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Examination and screening of the perceived toxicity influence of produced wastewater using Allium cepa assay and health risk model as monitoring tools

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ABSTRACT

This research work evaluated the likely cyto-genotoxic influence of produced effluent using *Allium cepa* assays and the health risk models. The physical and chemical analysis carried on the effluent showed no significant variation at p>0.05. The results from the microscopic assessment revealed that the effluent induced clastogenic influence; chromosomal induction and mitotic impact on the *A. cepa* cell. The results of the macroscopic analysis showed that the effluent induced root inhibitions on *A. cepa* roots, resulting in the various deformations; crotchet, c-tumor, twists and spiral roots in all the concentrations exempting the control. Findings from the study showed that there were concentration-reliant inhibitions of the root development of the *Allium cepa*, the index mitosis and stimulation of the chromosomal irregularities. The results obtained for the assessment of the health risk, exhibited that the hazard quotient (HQ), hazard index (HI) and chronic daily intake (CDI) for the ingestion and dermal non-carcinogenic pathways of metals in the effluent were < 1, demonstrating no health danger. However, the result of the carcinogenic assessment for Pb was 2.55E-04, which was slightly above the slated unity limit and revealed serious risk. The findings of this research work, exposed that the effluent was possibly cyto-genotoxic mediator and germ-cell disruptor, which may elicit antagonistic healthiness influence on unprotected persons. There is the need to treat the effluent in order to protect humans and aquatic lives from the deleterious impacts it portends.

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1.0 Introduction

The rate of environmental degradation as a result of intense human activities (pesticides and herbicides from farming, industrial spills of crude oil, road construction) and natural occurrences such as volcanic eruption and earthquake, can increase the background concentration of natural chemicals in the environment. Intense technological, population growth and economic progress have also been associated with the increase of pollutants in the environment. Of recent, the increase rate of pollutants from source points into terrestrial soil and aquatic ecosystems, have gone beyond unquantified safe limits as set by international and national regulatory bodies.

The oil rich regions of Southern Nigeria have been faced with serious economic problems, health and social concerns for the past 40 years. The greatest pollution threats have been sourced from crude oil pollution (Akpojivi and Akumagba, 2005). The terrestrial and aquatic bodies are not only impacted by this crude oil pollutant but also the lives therein are influenced by the contaminants. Some of the species that are not able to migrate from the pollution sources accumulate the toxic compounds into their bodies while others are killed by them (Bagchi, 2004; Adeyemi et al., 2009). The biotic communities experience the natural and human impact that results in environmental degradation, deterioration and depletion of the natural resources, which have resulted in the substantial influence of the chromosomal makeup of flora and fauna therein (Harwell et al., 1994).

In crude oil exploration and production processes, to attain maximum crude oil reclamation output, injection of additional water is needed. However, the crude oil has water that comes with it. So, both the injection and the formation water are term produced water (Veil et al., 2004). These waters contain high residues of pollutants such as total dissolved substances (TDS), total suspended substances (TSS), sodium chloride, sulphates, grease, oil, heavy metals, bacteria and some associated chemicals (USEPA, 2000; Isehunwa and Onovae, 2011; Ozgun et al. 2013; Olorunfemi et al., 2014 and 2015a and b; Martel-Valles et al. 2016; Hedar and Budiyono 2018; Danforth et al. 2020; Rodriguez et al. 2020). Produced water ejection into the environment has been known to occur indiscriminately and has not yet met the required statutory standards in Nigeria water bodies and land (Onojake and Abanum, 2012).

The purple *Allium cepa* bulb (L., 2n=16) has been used for decades in the screening of environmental toxicants (Fiskesjö, 1997; Olorunfemi et al. 2011, 2015b; Fadoju et al, 2020). It has been recounted to stand first amongst the 7 flora bioassays commonly used as standard protocol for testing and monitoring wastes toxicants in the environment (Olorunfemi et al. 2015b). More so, it is widely used in the screening of not only toxicants but in the quality validation of rain and river water for effective consumption.

