles were measured in each section, making ten thyroid follicles in each tadpole. The data was then combined for all the tadpoles per exposure concentration group.

2.7. Data analysis

A non-parametric Kruskal-Wallis test was used to assess variance in median NF-stage among concentration (since developmental stage constitutes ordinal data), followed by Dunn's multiple comparison test (DMCt) to identify significant pairwise differences in stages (Shi *et al.*, 2012). Normality and homogeneity of variance in WBM, WBL and SVL data were analyzed with Shapiro-Wilk's and Levene's tests respectively. One-way ANOVA or Kruskal-Wallis ANOVA test (K-W ANOVA) for non-parametric data was subsequently used to analyse for variations among concentration groups. Front limb length (FLL) and hind limb length (HLL) were normalized to snout-vent length in order to correct for the growth effect (or size related NF stages) (Coady *et al.*, 2010). Normality of the normalized FLL and HLL was evaluated using residuals' normal

probability plots and the Shapiro-Wilks test, whereas Levene's test was applied to test for homogeneity of variance. The treatment's effect (i.e. specific pesticide concentration), developmental stage and the treatment stage interaction on FLL and HLL was tested using mixed model ANOVA, using individual tadpoles as random factor. Pairwise differences in WBM, WBL, SVL and the normalized FLL and HLL between pesticide treatments and the control groups were assessed using the Tukey HSD test with Spjotfoll/Stoline correction for parametric data or the Dunn's test for non-parametric data. Significant differences were taken at $P<0.05$. All statistical analyses were done using Sigma Statistica V12 (Statsoft Inc., USA).

III. RESULTS

3.1 - Mortality

No mortality incidence was observed in all the experimental tanks throughout the 21-day exposure period.

3.2. -Kilo max

3.2.1.-Variation in Developmental Stages

Following the 21-day treatment with the Kilo Max formulation, the frequency distribution of developmental stages ranged from NF-stage 54 to 64 (Fig 1).

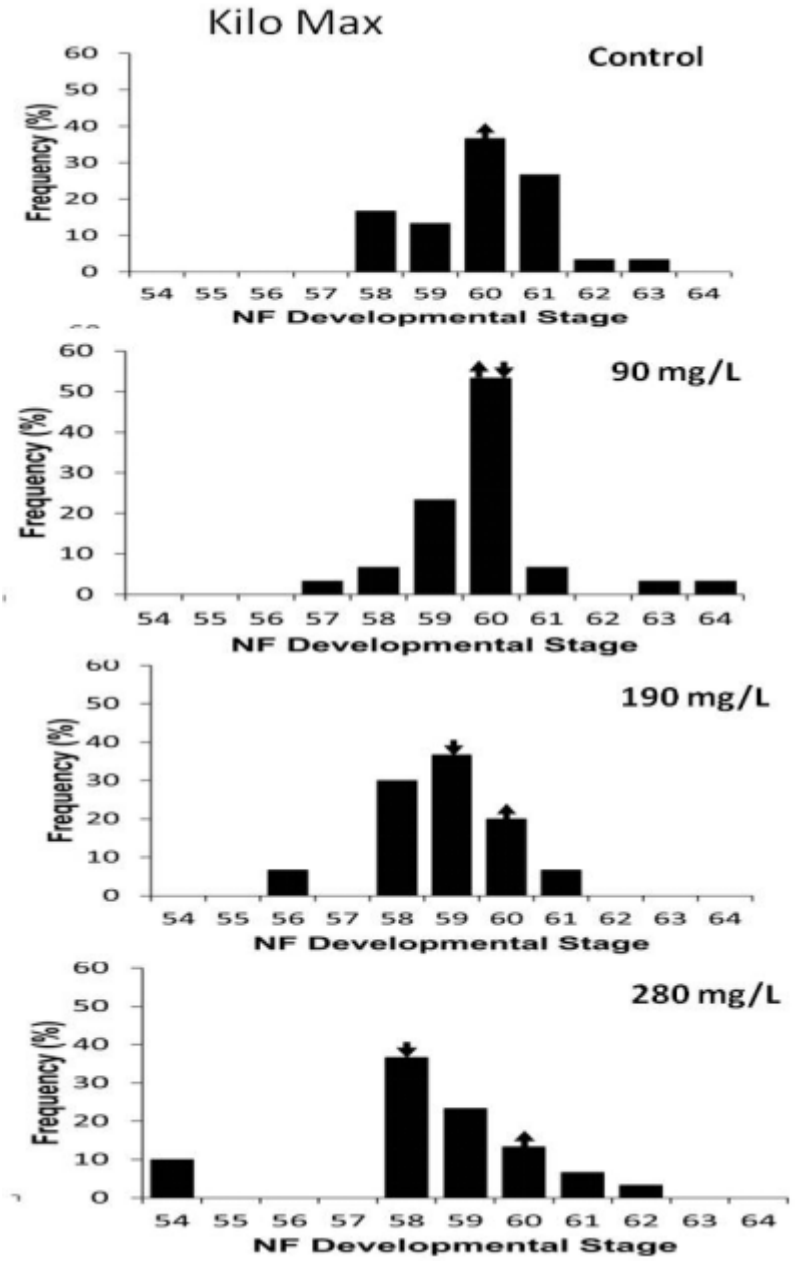

Fig. 1: The frequency distributions (n= 20) of developmental stages (Nieuwkoop and Faber, 1958) attained by *X. laevis* larval exposed to concentrations series of Kilo Max formulation (a) control, (b) 90 mg/L, (c) 190 mg/L and (d) 280 mg/L. The asterisk indicates significant difference (Post -HOC; P< 0.05) relative to the median stage in the control. The upward arrow showed the median at the control relative to the downward arrow that showed the median distribution of developmental stage at the various concentrations.

At both the control group and concentration of 90 mg/L, the median was NF-stage 60. The exposure at concentrations of 190 mg/L and 280 mg/L showed a significant delay in development with a median NF-stage 58. The exposure impact of the Kilo Max formulation delayed the NF developmental stages of the exposure in a dose dependent manner across all the exposure concentrations.

The exposure impact of the Roundup formulation on the treated tadpoles produced a developmental stage that ranged from NF-stage 57 to NF- stage 64 (Fig. 2).

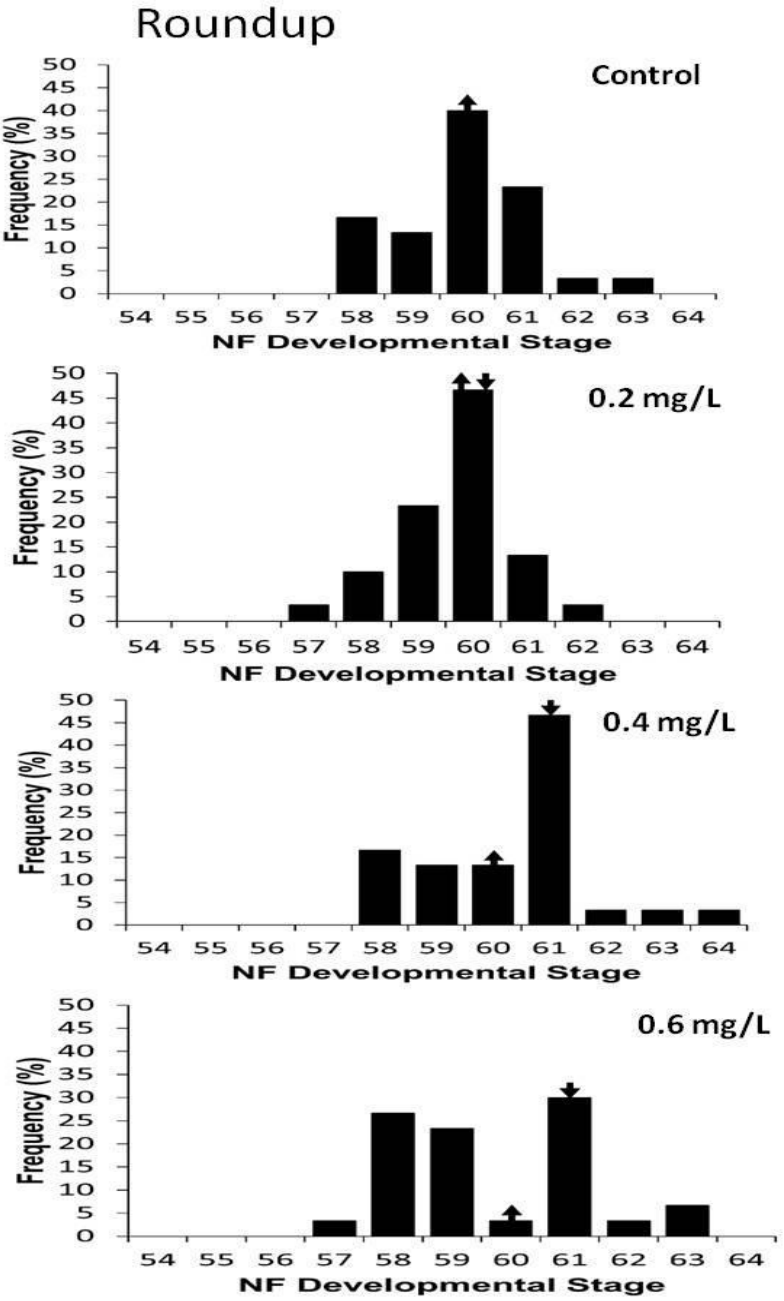

Fig. 2: The frequency distributions (n=20) of developmental stages (Nieuwkoop and Faber, 1958) attained by *X. laevis* larval exposed for 21-day to graduated concentrations of Roundup (b) 0.2 mg/L, (c) 0.4 mg/L (d) 0.6 mg/L relative to the control (a). The upward arrow showed the median at the control relative to the downward arrow that showed the median of developmental stages at the various concentrations.

