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Buffering Efficiency of Bitterleaf (*Vernonia Colorata*) on Acidic Borehole Water for Fish Production in Port Harcourt, Rivers State, Nigeria

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Abstract

Low pH in sediments and waters has been a constrain for a successive culture of fishes in Port Harcourt. The use of organic buffers has existed to remedy this constrain. This study was carried out to evaluate the buffering efficiency of bitter-leaf (*Vernoniacorolata*) parts on acidic borehole water for the culture of fish in remediation to this constrain. It was done using the Whole Method (for 14 Days) having Five, 18 Litres Plastic tanks (14 litres mark filled with water having a pH of 4.96) which was triplicated for each of the treatment. Contained in the Treatment tanks are Control (T0), Dried Leaves (T1), Fresh Leaves (T2), Dried Stems (T3) and Fresh Stems (T4). Water quality monitoring (pH, oxidative reduction potential, total alkalinity, electric conductivity and temperature) was carried out on daily basis except for calcium and turbidity which were carried out once in a week. Data generated from this study were collated and subjected to microsoft excel and IBM statistical package for social science to get the descriptive and inferencial statistical values (mean, barchats, standard deviation, standard error, maximum, minimum, range, and analysis of variance). All treatments increased with T1 having the highest buffering efficiency with a mean pH of 6.54(Day 3). From the study, the pH had an inverse relationship with the Oxidative-reduction potential and a positive relationship with total alkalinity, total dissolved solids, electric conductivity, calcium and turbidity while temperature was not controlled .The bitter-leaf parts is easily affordable and available for the culture of fish for consumption with the dried leaves having the best result in shorter time than other bitter leaf parts.
Keywords: Organic buffers; Potency; pH; Whole method; Vernonia; Colorata.

1. Introduction

A buffer is like a chemical cushion which can neutralize an acid or a base or an acid when it is added into water [1]. It can be natural (formation of carbonic acid buffer when Carbon (IV) oxide is dissolved into water, rocks like limestone producing calcium and magnesium) or man-made [2]. A buffer is a solution which has the ability to resist change of pH upon the addition of an acidic or basic component. It is able to neutralize small amounts of acids or bases that are been added, thus maintaining the pH of a solution relatively stable [3]. Low pH of water and sediment has been a major constrain which possesses effects on the growth and profitability of aquaculture in Port Harcourt, Nigeria. Ground water (e.g. borehole) is considered more desirable for aquaculture because it possesses consistent quality of water than the surface water, and is less likely to contain pathogens or fish but it has low pH.Acidic water affects fish growth hence the low hatchery, nursery, grow-outs and brood-sstock productions, and profitability of fish farming in this region [3]. There has been designed efforts being made by Davies, *et al.* [4] and Davies and Ansa [5] to boost production and reduce cost of, and stopping the use of synthetic chemicals in fish production for safe and healthy food. In recent times, organic agriculture is being advocated for safe and healthy food thus alternative buffer agent is necessary to fight this challenge in order to boost the production fish Davies, *et al.* [4]; Davies, *et al.* [6]. Organic materials can serve as alternatives to chemicals for safe and healthy production of fish. According to Burkill [7], the bitter leaf (*Vernonia colorata*{wild}) is a useful plant of West Africa which is from the family Compositae is known commonly as the English Bitter leaf. It is a variable plant species of the savannah region but has been dispersed by man in vicinity of villages and has taken it into the forest zone for its medicinal purposes.The fruits of *V.colorata* are glabrous and its florets are packed together. It is a multipurpose shrub or small tree found among other parts of Africa, especially in West Africa. Some chemical compositions of *V. colorata* carried out by Fube and Djonga [8] are Crude protein- 26.50 Mg/100g dry matter, Ash-19.60 Mg/100g dry matter, Cellulose- 10.50 Mg/100g dry matter, Iron (Life Ionizers) - 200 ppm/100g dry matter, Copper (United Nations Educational)- 16 ppm/100g dry matter, Zinc (Zn)- 82 ppm/100g dry matter and Manganese (Mn)- 885 ppm/100g dry matter [9].