Nonetheless, recent and current studies have documented the utilization of *Allium cepa* in the screening of toxic effluents in the environment Fiskesjö (1997), Leme and Marin-Morales (2009) Olorunfemi et al. (2015 a, b), Rosculete et al. (2019). The valuation of the probable exposure of concerned environmental contaminants in humans has been certified by US EPA (2001) and Crentsil and Anthony (2016).

This study aims at the screening of the possible toxicity of produced water with *Allium cepa* assay and its potential health risk evaluation.

2.0 Methodology

2.1 Area of Study

2.1.1 Contextual of the Area Studied

Ogboinbiri is one of the communities in Bayelsa state. Ogboinbiri is located at latitude N 50’ O”N and longitude 4 58’O”E Figure 1. The study area has little life forms (plants and animals) sparsely distributed around the oil field onshore and offshore. The company produces crude oil and gas. The collected materials were gotten from three sampled reservoir sites or stations which were located close to a water body. The social-economic activities in Ogboinbiri are mainly; fishing and farming because of the rich water vegetation body surrounding the community.

2.1.2 Climatic conditions

The climate of Ogboinbiri has an average rainfall of 1500–4000 mm per year during the wet and the dry seasons. The average mean temperatures vary between 23-33 °c.

2.2 Sampling technique

The protocol of Olorunfemi et al. (2014) was employed in the collection of the effluents.

2.2.1 Digestion and analysis of the chemical and physical constituents of produced effluents

The procedures of the US EPA, (2009) and NESREA, (2009) were used in the digestion and analysis of 8 physical and chemical, as well as 13 metal parameters.

2.3 Biological materials

Allium cepa (brown onions) belonging to the family *Amaryllidaceae* (2n=16), was used for this study. They were sun-dried for about two weeks before being used for the assay. During that period, rotten bulbs were thrown away and the healthy ones were used.

2.3.1 Macroscopic and microscopic evaluation

The improved *Allium cepa* assay protocols by Fiskesjo (1997), Bakare et al. (2009), Babatunde and Anabuike (2015), Olorunfemi et al. (2015 a, b), Fadoju et al. (2020) were utilized in this study. Hundred millilitres (100 ml) of glass holding 0.5, 1.0, 2.5, 5 and 10 % concentrations (volume/volume), tap/effluent water were utilized for the test trial set up. The squashing technique for chromosomal analysis (microscopic evaluation) by Olorunfemi et al. (2011 a, b) was employed on the second day (48h) to the root *Allium cepa* tips for the control and experimental setup. The various stages of mitosis as well as the chromosomal aberrations were scored using a light microscope (Nikon Eclipse E400) at the 1000* power of magnification of the sum 5000 from five slides (Olorunfemi et al., 2015 a, b). On day four (96h) of the trial set up, the root growth inhibition was estimated for both the control and treatment groups using the method set by Olorunfemi et al. (2012). 12 *Allium cepa* bulbs were grouped in all concentrations, out of which the best ten presenting excellent root development were carefully chosen for examination of root-growth deformities. The influence of the effluent on the root growth morphology was also scrutinised. The Bresser model was used in the photomicrographs with a Sony Mavica Digital camera,

2.4 Biological and data analysis

The MI (mitotic index) and the incidence of CA (chromosomal aberrations) was estimated using the method established by Fiskesjö (1997) and Bakare et al. (2000).

Numerical data were estimated as mean ± SE (standard errors) and later exposed to one-way ANOVA (Analysis of Variance) and DMRT (Duncan multiple regression Test) to ascertain the level of significant differences at 0.05 using SPSS version 23.0 for windows. The Microsoft Excel package 2013 edition was used to estimate the health risk.