The median of developmental stages shifted from NF-stage 60 at 0.2 mg/L to NF-stage 61 at concentrations of 0.4 mg/L and 0.6 mg/L (Fig 2). However, Kruskal-Wallis ANOVA test showed that these observed shifts were not significant compared to the control ($P > 0.05$).

For Enviro glyphosate treated tadpoles, the rate of stage development varied between NF-stage 57 through NF-stage 62. Using the Kruskal-Wallis ANOVA test and Dunn's multi-comparison test there was no significant variation in developmental stage distribution of the treated

tadpoles compared to the control ($P > 0.05$). (Fig 3).

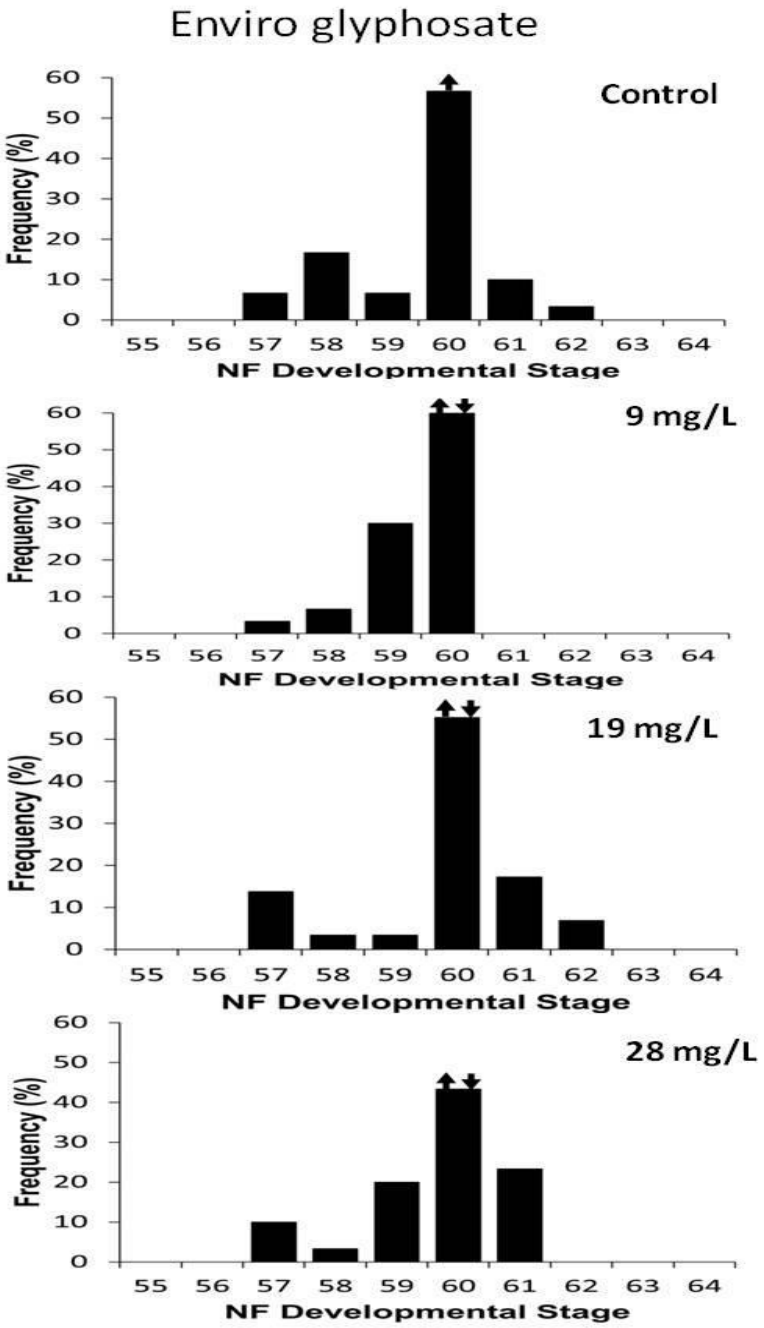

Fig. 3: The frequency distributions (n=20) of developmental stages (Nieuwkoop and Faber, 1958) attained by *X. laevis* larvae are exposed for 21-day to graduated concentrations of Enviro glyphosate (b) 9 mg/L, (c) 19 mg/L (d) 28 mg/L relative to the control (a). The upward arrow showed the median at the control relative to downward arrow that showed the median of developmental stage at the various concentrations

For developmental stages, comparing the NF developmental stages medians between the Kilo Max treated tadpoles and that of the control group, the Kruskal-Wallis ANOVA test showed significant variation in the median of developmental stages, this was further confirmed with multi-comparison analysis, which showed a significant delay in developmental medians at 190 mg/L and 280 mg/L relative to the control tadpoles (Fig. 4).

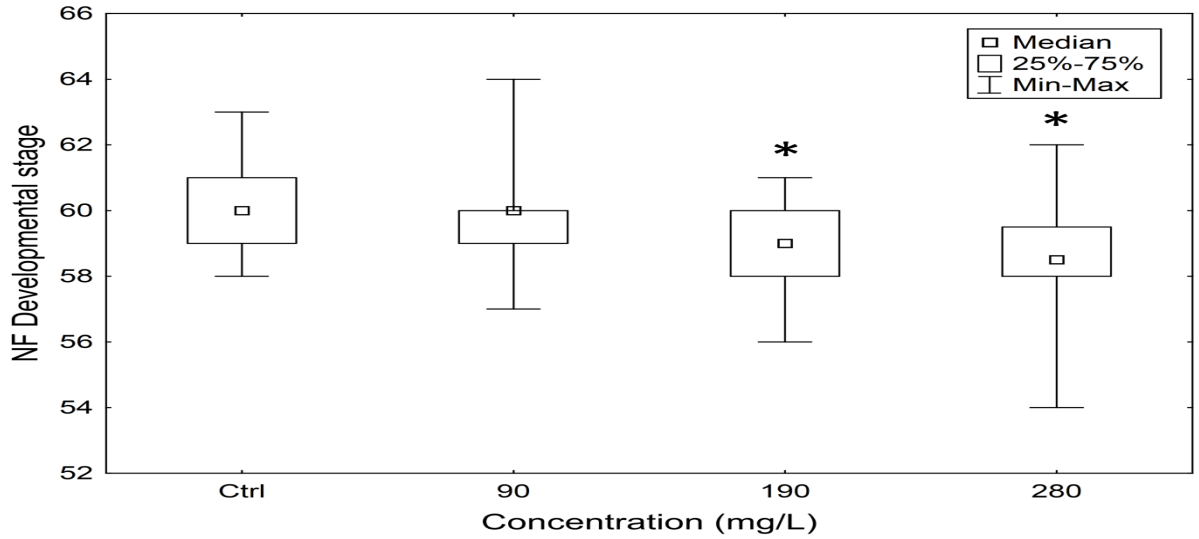

Fig. 4: Stage differentiation of *X. laevis* after 21-day exposure to graded concentrations of Kilo Max compared to the control. Asterisks indicate significant difference (DMCt; P< 0.05) from the control.

For Roundup formulation, the exposure showed no variation in the rate of the tadpoles development, as there was no variation in the median of developmental stages at all the exposure concentrations compared to the control exposure (Fig 5).

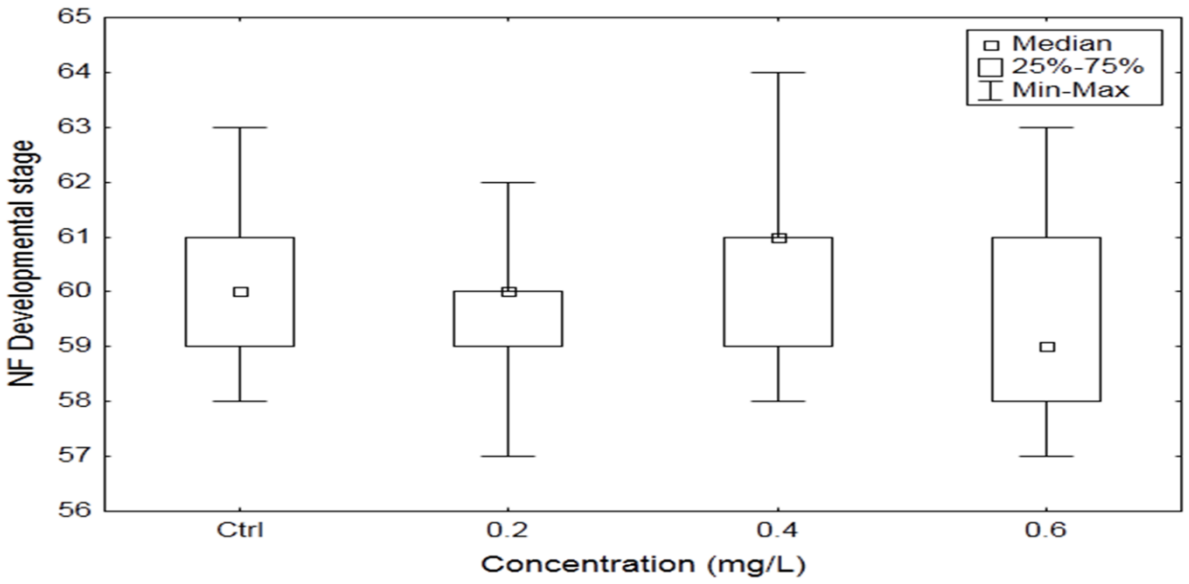

Fig. 5: Stage differentiation of *Xenopus laevis* larval following 21-day exposure to graded concentrations of 0.2, 0.4 and 0.6 mg/L of Roundup formulation compared to the control (Ctrl).

In the case of Enviro glyphosate, the rate of the development of the treated tadpoles showed no variation as the median of developmental stages remained constant at all the exposure concentrations as well as the control (Fig 6).