Acidic water is water with a potehtial hydrogen (National Oceanic and Atmospheric Administration) which is less than 7 [10]. The pH of a water is the negative common logarithm of the hydrogen ion: pH=-log (H^+). It is the measure of the acid-base equilibrium and, in most natural waters, is controlled by carbon dioxide-bicarbonate-carbonate equilibrium system [11]**.** The standard acceptable range of pH for fish culture is between 6.0 and 9.0 [12]. Boyd also suggested a pH range of 6.5-9.0 as optimum pH for aquaculture. In Nigeria the most cultured fish species are the Catfish species and the tilapia fish species [13]. Low pH affects fish growth hence the low hatchery, nursery, grow-outs and brood stock productions, and profitability of fish farming in this region [5]. Aquaculture is referred to

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the act of breeding, rearing, and harvesting of fish, shell fish, plants, algae, and other organisms in all types of water environments [14]. Aquaculture as defined by Food and Agricultural Organization (FAO) (2015) is the farming of animals and plants that are aquatic in freshwater, brackish water and salt water in all forms. To the best of my knowledge no report has been made using this particular specie of bitter leaf (*V. colorata*) to buffer water for fish production, hence the need to carry out this research. The aim of this study is to assess how efficient bitter leaf (*V.corolata*) can be used to buffer the pH of water to a suitable level of tolerance for fish production in Port Harcourt. The specific Objectives are todetermine the pH of acidic borehole waters and evaluate other water quality parameters (Temperature, Oxidative-reduction potential {ORP}, total dissolved solids {TDS}, Electric conductivity {EC}, Total Alkalinity, Calcium and Turbidity) of the borehole water for suitable fish culture which have relationships with pH.

2. Materials and Methods

2.1. Study Area

The study was carried out at the Aquaculture Centre of the Department of Fisheries and Aquatic Environment of the Faculty of Agriculture, Rivers State University Nkpolu-Oroworukwo, Port Harcourt, and Rivers State, Nigeria otherwise geo-located to Latitude 4.7882 ° E to longitude 6.9813° N The experiment was carried within the duration of 2 weeks (14 days).Various parts of the bitterleaf (*V. colorata*) which are the leaves and the stem were collected from Roone Farm at No. 2B Jetty offAbuloma Road, Port Harcourt, Rivers State, Nigeria. The design of the experiment was 5x3 which represents Bitter leaf parts (Dried and fresh leaves and stems) (Plate 1) and three replicates. It was carried out using a method called the **Whole Method (**which is putting the whole bitter leaf directly into the water without tying it into anything).

2.2. Determination of Bitter Leaf Parts Density

The standard dosage is 27g of the different parts of the bitter leaf/100 Liters of acidic bore-hole water [4]. For 14 liters used, it was calculated as:

27g of each part of bitter leaf=100 Liters of Acidic water

$$14 \text{ Litres of acidic water} = \frac{14 \times 27}{100}$$

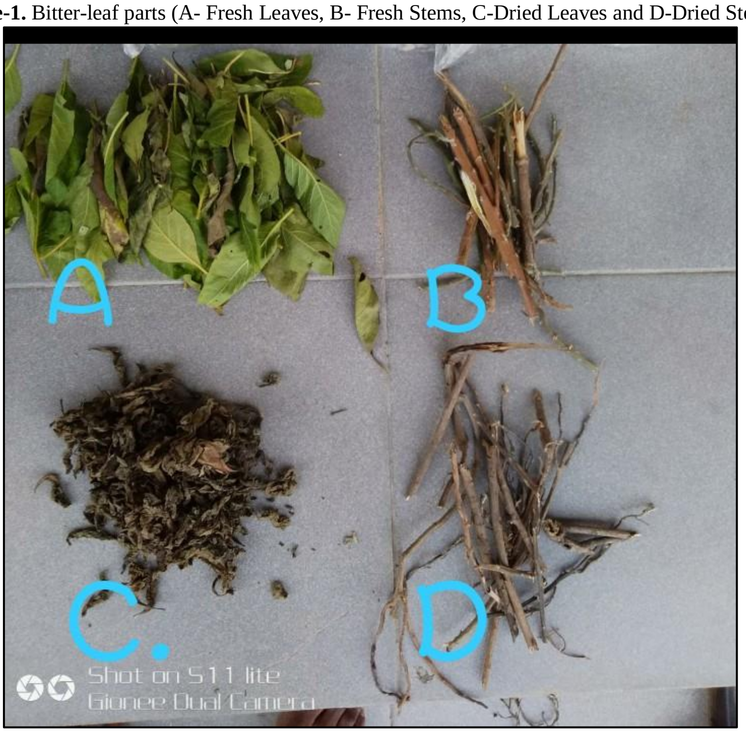
=3.8g of each part of bitter leaf

Therefore, Every 14 Liters of water = 3.8g of each part of bitter leaf.

Fifteen (15), 18 liters plastic tanks were been used to carry out this experiment. The labels on the plastic tanks were T0 (Control), T1 (Dried leaves), T2 (Fresh leaves), T3 (Dried stem), and T4 (Fresh stem) all in three replicates. Measurement of pH from the overhead bore-hole tank was be carried out. All of the 15 liter tanks were filled up to the 14 liter mark.

For T1 tanks, 3.8g of dried leaves of the bitter leaf each was weighed in triplicate and put into the waters. Into tanks T2, 3.8g of fresh leaves of the bitter leaf each was weighed in triplicate and put into the waters. Into tanks T3, 3.8g of dried stem of the bitter leaf each was weighed in triplicate and put into the waters. In tanks T4, 3.8g of Fresh stem of the bitter leaf eachwas weighed in triplicate and put into the waters. While for T0 which was the control nothing or no bitter leaf part was added to it.

Plate-1. Bitter-leaf parts (A- Fresh Leaves, B- Fresh Stems, C-Dried Leaves and D-Dried Stems)



Source: Field Work (2019)

2.3. Evaluation of Water Quality Parameters

Physico-chemical parameters (pH, Temperature, Oxidative-reduction potential (ORP) and Total Alkalinity) were measured on a daily basis for 14 days. Restriction of the measurement of these physico – chemical parameters was to 14 Days adopting Viveen, *et al.* [15] recommendation of minimum duration of 14 days to obtain pH 6.5 if selected rate of liming materials is used.

The pH was been measured using the pH meter of which the electrode of the pH meter was been inserted into the water up till the marked point for two (2) minutes after which the value was been taken once stable. The brand of meter that was used is the pHep meter (H19127) produced by Excelvan Ltd, China.The TDS and EC and Temperature meter electrode was been inserted into the water up to the marked point (3.5cm) on the meter for two (2) minutes. The meter was agitated slightly at interval so that the probe (electrode) could read the Total Dissolved Solids. (TDS), Temperature and Electrical Conductivity (EC). Reading was taken once the displaced value is stable for few seconds.The total alkalinity (mg/L) was been measured by Aquacare 2000.3 Para test. One (1) drop of indicator B was added to 5 ml of the experimental water and mixed well. The sample water turned greenish-blue. Titrant Solution A was added, drop by drop and whirled test vessel gently after each drop until the solution changed to lavender-gray (right before end point). One (1) more drop was added into the solution to turn pink. The number of drops used from the Titrant Solution A were multiplied by 17 to give the total alkalinity level in mg/L.TheOxidative-reduction potential (ORP) meter electrode was been inserted into the water up to the marked point (3.5cm) on the meter for two (2) minutes. The meter was agitated slightly at interval so that the probe (electrode) could read theOxidative-reduction potential (ORP). Reading was taken once the displaced value is stable for few seconds.The calcium and Turbidity were been measured using the Solar Thermo-Elemental Flame Atomic Absorption Spectrophotometer; Model – SG71906, at the beginning and on Day 8 of the study (once a week).

2.4. Statistical Analysis

Data generated from this study, were been collated and subjected to Microsoft Excel (MS Excel) and IBM Statistical Package for Social Sciences (SPSS) Statistics 23 to get the descriptive (mean and bar charts, maximum, minimum, standard deviation,standard error and range) and inferential (single factor Analysis of Variance (ANOVA)) statistical values.