3.0 Results

3.1 The outcomes of the physical, chemical and metal characteristics of produced effluent

Table 1 shows the outcomes of the physical, chemical and metal characteristics of produced effluent. It was observed that there was a significant difference in the mean values of all the characteristics of the effluent at P>0.05. There were also high variability of the mean, minimum and maximum values of sulphates [50.17 (25.89-84.00) mg/l], conductivity [2793.33 (1560.23-6780) µs/cm], alkalinity [274.53 (1.10-819.4) mg/l], phosphate [44.00 (33.30-79.5) mg/l], sodium [60.30 (65.25-110) mg/l], calcium [48.80 (20.08-65.8) mg/l] and potassium [55.57 (35.33-85.5) mg/l] contents in the effluent. Most of the mean values slightly surpassed the set standards.

3.2 The outcomes of the macroscopic evaluation of produced effluent

The outcomes of the assessment of the root-growth of *A. cepa* tubers grown in produced effluent as related to the control, are shown in Table 2 and Figure 4. The root-growth reduction was concentration dependent. The *A. cepa* exposed to produced effluent displayed slightly root reduction as related to the control. This can be observed in the average length of *A. cepa* root cultivated in 0.5 % (3.72 ± 0.21 cm) and 10 % (2.34 ± 0.10 cm). The roots were characterized mainly by crochets (roots twisted up similar to hooks) and twists Figure 2a-e. Others aberrations are spiral, c-tumor and crotchet roots Table 3.

3.3 The outcomes of the morphology, colour and of shape *A. cepa* roots exposed to produced effluent

Table 3 shows the morphological, colour and shape characteristics of *A. cepa* roots to the various effluents concentrations at 0.5, 1.0, 2.5, 5.0 and 10%. No change in colour of roots were observed. However, the colour of roots turned pale and black during the range finding test at 25 and 30% effluent (produced water) concentration.

3.4 The outcomes of the microscopic evaluation of produced effluent

The outcomes of the evaluation of the exposure of the effluent in the microscopic root cells of the onion are shown in Table 4. The tips of *A. cepa* exposed to produced water were chockfull with cells that are anomalous in all the produced effluent concentrations.

The results of the comparison of *A. cepa* bulbs exposed to produced effluent in relation with the control are presented in Table 4 and Figure 5. All the tested effluents elicited chromosomal abnormalities in all the concentrations and they were not statistically significance at (p>0.05). Frequent irregularities observed, were stickiness, bridges, C-mitosis and vagrants (Figure 3a-e).

3.5 The outcomes of the health risk evaluation of the produced effluent

The outcomes obtained in the health risk assessment, showed that the non-carcinogenic evaluation; HQ, HI and CDI for the ingestion and dermal pathways of metals in the effluent were <1 Table 5. However, the result of the carcinogenic assessment for Pb was 2.55E-04, which was slightly above the slated unity limit Table 6.

4.0 Discussions

4.1 The characteristics of the chemical, physical and metal in produced effluent

In this study, the chemical, physical and metal characteristics of produced effluent analysed, were found in appreciable level for lead, copper and zinc. Sulphate, conductivity, alkalinity, phosphate, sodium, calcium and potassium had very high varied mean values. Phosphates were present in concentrations above the permissible limits. The high conductivity level showed that the effluent can conduct electrical current. Similar findings have also been recorded by Ozgun et al. (2013), Martel-Valles et al. (2016), Hedar and Budiyono (2018), Danforth et al. (2020) and Rodriguez et al. (2020).

This can also be related to the high presence of ions of metals and non-metals (Cl^-, SO_4^{-2}, Na^+, Ca^{2+} and K^+) and high concentration of alkaline substances. The high presence of Ca^{2+}, reveal that the waste water cannot form lather. The implication of this is that aquatic animals can be hindered or influenced greatly negatively by the presence of the contaminants and will not thrive better therein.

4.2 The characterization of the microscopic assay

The microscopic evaluation of the effluent showed that there were mitotic depressions in the chromosomes of the *A. cepa* consequence of its clastogenic influence. There were evidences of chromosomal induction and mitotic impact of the *A. cepa* cell, resulting to various aberrations; C-mitosis, vagrants, bridges and stickiness. Similar findings were documented by Mangalampalli et al. (2017), Bonciu et al. (2018), Rosculete et al. (2019) and Sabeen et al. (2020) in different effluents. Findings from the study showed that there were concentration-dependant inhibitions of the onion root development, mitotic index and initiation of chromosomal anomalies.