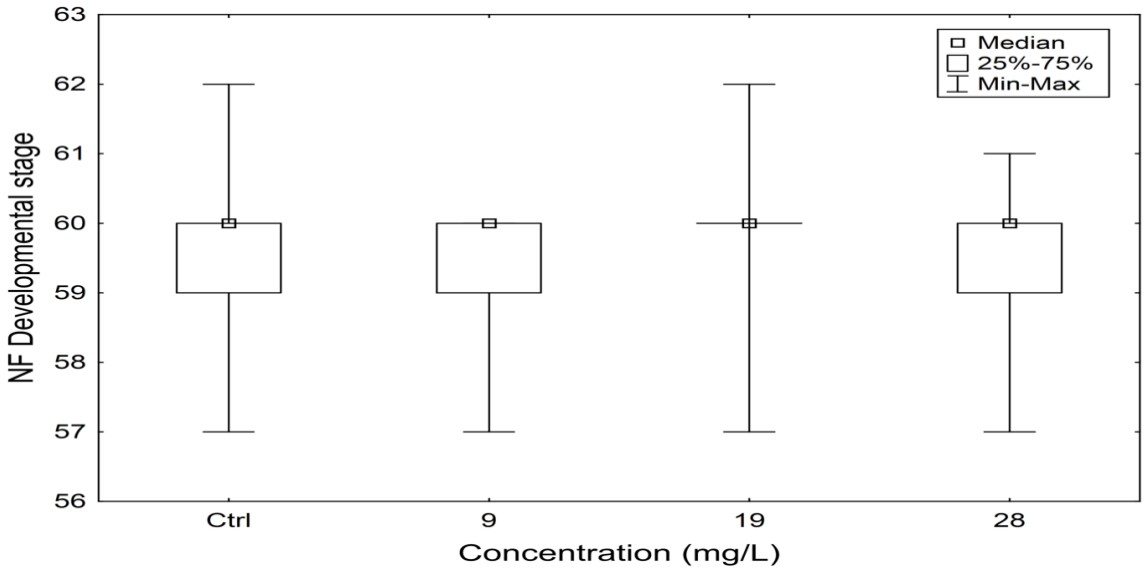

Fig. 6: Stage differentiation following 21-day exposure to graded concentrations of 9, 19, and 28 mg/L of Enviro glyphosate formulation compared to the control (Ctrl).

3.1.3. Morphometric Analyses in Kilo max Treated Tadpoles

The exposure of tadpoles to Kilo Max formulation significantly reduced the mean WBL (Fig. 7a) and SVL (Fig. 7b) at the two highest concentrations of 190 mg/L and 280 mg/L compared to the control (Tukey HSD test; $P < 0.05$). The Kilo Max formulation at all the exposure concentrations also showed a significant concentration dependent reduction in WBM compared to the control (Fig. 7c). The mean FLL (normalized) (Fig. 7d) also significantly increased at 90 mg/L compared to the control (Tukey HSD Test; $P < 0.05$).

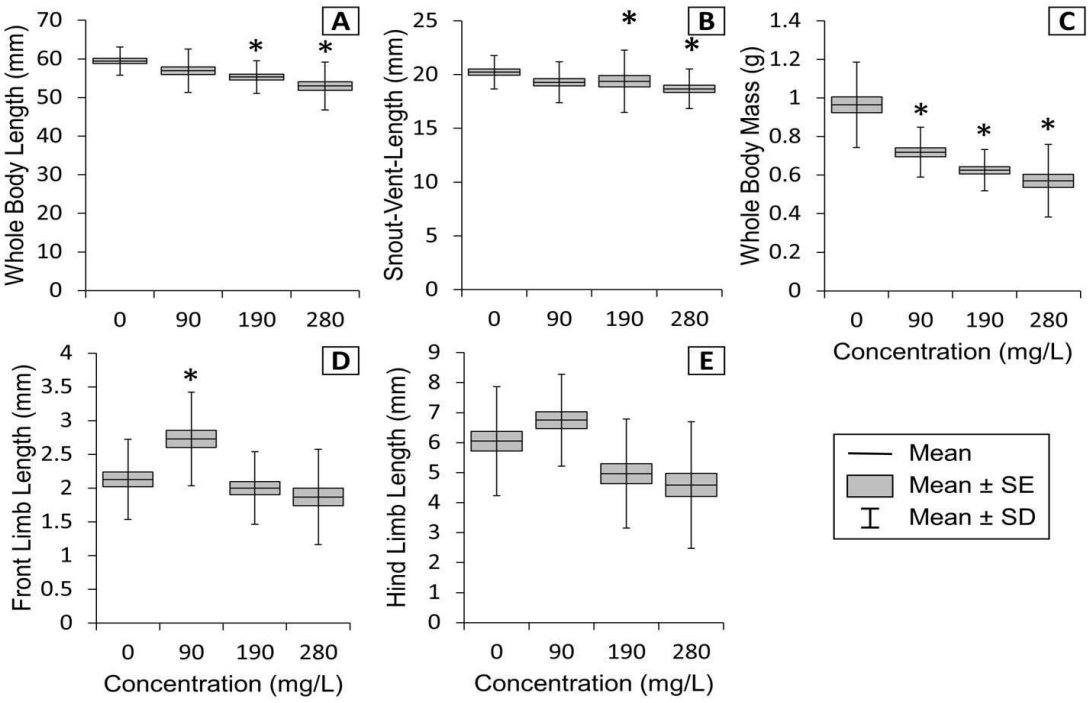

Fig. 7: Exposure impacts of graded concentrations of Kilo Max formulation on treated *Xenopus laevis larval* (a) Whole Body Length, (b) Snout-Vent Length (c) Whole body Mass, (d) Front Limb Length, (e) Hind Limb Length. Asterisks indicate significant difference ($P< 0.05$) from the control.

The exposure of the tadpoles to Roundup formulation resulted in a higher mean WBL (Fig.8a) and SVL (Fig. 8b). But using the Kruskal-Wallis ANOVA test, followed by Tukey HSD multiple comparison test, only the mean WBL was significantly different (P< 0.05) at the lowest exposure concentration of 0.2 mg/L compared to the control. The Roundup treated tadpoles showed concentration dependent reduction in mean WBM (Fig. 8c), which was confirmed with the Kruskal-Wallis ANOVA test followed by Tukey HSD multiple comparison test at concentrations of 0.4 and 0.6 mg/L (P < 0.05) compared to the control.

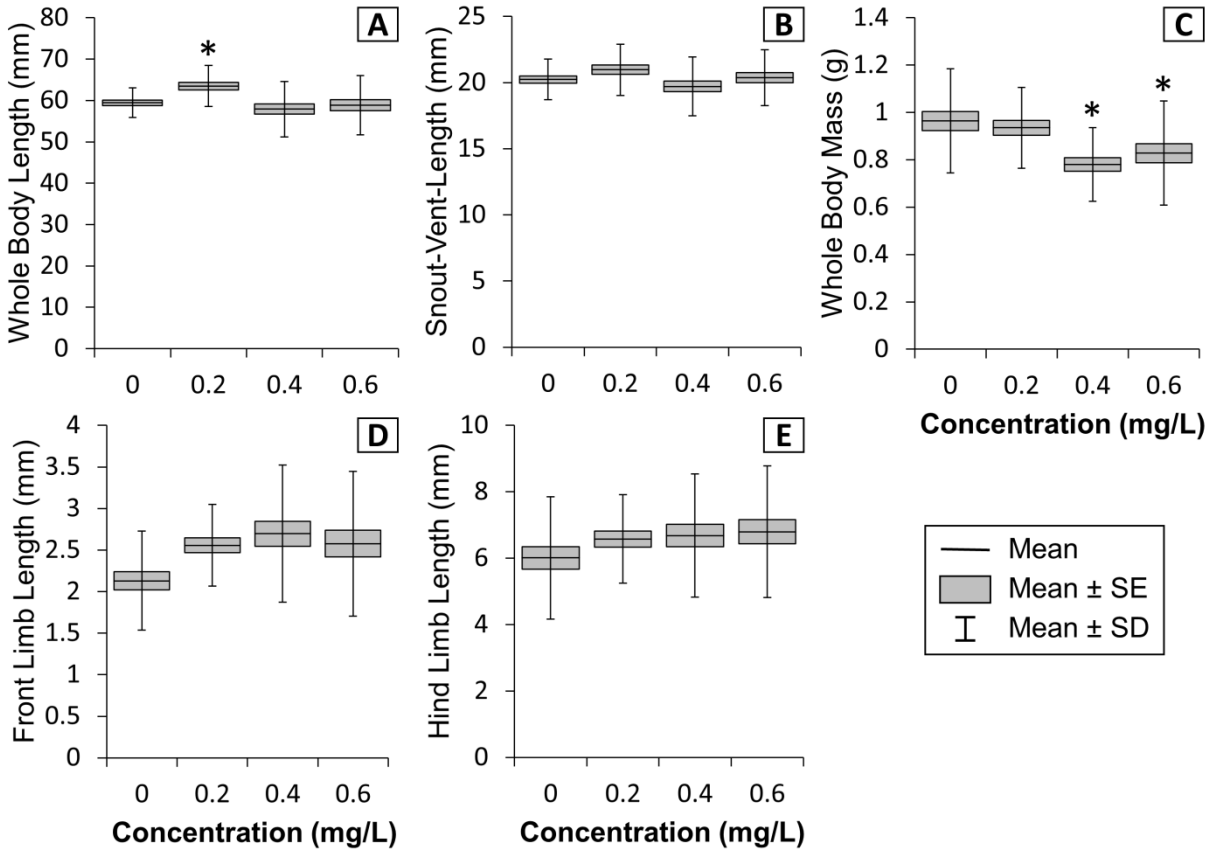

Fig 8: Exposure impacts of graded concentrations of Roundup formulation on treated *Xenopus laevis tadpoles* on (a) Whole Body Length, (b) Snout-Vent Length (c) Whole body Mass, (d) Front Limb Length, (e) Hind Limb Length. Asterisks indicate significant difference (P< 0.05) from the control.