Chromosomal stickiness may be as a result of intensification of the chromosome condensation and contraction or the depolymerization of the hereditary structure Bakare et al. (2009) as well as the disbanding of the nucleoproteins Olorunfemi (et al., 2015 a, b). The presence of C-mitosis may designate the inhibitory impact of the effluent on the establishment of the spindle fibers or tubargenic influence as a result of the clastogenic impact (Shahın and El-Amood, 1991). At times, induced stickiness and C-mitosis on chromosomal structures cannot be reversed Fadoju et al. (2020) and might result to subsequent demise of the cell. In other words, aberration caused by influence of environmental stressors can either be passed on from generation to generation or been repaired depending the level of impact by the clastogens. Dislocation and asymmetrical separation of the chromosomes, which might constitute a hazard of aneuploidy might be as a result of vagrant aberration (Babatunde and Anabuike, 2015; Olorunfemi et al., 2015 a; Fadoju et al., 2020).

4.3 The characterization of the macroscopic assay

The results of the macroscopic analysis after 4 days (96 hours), showed that the effluent induced root inhibitions on *A. cepa* roots, resulting in the various deformations; crotchet, c-tumor, twists and spiral roots in all the concentrations exempting the control. The screening of the effluent with *A, cepa* in this study, revealed that effluent is highly clastogenic. The findings from this study indicate that there were slight mitotic index and more abnormalities of the *A. cepa* cell as the concentration increase. The higher the dividing cells the lower the percentage of aberrant cells, while the lower the dividing cells the higher the percentage of the aberrant cells. This is in consonance with what was obtained by Fiskesjo (1997), Bakare et al. (2009), Babatunde and Anabuike (2015), Olorunfemi et al. (2015 a, b), Fadoju et al. (2020).

4.4 The characterization of the probable risk associated with health

In this study, the results obtained from the probable risk associated with health, showed that the ingestion and dermal non-carcinogenic pathways of metals in the effluent were < 1, demonstrating no health risk. However, the result of the carcinogenic assessment for Pb was 2.55E-04, which was slightly above the slated unity limit and revealed serious risk. The findings from this research work, exposed that the effluent was possibly cyto-genotoxic mediator and germ-cell disruptor, which may elicit antagonistic healthiness influence on unprotected persons. Similar findings have also been reported in several toxic media by Crentsil and Anthony (2016), Anani and Olumokoro (2018 a, b) and Enuneku et al. (2018 and 2019).

Table 1: Summary of the chemical and physical characteristics of produced effluent

Parameters N=3	Units	Produced water Mean± S.E	Min	Max	NESREA (2009) LIMIT	USEPA (2009) LIMIT	P-Value
pH		7.71±0.15	7.5	8.00	6-9	6.5.-8.5	P>0.05
Ammonia	mg/l	0.37±0.19	0.00	0.60	1	0.03	P>0.05
Sulphates	mg/l	50.17±25.59	25.89	84.00	NS	NS	P>0.05
Hardness	mg/l	8.90±8.06	10.60	25.00	NS	0-75	P>0.05
TDS	mg/l	0.03±0.02	0.00	0.07	500	500	P>0.05
Conductivity	µs/cm	2793.33±2046.2	1560.23	6780	NS	NS	P>0.05
Nitrates	mg/l	1.01±0.95	0.05	2.90	10	10	P>0.05
Alkalinity		274.53±272.43	1.10	819.4	150	20	P>0.05
Chlorine	mg/l	65.43±48.93	16.3	163.3	250	250	P>0.05
Phosphates	mg/l	44.00±23.34	33.30	79.5	2	5	P>0.05
Sodium	mg/l	60.30±32.19	65.25	110	NS	NS	P>0.05
Calcium	mg/l	48.80±14.43	20.09	65.8	NS	NS	P>0.05
Magnesium	mg/l	3.24±3.24	0.00	9.72	NS	NS	P>0.05
Potassium	mg/l	55.57±27.81	35.33	85.5	NS	NS	P>0.05
Iron	mg/l	0.17±0.07	0.10	0.30	NS	0.3	P>0.05
Copper	mg/l	0.07±0.04	0.00	0.11	0.5	1.3	P>0.05
Zinc	mg/l	0.69±0.26	0.30	1.18	NS	5	P>0.05
Lead	mg/l	0.07±0.07	0.00	0.20	0.05	0.02	P>0.05
Cadmium	mg/l	0.00±0.00	0.00	0.00	0.2	0.01	P>0.05
Manganese	mg/l	0.37±0.19	0.00	0.60	0.2	0.05	P>0.05
Aluminum	mg/l	0.00±0.00	0.00	0.00	NS	NS	P>0.05
Chromium	mg/l	0.00±0.00	0.00	0.00	0.05	0.1	P>0.05
Nickel	mg/l	0.03±0.03	0.00	0.10	NS	NS	P>0.05