The mean WBL (Fig 9a), WBM (Fig 9c) and SVL (Fig 9b) of the Enviro glyphosate treated tadpoles were lower compared to the control. This significant reduction was confirmed by the Kruskal-Wallis ANOVA test, followed by Tukey HSD multiple comparison tests at only 28 mg/L compared to the control (P< 0.05).

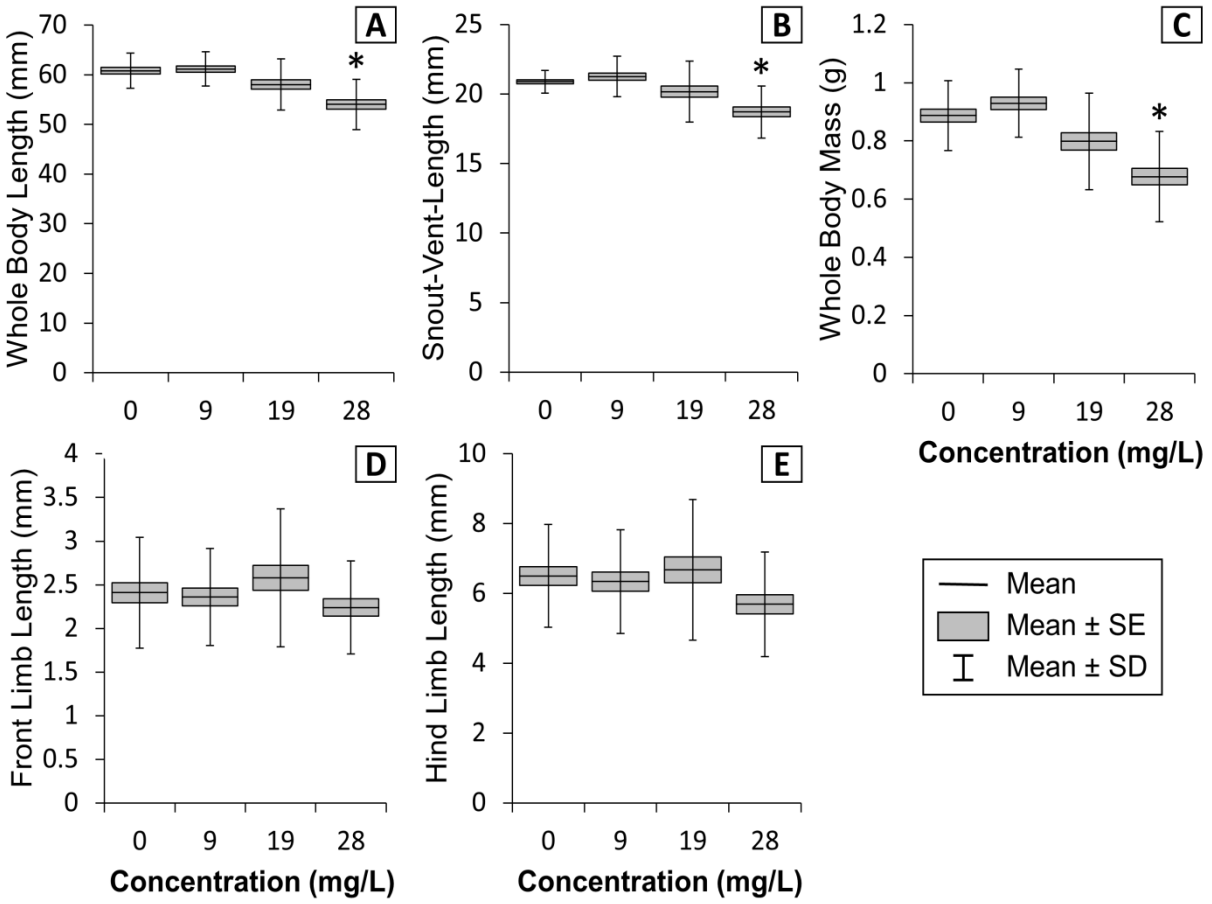

Fig 9: Exposure impacts of graded concentrations of Enviro glyphosate on treated *Xenopus laevis* tadpoles (a) Whole Body Length, (b) Snout-vent Length (c) Whole body Mass, (d) Front Limb Length, (e) Hind Limb Length. Asterisks indicate significant difference (P< 0.05) from the control.

3.1.4. Histopathological Endpoints

The exposure of developing tadpoles to Kilo Max formulation showed slight atrophy of both the gland area and the Colloidal area (Fig 10 b,c,d; Table 2)., which were not significantly different compared to the control (Fig 10 a). But the follicle epithelium showed evidence of hypertrophy, with a significant increase at all exposure concentrations compared to the control (K-W ANOVA Test; P< 0.05). Following the exposure to the Roundup formulation, the gland area (Fig 10e) and colloidal (luminal) area of the thyroid gland of the treated tadpoles showed significant increase in hypertrophy (P< 0.05) at the concentration of 0.6 mg/L compared to the control (Fig 10 a). The height of the follicle epithelium also showed hypertrophy with a significant increase (P< 0.05) at all exposure concentrations compared to the control (Table II). The exposure of tadpoles to Enviro glyphosate formulation showed evidence of atrophy of the colloidal area, with a significant reduction at concentrations of 9 and 19 mg/L (Fig 10 f and g) compared to the control (Fig 10 a) (K-W ANOVA Test; P< 0.05) (Table 2). The follicle epithelium also showed evidence of hypertrophy with a significant increase (K-W ANOVA Test; P< 0.05) at the two exposure concentrations of 19 and 28 mg/L (Fig 10 g and h) compared to the control (Fig 10a).. For the gland area, there was no significant difference in the treated tadpoles (P> 0.05) relative to the control (Fig 10 a).

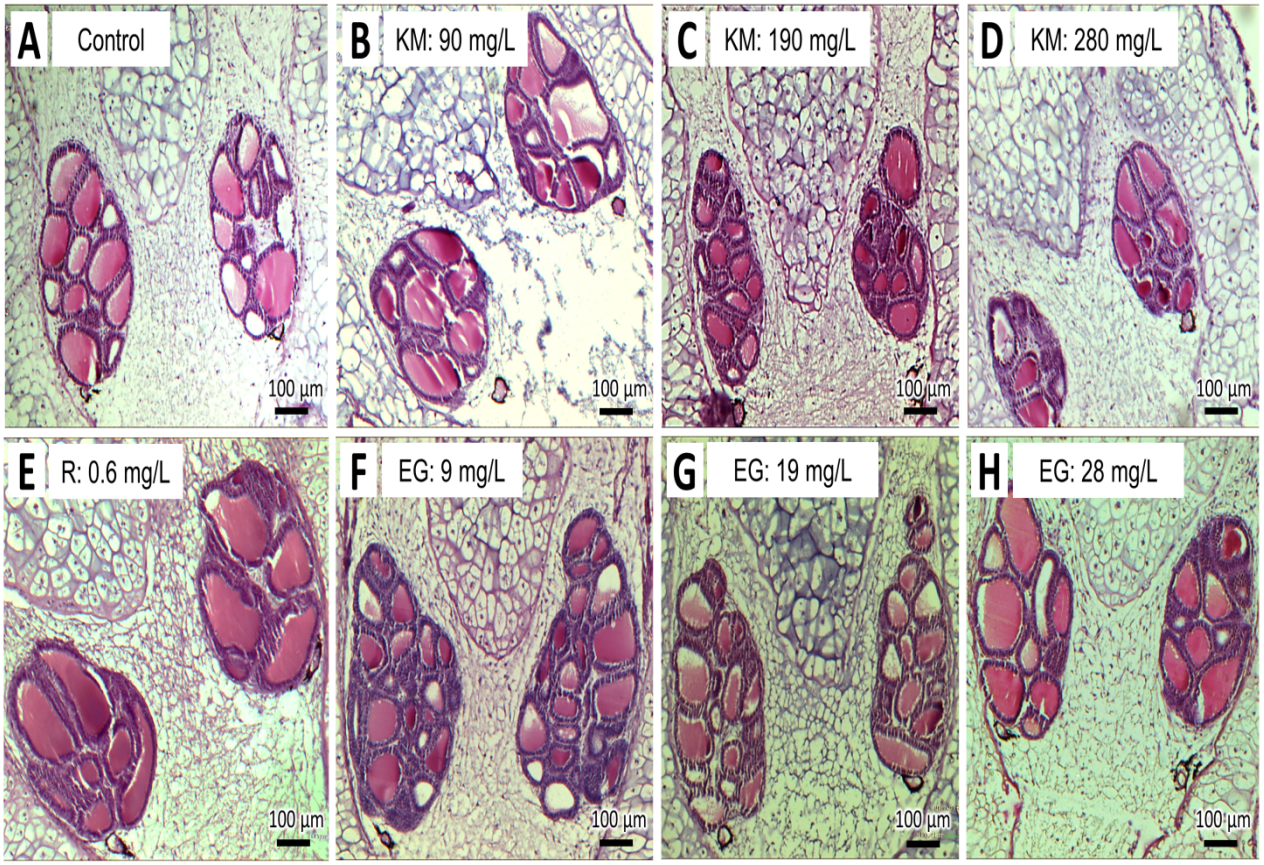

Fig 10: Histological cross-sections of the *X. laevis* thyroid glands exposed to graded concentrations (90-280mg/L) of the Kilo Max (10 b, c, d), and concentration (0.6 mg/L) of Roundup (10 e) as well as graded concentrations (9-28 mg/L) of Enviro glyphosate (EG) (10 f, g, h) relative to the control (10 a) (Mag 100X).