TDS: Total Dissolved Solids in mg/l, NS: Not specified. All metals and non-metals are expressed in mg/l while conductivity is expressed in µs/cm. P>0.05 mean no significant difference. N=3; means sample in triplicates.

Table 2**:** Comparison of root lengths of onion bulbs grown in produced water

Conc. (%)	Produced water	P-Value
Control (tap water)	4.96± 0.51	P>0.05
0.50	3.72±0.21	P>0.05
1	3.37±0.28	P>0.05
2.50	2.89±0.23	P>0.05
5.0	2.28±0.19	P>0.05
10	2.34±0.10	P>0.05

NB: All values are communicated as Mean ± S.E. P>0.05 mean no significant difference.

Table 3: Morphology, colour and shape of *A. cepa* root grown in produced effluent

Effluent	**Morphology of Root tips**						**Colour of Roots tip**		
	Abnormal					**Normal**		**Abnormal**	
Concentration (%)	**CH**	**T**	**CT**	**BT**	**SP**	**Straight**	**White**	**Pale**	**Dark Brown/Black**
0.5 – Produced	Yes	Yes	Yes	No	Yes	Yes	Yes	No	No
1.0 – Produced	Yes	Yes	Yes	No	Yes	Yes	Yes	No	No
2.5 – Produced	Yes	Yes	Yes	No	Yes	Yes	Yes	No	No
5.0 – Produced	Yes	Yes	Yes	No	Yes	Yes	Yes	No	No
10 – Produced	Yes	Yes	Yes	No	Yes	-	Yes	No	No

Roots were cultivated in effluents for 96 hours. The negative sign (-) indicates very minutes roots. CH: Crochet hooks, BT: Broken tips, CT: C-Tumor, SP: Spiral, T: Twist.

Table 4: Cytological influence of produced effluent on A cepa cells

Produced water Conc. %	No of dividing cells	Mitotic Index (Mean ±SE)	Mitotic inhibition (Mean ±SE)	% of aberrant cells
0	311	62.20±2.27	-	-
0.5	292	58.40±2.73	6.11	5.8
1	279	55.80±1.88	10.3	9
2.5	269	53.80±1.74	13.5	11.9
5	260	52.00±1.97	16.4	15
10	250	50.00±0.71	19.6	18.8

*6000 cells per concentration of all produced effluent (treated group; 5 slides)) and control (1 slide)

Table 5: The non-carcinogenic risk assessment of produced water

Elements	Rf ingestion (mg/kg/d)	Rf dermal (mg/kg/d)	EXPing	Expderm	HQ ing/derm	HQ ingestion	HQ dermal	∑HQS	∑HI ing/derm	CDI
Fe	700	140	0.005	0.00040	0.00013	7.5E-06	2.87E-06	1.0E-05	2.6190	0.04
Zn	24	0.96	0.021	0.00010	9.47283	8.9E-04	1.02E-04	9.9E-04	8.7302	0.00
Mn	300	60	0.011	0.00009	0.00728	3.8E-05	1.46E-06	4.0E-05	26.1905	0.01
Cu	40	8	0.002	0.00002	0.40923	5.4E-05	2.07E-06	5.6E-05	26.1905	0.00
Pb	3	0.075	0.002	0.00007	145.50265	7.2E-04	8.84E-04	1.6E-03	0.8185	0.00
Ni	1.4	0.42	0.001	0.00001	111.35407	5.4E-04	2.75E-05	5.7E-04	19.6429	0.00
∑HI ing/derm					**HI**	**0.002**	**0.001**		84.1915	

*HQ (Hazard quotient), HI (Hazard index), Rf (reference dosage), Exping (Exposure via ingestion), Expderm (exposure via dermal) and CDI (Chronic daly intake)

Table 6: The carcinogenic risk assessment of produced water

Elements	EXPING	Sfing	CR
Pb	0.002	8.50E+00	2.55E-04

*Sfing (slope factors), CR (Carcinigenic risk), Exping (Exposure via ingestion),

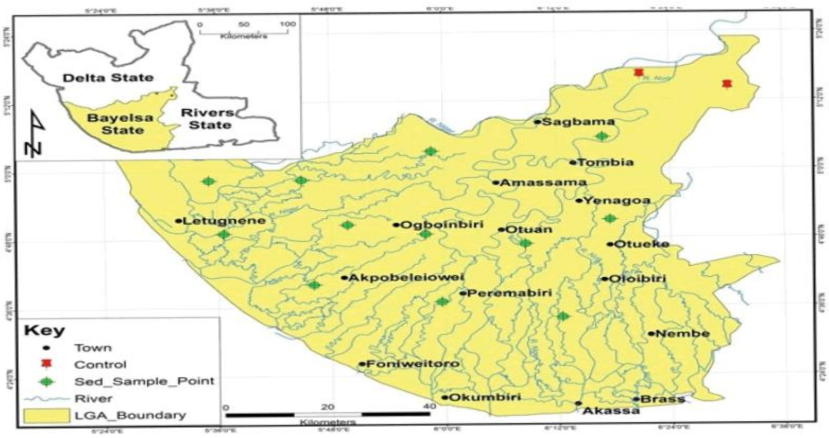

Figure 1: Map of Bayelsa showing Ogboinbiri community.

Figure 2a-d: Different forms of root anomalies; crochets and twists (a-d) in *A. cepa* when exposed to varied concentration of produce effluent.

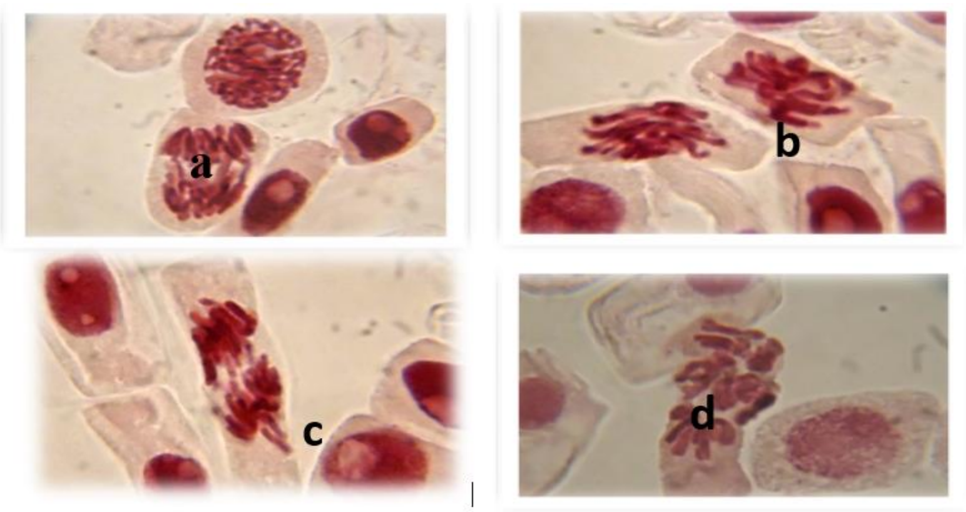

Figure 3a-d: Different forms of chromosomal anomalies; a: multiple bridges, b: sticky chromosome at anaphase, c: vagrants at anaphase and d: C- mitosis.in *A. cepa* when exposed to varied concentration of produce effluent at *1000 microscopic power.