IV. DISCUSSION

All the tadpoles selected for this study were healthy. The control tadpoles passed through the metamorphosis at the expected international standard. The treated tadpoles at all the exposure concentrations of the three formulations showed no acute toxicity effect on the survival of the *X. laevis,* as no single tadpole died during the studies. The overall mean body mass and snout-vent length (± SD) for the control tadpoles after 21-day were 0.96 ± 0.23 and 20.23 ± 1.54 for Roundup, 0.96 ± 0.22 and 20.22 ± 1.56 for Kilo Max, and 0.99 ± 0.23 and 20.24 ± 1.78 for Enviro Glyphosate. These size and length values were even slightly higher than the recommended international standard as spelt out in OECD's phase 1 pre-validation study with NF stage 51 tadpoles, which has 0.94g and 19.5 mm as the mean WBM and mean SVL respectively (OECD, 2007). The control tadpoles were distributed between NF-stages 58-63 for Roundup and Kilo Max, and between NF 57-62 for Enviro glyphosate, with all their median developmental stage at NF-stage 60 at exposure termination. These stages are consistent with OECD, 2007 pre-validation guidelines recommendations that control tadpoles should reach a minimum median stage of NF 57 at the termination of the study (OECD, 2007).

The Kilo Max formulation significantly inhibited developmental stages at 190 and 280 mg/L but without impacting on the histopathology of the thyroid gland. The inhibition of developmental stages in this current study supported the earlier result of Babalola *et al.* (2019), where they showed concentration dependent growth inhibition of Kilo Max formulation on early larval stages of *X. laevis* at concentration of 190- 280 mg/L. This no impact on the thyroid gland, coupled with reduction in the tadpoles' sizes,

body mass and whole-body length suggests a non-thyroidal action, and extra-thyroidal toxic activities (Coady *et al.*, 2014). As noted by Fort *et al.* (2011), non-thyroidal action occurs when a toxicant affects whole body length, snout-to-vent length and whole-body mass, but not hind limb length and thyroid gland histology. As also pointed out by Optiz *et al.* (2005), total blockage of thyroid hormone (TH) synthesis and thus complete inhibition of metamorphosis does not necessarily inhibit growth of the tadpoles. This means that the concentration dependent growth inhibition observed in tadpoles treated with the Kilo Max is non-thyroidal at the current exposure concentrations. These non-thyroidal effects supported the findings in Babalola *et al.* (2019), where they noted that Kilo Max formulation is not a growth disruptor as its minimum concentration inhibiting growth (MCIG) ratio of 0.82 exceeded that of 0.32 benchmark ratios for growth inhibiting potential (Bantle *et al.,* 1999).

The tadpoles exposed to Roundup formulation showed a significant reduction in MBM at concentrations of 0.4 and 0.6 mg/L. This finding is consistent with the result of Howe *et al.* (2004), who noted that Northern leopard frog (*R. pipiens)* tadpoles were significantly smaller compared to the control tadpoles when exposed to Roundup formulation. This reduction in body mass in both *R. pipiens* and *X. laevis* species is possibly a physiological response to toxic effects of the Roundup formulation, as the same effects were observed in tadpoles exposed to the POEA surfactant alone (Howe *et al.*, 2004). This current reduction in body mass, particularly at concentration below the expected environmental concentration of Roundup at 1.43 mg/L (Govandarajulu, 2008) could have serious implications on the growth and reproduction of the amphibians in the wild. Several researchers have shown numerous implications of the size reduction, including increased chance of predation, and possible influence for lower survival rate and later reproductive fitness (Howe *et al.*, 2004; Gupta, 2012). The size reduction may also have strong downstream effects on adult phenotype and fitness (Dmitriew and Rowe, 2011). This means that the reduction in *X. laevis* body mass in tadpoles treated with Roundup formulation will have negative impacts on reproductive fitness and subsequently wider effects on the wild population.

The developmental stages of tadpoles treated with Roundup formulation was not significantly affected when compared to the control group. This result is consistent with the previous findings from our research group (Babalola *et al.*, 2019), where the Roundup formulation at concentrations of 0.5 -1.3 mg/L showed no significant inhibitory effects on the growth of embryo and early larval stages of *X. laevis*. The current results are also consistent with the findings of Lanctot *et al.* (2013), where they reported that Roundup formulation did not affect the growth of wood frog (*Lithobates sylvaticus)* tadpoles. However, the histopathological evidence in the thyroid gland showed inhibiting activities. The hypertrophy of the thyroid gland with a significant increase at concentration of 0.6 mg/L, coupled with hypertrophy of the follicle epithelium height at all exposure concentrations, as well as hypertrophy of colloidal area (goitre phenotype), at a concentration of 0.6 mg/L, showed the interaction of the Roundup formulation with the thyroid pathway. This occurrence of goitre phenotype may be an indication of thyroid hormone synthesis, either by modulating iodine uptake or biosynthesis of THs through hyperthyroidism (Mirata and Ose, 2012). That there is inhibitory effect on thyroid at the current highest exposure concentration of 0.6 mg/L suggests the initiation of inhibitory processes which normally commences with alteration in thyroid gland at concentrations below the onset of reduction in tadpoles developmental stages, majorly due to the compensatory growth responses (Carlsson *et al.,* 2019). This is consistent with the result of Howe *et al.* (2004), who noted the inhibitory impacts of Roundup formulation on the *R. pipiens* tadpoles. As noted by several researchers, low level of thyroid hormones (THs) usually activates the HPT for increased secretion of TSH from the pituitary gland, resulting in excessive stimulation, and an increase in thyroid gland size (OECD, 2008).

For Enviro glyphosate formulation, both the developmental stages and histological evidence

showed no significant difference between the treated tadpoles and the control. However, the reduction in body mass, SVL and WBL at concentration of 28 mg/L, also suggested the involvement of toxic properties of the formulation rather than the thyroidal activities (OECD, 2008; Fort *et al*., 2011). The reduction in body mass, SVL and WBL is consistent with the results of Babalola *et al*. (2019), where it was noted that exposure of embryo-larval to the Enviro glyphosate formulation resulted in a significant growth inhibition at concentration of 440 mg a.e. /L compared to the control. According to them, the Enviro glyphosate MCIG of 0.94 as against the benchmark of 0.30 makes the formulation a non-growth disruptor. Therefore, Enviro glyphosate formulation does not have thyroidal effects on the growing *X. laevis* at the current exposure concentrations.

From the exposure impacts of these three glyphosate formulations, it is clear that the exposure produced two varieties of actions. The first is the total growth inhibition that occurred in the Kilo Max and Enviro Glyphosate formulation, where the reduction in tadpoles' size, body mass, SVL and WBL, without gland alteration compared to the selective reduction in body mass and alteration in gland histology as shown in Roundup treated tadpoles. Several deductibles of interest can be obtained from this result including concentrations at exposure impacts, the potential contributions of active glyphosate and the potential role of surfactants .

The total growth inhibition observed in both Kilo Max and Enviro glyphosate formulations occurred at higher concentrations compared to the observed effects in Roundup formulation. This means that higher concentrations of active glyphosate are involved in the observed impacts of Kilo Max and Enviro glyphosate than in the Roundup formulation. This fact effectively rules out the active glyphosate as the direct cause of thyroidal activities as observed in Roundup formulation. This support the findings of Howe *et al*., 2004 and Turhan *et al*., 2020 that the toxic effects in glyphosate formulations is not a function of the active glyphosate, but rather the effects of the surfactants

On the role of added surfactants, relying on the two partitioning of the results again, the total growth inhibition as occurred in Kilo Max and Enviro glyphosate formulations without gland alteration compared to the selective body mass reduction and gland alteration in Roundup clearly indicates the role of added surfactants. That the Kilo Max (with an unknown surfactant and Enviro glyphosate with polyethylene alkalamine surfactant only caused general toxic action, without impacting the thyroid gland compared to the POEA in Roundup, which caused specific thyroid-driven body mass and gland alteration. This shows that the surfactants in Kilo Max and Enviro glyphosate are not thyroid active compared to the POEA in the Roundup formulation

It is evident from the current exposure study using the three formulations that the active glyphosate ingredient is not mediating the thyroid-disrupting activity. In addition, the surfactants in both Kilo Max and Enviro glyphosate only produced toxic impacts but are not thyroid active, unlike the POEA in Roundup that disrupted the thyroid-driven activities in the treated tadpoles. The issue of surfactants have always been very controversial for several reasons. First, the identity of the surfactants are usually shrouded in secrecy by the manufacturers of the pesticides under the guise of trademark protection. This makes it generally difficult to identify the surfactant, and access their exposure impacts as well as environmental effects. As noted by Mesnage *et al*., 2019, the issues of surfactant could be very confusing. According to them, hiding the identity of the surfactant by the producers usually makes it difficult to identify the toxicity of the co-formulas, and makes it even harder to specify their health effects both on human beings and the environment.

V. CONCLUSION AND RECOMMENDATIONS

In this study, it is very obvious that the three glyphosate formulations showed two opposing reactions on the growth and development of the treated tadpoles. The Roundup formulation, even at a low environmental relevant concentration of 0.6 mg/L, significantly increased the thyroid

gland, hypertrophy of the follicle epithelium height at all exposure concentrations, and hypertrophy of colloidal area at concentration of 0.6 mg/L, showing the inhibitory potential of the Roundup formulation on thyroid homeostasis. The Kilo Max formulation, even though significantly inhibited the developmental stages of treated tadpoles, shows similar no effects, just like Enviro glyphosate formulation on the histopathology of the thyroid gland, suggesting a no thyroid-disrupting activities, but rather other toxic impacts.

These observed impacts from these formulations clearly showed three important points, the active glyphosate play no role in thyroid activities and, therefore, is not thyroid active, the surfactants in Kilo Max and Enviro glyphosate formulations are not thyroid active, unlike the POEA surfactant in Roundup, which is thyroid active and not biologically inert as already pointed out by many studies This result confirms that the new emerging surfactants have differential characteristics compared to the old POEA surfactant. This study also showed that the activities of these new glyphosate formulations are likely the mirror of their surfactant, particularly in regard to the thyroid activities. Therefore, assessment of the thyroidal activities of aquatic herbicides should be mandatory before herbicides are approved for application.