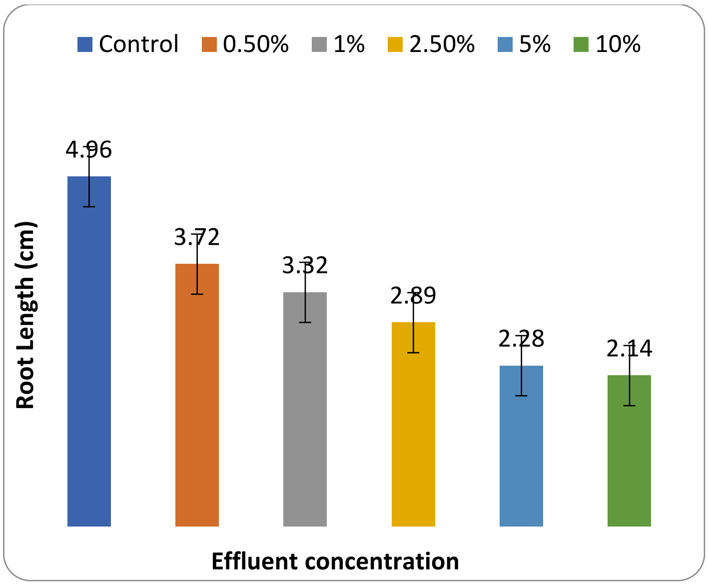

Figure 4: Various concentrations of produced effluent that provoked root reduction in *A. cepa* when exposed to it as related to their control

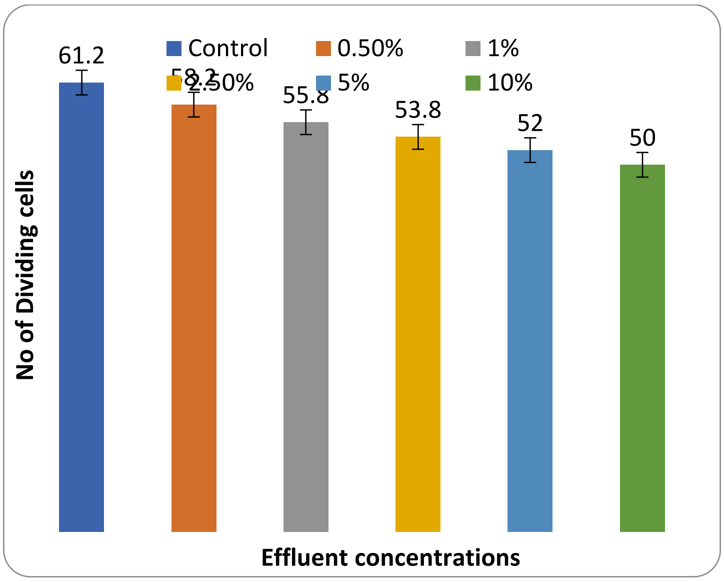

Figure 5: Various concentrations of produced effluent that provoked mitotic index in *A. cepa* when exposed to it as related to their control

Conclusions

Although, there were no statistical differences of the physical, chemical and metal characteristics of the effluent, there were enough indications of serious chromosome anomalies, root reduction in terms of development and noxiousness on *A. cepa* in all concentrations tested. This might be as a result of the chemical enrichment in the effluent. This also elucidates the influence as well as the interaction of the toxic complexes from the effluent test on the onion roots and cells. On this note, there is the need to treat the effluent with the sustainable green method in order to protect humans and aquatic lives from the deleterious impacts it portends.

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