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Data Accessibility

The data are available online in the doctoral thesis of the lead author.

Ethical care Statement

Xenopus laevis used for this study were collected, cared for, and treated under strict compliance with all ethical practices and law.

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Formal and Informal Foraminiferal Biozonation Framework for Tertiary Sedimentary Succession in F- Well, Niger Delta

Ononeme, O E, Fregene, TJ & Lucas, F A

ABSTRACT

Foraminiferal biozonation of tertiary sediments in F-Well Niger Delta were carried out with the aim of establishing a formal and informal biozones. Fifty (50) ditch cutting samples, sampled at varying intervals were used to establish the biozonation framework for the studied intervals. A total of eighty (80) foraminiferal species were recorded, most of the species recorded are calcareous and arenaceous benthic foraminiferal species. Two planktic zones (*N4-N3 and N3 – N2*) and nine informal assemblage zones ranging from A to I were established. *N4-N3 Planktic zone* has a reference interval of 8,000ft – 9,400ft. The top of this zonal interval which ought to be marked by the FDO of *Ammonia becarril* was absent but was estimated to be 8,000 ft. The base of this zonal interval is marked by the LDO of *Epistominella Vitrea* at 9,400 ft. *N3 – N2 Planktic zone* has *a reference interval* of 9,400ft – 10,000 ft. The top of this zonal interval is marked by the FDO of *Bolivina imperatrix* at 9,400 ft. The base of this zonal interval is marked by the LDO of *Spirosplectaminawrightii* at 10,000 ft. The presence of twenty-eight (28) diagnostic species aided in establishing nine informal biozones. These are *Haplophragmoides Sp Assemblage* zone A, *Bathysiphon sp.* Assemblage zone B, *Trochamminasp*assemblage zone C, *Ammo- baculites Sp Assemblage* zone D, *Arenaceous indeterminate* assemblage zone E, *Uvigerina Sparsicostata* assemblage zone F, *Haplophra- gmoides Nariva Ensis Assemblage* zone G, *Epistominella Vitrea Assemblage* zone H and *Cassigerinellachipollensis Assemblage* zone I.

Keywords: miocene – oligoocene, biozone, arenaceous, foraminifera, niger delta.

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Keyward: miocene – oligoocene, biozone, arenaceous, foraminifera, niger delta.

I. INTRODUCTION

The study area lies within the Niger Delta Basin, which is situated on the continental margin of the Gulf of Guinea in equatorial West Africa (Klett et al., 1997). The Niger Delta ranks amongst the world's most prolific petroleum producing Tertiary deltas that together account for about 5% of the world's oil and gas reserves and for about 2.5% of the present day basin areas on earth. Biostratigraphy is defined as the classification of sediment units according to observable variations in fossil content (Lowe & Walker, 1997). This enables sediment sequence to be divided into biostratigraphic units or biozones, each characterised by a distinctive fossil assemblage. Foraminifera has a small size, global ecological extent and rapid evolutionary turnover provide an excellent means of biozonation study. It is necessary for correlation, paleoenvironmental reconstruction etc. It is essential to the petroleum industry as a tool for defining geologic constraints on prediction of exploration risk and modeling reservoir simulation. Deep-water agglutinated foraminifera have been used during hydrocarbon explorations since the 1970's when the first Deep Sea Drilling Programme (DSDP) established their value for both biostratigraphical and palaeoenvironmental studies (Gradstein & Berggren, 1981). Planktonic foraminifera are good stratigraphic indicators of the interval covering the Jurassic to present, while benthic foraminifera are found since the Cambrian (Ordovician to Present for calcareous species). They are very good biostratigraphic markers within marine environment. The use of foraminiferal analysis in this study enables us to determine biostratigraphic zonation of the sediments penetrated by the drill (Lucas & Ononeme, 2019; Fregene et al., 2021).

II. STRATIGRAPHY OF NIGER DELTA

2.1 Benin Formation

This is the uppermost unit in the basin and predominantly (over 90%) sandy with isolated clay/shale intercalations. The sands are coarse grained, granular, poorly sorted, subangular to well rounded. They are white or yellowish-brown and contain thin lignite streaks and wood fragments. The sediments are of continental to deltaic plain origin. The sands and sandstones may represent point bar deposits, channel fills and natural levees, whereas the shales may be interpreted as back swamp deposits or ox-bow fills. The Benin Formation is thicker in the central onshore part of the delta where it reaches about 2,000m (Avbovbo, 1978) and thins outwards towards the delta margins. It ranges in age from Oligocene to Recent.

2.2 Agbada Formation

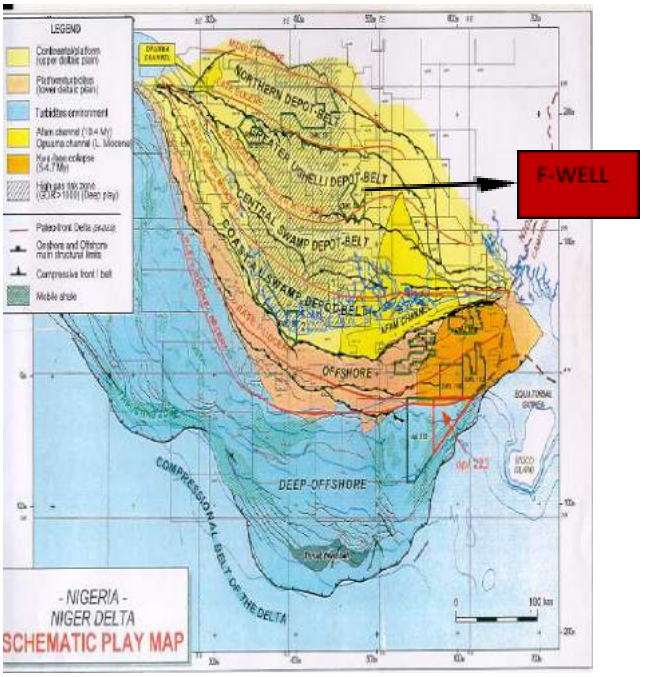
This unit comprises cyclic sequences of alternating sands (fluviatile, coastal, and fluviomarine) and marine shales. Two (2) distinct intervals are easily recognizable: an upper sandy unit with minor shale intercalations and a more marine lower unit in which the shaly sections become prominent. The sandstones and sands are very coarse to very fine grained, unconsolidated or slightly consolidated and poorly sorted. Lignite streaks are common. The shales are grey and dense at the base becoming markedly sandy and silty upward. The Agbada Formation is up to 4,000m thick in the central part of the delta, thinning seaward and towards the delta margins. It is Eocene to Recent in age.

2.4 Akata Formation

This is the basal unit of the Niger Delta complex. It consists of uniformly developed shales deposited in an open marine environment. There is the presence of some sand beds considered to be of continental slope, channel-fills and turbidites (Weber & Daukoru, 1975). The formation is largely under compacted (overpressured). The actual thickness is not known due to inability to penetrate the formation fully except on the basin flanks. The age span is Eocene to Recent.

III. LOCATION OF WELL

F-Well is a well drilled to a total depth of 10,185 feet. It is located in the Greater Ughelli Depo belt of Niger Delta basin defined by the following coordinates: Between Longitude 6°E and 7° E and Latitude 5°N and 6° N.



Source: Nwozor et al., 2013

Figure 1: Location Map of F-Well

IV. MATERIALS AND METHOD

4.1 Foraminifera Slide Preparation

Labeling and Weighing: 20g of each collected sample was weighed, packaged and labeled accordingly indicating the well name, sample type and depth.

Soaking: Bowls were labeled for indicated sample depths contained and soaked with kerosene for about four (4) hours after which the samples were decanted. Water was later added to the labeled samples and allowed to stay/ soak overnight.

Wet sieving and Drying: Samples were washed through 230mesh sieve with 63 micron (um) aperture under running tap water with a shower head. Washedsamples were dried on hot plate at about 60°C for about 45minutes.

Dry sieving and Bottling: A set of micro sieves (coarse, medium and fine) was stacked on each other and dried residue for each sample was run through them and sieved manually. The respective fractions were collected and bottled in three (3) already cleansed and properly labeled bottles.

Picking: Each fraction was spread on a gridded foraminifera tray of 4.5 by 6.0cm and moved along definite traverses to pick observed foraminifera under centered binocular microscope. Using a picking needle recognized fossils were picked and placed in the cavity of appropriately labeled slide. The recovered foraminifera were recorded in a picking sheet.

Splitting: This is the sorting/separation and grouping of fossils according to their morphological similarity. Different species are grouped together with the tip of a moistened fine brush and stocked in 10s, 20s, and 50s depending on the richness of the interval on the slide and glued onto the slide with a gum.

Analysis: Identification of the picked foraminifera was done with the aid of type collection and foraminifera album considering the test composition, chambers arrangement, sutures, aperture, habits and ornamentation. The results of the micro fauna analysis are plotted on range and distribution charts to show the sequence of occurrences of the species. The groups of species identified will be described systematically later.

Dating and Biozonation: Age was determined based on the presence of marker species and correlated with the published chronos-tratigraphy of Haq*et al.,*(1988) and Harland *et al.,* (1990). The F-zones are of immense help in recognizing MFS and in understanding the cycle concept as well as sequence stratigraphy.

Zones were delineated in the well based on the recognition of the last appearance datum and first appearance datum of important diagnostic species. Furthermore/ maximum/minimum fauna abundance/diversity peaks were also employed to assist in the correlation of the determined horizons to global bioevents.

V. RESULT AND DISCUSSION

5.1 Sedimentology

The sedimentologic description of F-Well was carried out on six hundred and seventy nine (679) Ditch Cutting samples with the aid of both visual and a reflected light microscope with the guide of a standard textural comparison chart showing grain sizes, shapes and degree of sorting. The Sedimentological analysis allowed the erection of one hundred and three (103) lithozones of Clayey Sandstone, Sandstone, Sandy Shale, Shale and Shaly Sandstone lithofacies based on the textural properties observed. Associated minerals which include: Quartz, Iron oxide, and Mica were identified. Fifty (50) Shale and Sandy Shale lithofacies were collected at various intervals for Standard foraminifera analysis.

Table 1: Lithology and Lithozones with Depth

DEPTH(FT)	LITHOLOGY	LITHOZONES
15 – 1,995	Sandstone	1
2,010 – 2,175	Clay	2
2,190 – 2,535	Clayey sandstone	3
2,550 – 2,595	Shaly sandstone	4
2,610 – 2,970	Sandstone	5
2,985 – 3,015	Sandy shale	6
3,030 – 3,105	Shaly sandstone	7
3,120 – 3,150	Sandstone	8
3,165 – 3,315	Shaly sandstone	9
3,330 – 3,345	Sandstone	10
3,360 – 3,405	Shaly sandstone	11
3,420	Sandstone	12
3,435 – 3,495	Shaly sandstone	13
3,510	Sandy shale	14
3,525 – 3,600	Shaly sandstone	15
3,615 – 3,660	Sandstone	16
3,675 – 3,705	Shaly sandstone	17
3,720 – 3,765	Sandy shale	18
3,780 – 3,795	Shaly sandstone	19
3,810	Sandy shale	20
3,825 – 3,885	Sandstone	21
3,900 – 3,930	Shaly sandstone	22
3,945 – 4,155	Sandstone	23
4,170	Clay	24
4,184 – 4,785	Sandstone	25
4,800 – 4,845	Shaly sand	26
4,860	Sandstone	27
4,875	Shaly sandstone	28
4,890	Sandstone	29
4,905 – 5,280	Sandstone	30
5,295	Shaly sandstone	31
5,310 – 5,385	Sandy shale	32
5,400 – 5,505	Sandstone	33
5,520 – 5,550	Shaly sandstone	34
5,565 – 5,595	Sandstone	35
5,610 – 5,715	Shaly sandstone	36
5,730 – 5,985	Sandstone	37
6,000	Shaly sandstone	38
6,015 – 6,030	Sandstone	39
6,045	Shaly sandstone	40
6,060	Sandy shale	41
6,075 – 6,090	Sandstone	42

6,105 – 6,180	Shaly sandstone	43
6,195 – 6,225	Sandstone	44
6,240	Shaly sandstone	45
6,255 – 6,330	Sandstone	46
6,345	Shaly sandstone	47
6,360 – 6,525	Sandstone	48
6,540 – 6,600	Shaly sandstone	49
6,615	Sandy shale	50
6,630 – 6,690	Shale	51
6,705 – 6,735	Sandstone	52
6,750 – 6,810	Shaly sandstone	53
6,825 – 6,900	Sandstone	54
6,915 – 6,960	Shaly sandstone	55
6,975 – 6,990	Sandstone	56
7,005 – 7,020	Shale	57
7,035	Sandy shale	58
7,050 – 7,065	Shaly sandstone	59
7,080	Sandstone	60
7,095	Shaly sandstone	61
7,110	Sandstone	62
7,125 – 7,170	Shaly sandstone	63
7,185	Sandstone	64
7,200 – 7,260	Shaly sandstone	65
7,275 – 7,305	Sandstone	66
7,320 – 7425	Shaly sandstone	67
7,440 – 7,500	Sandstone	68
7,515	Shaly sandstone	69
7,530 – 7,560	Sandstone	70
7,575 – 7,590	Shaly sandstone	71
7,605 – 7,665	Sandstone	72
7,680	Shaly sandstone	73
7,695 – 7,710	Sandstone	74
7,725 – 7,755	Shaly sandstone	75
7,770 – 7,830	Sandy shale	76
7,845 – 7,860	Sandstone	77
7,875	Shaly sandstone	78

7,890 – 7,905	Sandstone	79
7,920 – 7,965	Shaly sandstone	80
7,980 – 8,010	Sandstone	81
8,025 – 8,100	Sandy shale	82
8,115 – 8, 130	Sandstone	83
8,145	Sandy shale	84
8,160 – 8,235	Shaly sandstone	85
8,250 – 8,325	Sandy shale	86
8,340 – 8,550	Shale	87
8,565	Sandy shale	88
8,580 – 8,595	Shaly sandstone	89
8,610 – 8,670	Shale	90
8,685 – 8,730	Sandy shale	91
8,745 – 8,760	Shale	92
8,775 – 8,865	Sandy shale	93
8,880 – 9,210	Shaly sandstone	94
9,225 – 9,240	Sandy shale	95
9,255 – 9,450	Shaly sandstone	96
9,465 – 9,780	Shale	97
9,795	Sandy shale	98
9,810	Shaly sand	99
9,825	Sandy shale	100
9,840 – 10,005	Shale	101
10,020 – 10,095	Sandy shale	102
10,110 – 10,185	Shale	103

5.2 Foraminiferal Abundance

A total of Eighty (80) foraminiferal species were recorded, most of the species recorded are calcareous and arenaceous benthic foraminiferal species. Planktic foraminiferal species are generally scarce in the well. The non-recovery of planktic and the general poor recovery of foraminiferal species might be due to environmental factor.

Species recorded include: *Haplophragmoides sp, Bathysiphon sp., Pori Textularia Panamensis, Haplophragmoides Compressa, Trochamminasp, Floriluscostiferum, Calcareous indeterminate, Ammobaculites sp., Pori Textularia Panamensis, Bolivina sp., Arenaceous indeterminate, Calcareous indeterminate, Pori Textularia Panamensis, Uvigerina Sparsicostata, Haplophragmoides Arvensis, Arenaceous indeterminate, Spirosplectamina Wrightii, Hanzawaia concentric, Hopkinsinabemoniensis, Brizalina imperatrix, Ostracod, Epistominella Vitrea, Hanzawaia concentric, Haplophragmoides Arvensis, Fursenko Punctata, Valvulineria sp., Fissurina sp., Bathysiphon sp., Hanzawaia concentric.*

Table 2: Foraminiferal distribution chart for F - Well

S/N	DEPTH(FT)	*Haplophragmoides sp*	*Bathysiphon sp.*	*Poritextularia panamensis*	*Haplophragmoides compressa*	*Bathysiphon sp*	*Trochammina sp*	*Florilus costiferum*	*Calcareous indeterminate*	*Ammobaculites sp.*	*Bolivina sp.*	*Arenaceous indeterminate*	*Uvigerina sparsicostata*	*Haplophragmoides narivaensis*	*Spirosplectammina wrightii*	*Hanzawaia concentric*	*Hopkinsina bemoniensis*	*Brizalina imperatrix*	*Ostracod*	*Epistominella vitrea*	*Spiroplectammina wrightii*	*Ammobaculites sp*	*Fursenkoina punctata*	*Valvulineria sp.*	*Fissurina sp.*	*Ammobaculites agglutinans*	*Anomalina sp.*	*Bolivina dertonensis*	*Cassigerinella chipollensis*	*Biozones (This Study)*	*F-Zones (Blow 1969, 1979)*
1	2010ft																													BARREN
2	2055ft																														
3	2085ft																														
4	2100ft																														
5	2145ft																														
6	3015ft																														
7	3735ft																														
8	5325ft																														
9	5370ft																														
10	6645ft																														
11	7035ft																														
12	7770ft																														
13	7800ft																														
14	7830ft																														
15	8025ft	1																													N4 – N3
16	8040ft																												A		
17	8055ft		1																												
18	8070ft			3	1																								B		
19	8085ft																														
20	8100ft																														
21	8145ft																														
22	8250ft			1		1	1																						C		
23	8265ft							1	1																						
24	8295ft									1																			D		
25	8310ft																														
26	8340ft			1																											
27	8370ft																														
28	8385ft										1	1																			
29	8400ft								2																				E		
30	8430ft																														
31	8460ft	1		1																											
32	8490ft																														
33	8520ft																														
34	8535ft								1				1																		
35	8610ft																												F		
36	8620ft																														
37	8745ft	1																													
38	8835ft																														
39	8865ft																														
40	9240ft			1								1		1																	
41	9480ft			3						1				1	2	3													G		
42	9540ft													1	1	3	1	1	1	1											N3 – N2
43	9555ft		1	2																2	2	1	1	1	1	1					
44	9660ft	1		2								1		1				1			1						1		H		
45	9765ft			4				1		1						1					2					1					
46	9855ft	1															1											1	1		
47	9870ft	1																								1				I	
48	9975ft																														
49	10155ft																														
50	10170ft																														

5.3 Foraminiferal Zonation

The foraminiferal zonation of the well was guided by the works of Blow (1969, 1979). Though planktic foraminiferal species are generally scarce in the well but benthic foraminiferal species whose stratigraphic distributions have been well established in the Niger Delta and have been caliberated with planktic foraminiferal species were used to assign ages and zonation in this Well. The non-receovery of planktic and the general poor recovery of foraminiferal species might be due to environmental factor.

Important foraminiferal bio-events considered include:

- First Downhole Occurrence (FDO) of chronostratigraphically significant planktic/benthic foraminiferal species.
- Last Downhole Occurrence (LDO) of planktic/benthic foraminiferal marker species.
- Foraminiferal abundance and diversity peaks dated with foraminiferal markers species whose stratigraphic ranges are well established world wide.

Table 3: Foraminifera Biostratigraphic summary of the Well

(First Downhole Occurrence of stratigraphically important Foraminifera species)				
Depth (ft)	Epoch/Period	Age (Ma)	Zones (Blow 1969, 1979)	Significant Foraminifera data
2,010	*First sample analysed*			
2,010 – 8,000	Indeterminate	-	Indeter-minate	Interval barren of foraminifera species
8,000-9,400	Early Oligocene – Early Miocene	22.2 - 24.3	N4 – N3	Interval characterized by occurrences of *Spiroplectammina wrightii and Uvigerina parsicostata.*
9,400 – 10,000	Early Oligocene	24.3 - 33.0	N3 – N2	*Hanzawaiaconcentrica, Hopkinsinabemoniensis, Brizalina imperatrix and B olivinadertonensis*

Index species among the recovered foraminifera assemblages have been used in dating and zoning the intervals. Details are given below:

Planktic Zone n4 – n3

Early Miocene - Oligoocene
Interval: 8,000ft – 9,400ft
Estimated numerical age: 22.2 - 24.3Ma

Definition

The top of this zonal interval which ought to be marked by the FDO of *Ammonia becarril was absent* but was estimated to be 8,000 ft.
The base of this zonal interval is marked by the LDO of *Epistominellavitriea* at 9,400 ft.

Features

- ✔ Interval is characterized by benthic foraminifera species.
- ✔ Interval characterized by the co-occurrence of *Spiroplectamminawrightii and Uvigerinasparsicostata*. Signifying (N4-N3) Early Miocene - Oligocene age.

Planktic Zone n3 – n2

Interval: 9,400ft – 10,000ft
Estimated numerical age: 24.3 - 33.0Ma

Definition

The top of this zonal interval is marked by the FDO of *Bolivina imperatrix* at 9,400 ft.
The base of this zonal interval is marked by the LDO of *Spirosplectaminawrightii* at 10,000 ft.

Features

- ✔ Interval is characterized by benthic foraminifera species.
- ✔ Interval characterized by the co-occurrence of *Hanzawaiaconcentrica, Hopkinsinabemoniensis, Brizalina imperatrix and Bolivinadertonensis* signifying (N3-N2) Oligocene age.

VI. INFORMAL FORAMINIFERA BIOZONATION

A total of nine foraminifera assemblage biozones have been identified. These are compared with works of Blow (1969, 1979). The biozones are coded and discussed alphabetically in ascending order below from A-I.

1. Haplophragmoides Sp Assemblage Biozone A

Reference section: F-Well 8,025ft-8,055ft
Definition: Selected species last appearing include Haplophragmoides Sp. and Bathysiphon Sp.

The top of the biozone is defined by the last stratigraphic occurrence of HaplophragmoldesSp and the base by Bathysiphon Sp.

2. Bathysiphon Sp Assemblage Biozone B

Reference Section: F-Well 8,055ft-8,250ft
Definition: Selected species last appearing include BathysiphonSp.andTrochammina Sp.

The top of this biozone is defined by the last stratigraphic occurrence of Bathysiphon Sp. and the base by the last appearing of Trochammina Sp.

3. Trochammina Sp. Assemblage Biozone C

Reference Section: F-Well 8,250ft-8,295ft

Definition: Selected species last appearing include Trochammina Sp. and AmmobaculitesSp,

The top of this biozone is defined by the last stratigraphic occurrence of Trochammina Sp. and the base by the last occurrence of AmmobaculitesSp,

4. Ammobaculites Sp Assemblage Biozone D

Reference Section: F-Well 8,295ft-8,385ft
Definition: Selected species last appearing include AmmobaculitesSp,and Arenaceous Indeterminate.

The top of this biozone is defined by the last stratigraphic occurrence of AmmobaculitesSp,and the base by the last occurrence of Arenaceous Indeterminate.

5. Arenaceous Indeterminate Assemblage Biozone E

Reference Section: F-Well 8,385ft-8,535ft
Definition: Selected species last appearing include Arenaceous Indeterminate and Uvigerina Sparsicostata.

The top of this biozone is defined by the last stratigraphic occurrence of Arenaceous Indeterminate and the base by the last occurrence of Uvigerina Sparsicostata.

6. Uvigerina Sparsicostata Assemblage Biozone F

Reference Section: F-Well 8,535ft-9,240ft
Definition: Selected species last appearing include Uvigerina Sparsicostata and Haplophragmoides Narivaensis.

The top of this biozone is defined by the last stratigraphic occurrence of Uvigerina Sparsicostata and the base by the last occurrence of Haplophragmoides Narivaensis.

7. Haplophragmoides NarivaensisAssemblage Biozone G

Reference Section: F-Well 9,240ft-9,540ft
Definition: Selected species last appearing include Haplophragmoides Narivaensis and Epistominella Vitriea.

The top of this biozone is defined by the last stratigraphic occurrence of Haplophragmoides Narivaensis.and the base by the last occurrence of Epistominella Vitriea.

8. Epistominella Vitriea Assemblage Biozone H

Reference Section: F-Well 9,540ft-9,855ft
Definition: Selected species last appearing include Epistominella Vitriea and last appearance of Cassigerinella Chipollensis

The top of this biozone is defined by the last stratigraphic occurrence of Epistominella Vitriea and the base by the last occurrence of Cassigerinella Chipollensis.

9. Cassigerinella Chipollensis Assemblage Biozone I

Reference Section: F-Well 9,855ft-10,170ft
Definition: Selected species last appearing include Cassigerinella Chipollensis

The top of this biozone is defined by the last stratigraphic occurrence of Epistominella Vitriea and the base could not be determined.

VII. CONCLUSION

This study was carried out on ditch cutting samples. The lithology of the well is composed of grey to dark grey shale and sandy shales. Biostratigraphic characteristic of the well were analysed using foraminifera. Two planktic forminiferal zones were recognized N4-N3 and N3-N2 zones while nine assemblage zones A - I according to the present study. The studied intervals in the well are dated Early Miocene - Oligoocene. Using the lithologic and foraminiferal studies, it is inferred that the intervals penetrated by the well correspond to Agbada Formation. The alternation of shales and sandy shales/mudstones within the sequence provides the combination of source, reservoir and cap rocks essential for hydrocarbon generation, accumulation and trap.

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INTRODUCTION

We read two articles authored by Mahato et al. 2020 [1] and Kumar S. 2020 [2] with great interest, which depict the status of air quality in metropolitan cities across India. The study conducted by Mahato et al. 2020 [1] principally deals with the air quality assessment in New Delhi, whereas Kumar [2] has evaluated the same in major cities like Mumbai, Ahmedabad, Kolkata, Hyderabad and Chennai. In the wake of COVID-19 pandemic, the Government of India had commenced 4 phase confinement across all the states. This continued from 25th March to 31st May 2020, during which the dramatic reduction in air pollution level has been reported due to diminished usage of vehicles and closed factories [3,4,5].

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Effect of COVID-19 on Air Pollution: The Indian Scenario

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I. INTRODUCTION

We read two articles authored by Mahato et al. 2020 [1] and Kumar S. 2020 [2] with great interest, which depict the status of air quality in metropolitan cities across India. The study conducted by Mahato et al. 2020 [1] principally deals with the air quality assessment in New Delhi, whereas Kumar [2] has evaluated the same in major cities like Mumbai, Ahmedabad, Kolkata, Hyderabad and Chennai. In the wake of COVID-19 pandemic, the Government of India had commenced 4 phase confinement across all the states. This continued from 25th March to 31st May 2020, during which the dramatic reduction in air pollution level has been reported due to diminished usage of vehicles and closed factories [3,4,5].

Mahato et al. [1] evaluated the concentrations of particulate matter (PM) and other gasses like CO and NO_2 (an indicator of $PM_{2.5}$) before and after lockdown in New Delhi. Later, it was revealed that PM_{10} and $PM_{2.5}$ concentrations were reduced by 50% compared to pre-lockdown conditions. Similarly, pollutants like CO (35% reduction) and NO_2 (52.68% reduction) have been decreased. The study also reported 40%-50% recovery in air quality just after 4 days of lockdown initiation. The National Air Quality Index (NAQI) has observed 31%-54% reduction around New Delhi [1]. Owing to lower power demand in manufacturing, use of fossil fuels or sources of renewable energy has been drastically reduced. In addition, Kumar S has reported effects of confinement on air quality in other major cities. In this study, steep decreases in aerosol concentration and PM_{10}, $PM_{2.5}$ and NO_2 were reported in Kolkata, Mumbai, Chennai, Ahmedabad, and Hyderabad. The concentration in aerosols (AOD) and NO_2 got reduced by 60% and 45% in these cities. In a key finding, he has revealed that meteorological factors like temperature and humidity do not play any role in the reduction of viral load. He has also stated the Government restrictions could only be able to reduce the effects in the absence of specific treatment options [2].

Further reduction in the level of air pollutants can lead to the reduction in several respiratory and cardiovascular problems being reported. India has been experiencing this problem since time immemorial, despite implementing several laws regarding the emission control from automobiles, factories and religious festivals [2,6]. Although several studies have reported the adverse effects and suggestions on health due to the air pollution, there have been no implementations of any restrictions on controlled usage of automobiles and running the industries [7,8]. But due to the inevitable confinement situation, the air quality has been improved substantially, supporting these studies. However, in their studies Mahato et al. 2020 [1] and Kumar S. 2020 [2] have focussed on urban population, which has been the main source of air pollution. But it remains unknown that the contribution from rural areas has also been significant. Surveillance on rural and suburban areas also needs to be conducted. Also, other major pollutants like SO_2 and CO need to be monitored [9,10,11]. Though it appears to be less compared to urban regions, factors like usage of firewood, charcoal, shifting cultivation, and forest

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fires ubiquitously lead to the air pollution in rural areas. Despite the promotion of pollution free measures like biogas production, air quality has been depleting in rural regions, which could be a major threat in future [12,13]. We conclude that, COVID-19 resultant lockdown has brought us a reminder of our detrimental activities on nature and their results on mankind and has shown us the path which can lead to a clear and better environment [14,15,16,17].

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