3. Results and Discussion

The morning mean pH values steadily increased daily from Day 1 to Day 14 in all treatments (T0-T4) (Fig.1). The recorded morning mean pH values of T1 ranged between 4.96(Day1) and 7.28 (Day 14). However, mean pH value of 6.59 was recorded on Day 3 for T1 while other treatments recorded mean pH values below 6.00. The pattern of buffering efficiency was T1>T2 and T4>T0>T3 (Table 4.1.1). The overall morning mean pH values (14 days) ranged between 6.24±3.60 (lowest, T3) and 6.85±0.37 (highest, T1) (Table 1). The daily variation of pH values (whole method, morning) within the 14 days was significant ($P<0.05$) except on day 12 & 13 (Table 3). The evening mean pH values for the Whole Method gradually increased also from Treatments T0-T4 within the period of 14 days (Fig. 2). For T1, the lowest (4.96) evening mean pH values was observed on Day 1 and highest (7.29) on Day 14. A mean value of 6.65 was recorded on Day 3 for T1 while other treatments recorded pH values below 6.00. The order of buffering efficiency at the end of Day 14 was T1>T4>T2>T0>T3 (Table .2). Within 14 days, the evening overall mean pH values ranged from6.25±0.32 (lowest, T3) to 6.89±0.36 (highest, T1) and (Table 2).The daily variation of pH values (whole method, evening) within the 14 days was significant ($P<0.05$) except on day 12 & 13 (Table 4) pH had a positive relationship with the Total Alkalinity, Electric Conductivity, Total Dissolved Solids from the Experiment that was been Carried out. Temperature fluctuated as it was not been controlled. Fluctuation on the Oxidative Reduction Potential levels could be as a result of the fluctuation in Temperature. Minimum and Maximum Values for Calcium and Turbidity(weekly) and other measured Water Quality Parameters across the Morning and Evening of the14 Days (Tables 4 & 5).The reported work been carried out indicated that the Bitterleaf (*V. colorata*) parts gradually buffered the water pH along the 14 days of treatment. The results indicated that after 14 days of treatment, T1 (Dried Leaves) buffered faster than other treatments having an observed order buffering efficiency as T1>T2 and T4>T0>T3. T1 had a mean pH values of 6.59 on day 3 morning. This result correlated to that of Boyd [13] which range between a pH of 6.5 to 9 and UNESCO/WHO/UNEP [12] which ranged between a pH of 6 to 9. Troy [16] also had a similar result using plantain plant to buffer acidic borehole water for aquaculture having a minimum (5.84±0.01) and maximum (7.70±0.02) pH values at the end of 16 days with the dried stem and leaves of plantain plant competingfavorably with the buffering efficiency of $CaCO_3$. Davies and Ansa [5] also carried out a similar work to evaluate the potency of paw-paw (*C. papaya*) plant parts having a similar pH values between 6.41±0.01 (T4) and 7.03±0.02 (T11) after 28 dayswith dried leaves buffering faster than other parts of the plant. Davies and Jaja [17], had a similar result using water hyacinth (*Eichhorniacrassipes*) having an initial pH value of 3.0 (minimum, Day 1) to a final value of 7.2 (maximum, Day 17) of Calcium carbonate with a mean value of 6.24±0.22 in T10. This was followed by dried whole roots and dried whole stem with maximum pH 6.9 with mean value of 6.07±0.23.The result of the study was achieved within 14 days following Viveen, *et al.* [15] and Davies, *et al.* [4] which recommends a minimum of 14 days to obtain a pH of 6.5 if the measured quantity of organic buffer is been used.The total alkalinity increased on treatment T1 gradually increased to 51mg/L on day 14. T2 had a total alkalinity level of 34mg/L on day 14 from a day1 reading of 17mg/L.This tallied with the report of Troy [16] on water

Fig-1. Daily mean pH values of treated acidic borehole water (Morning)

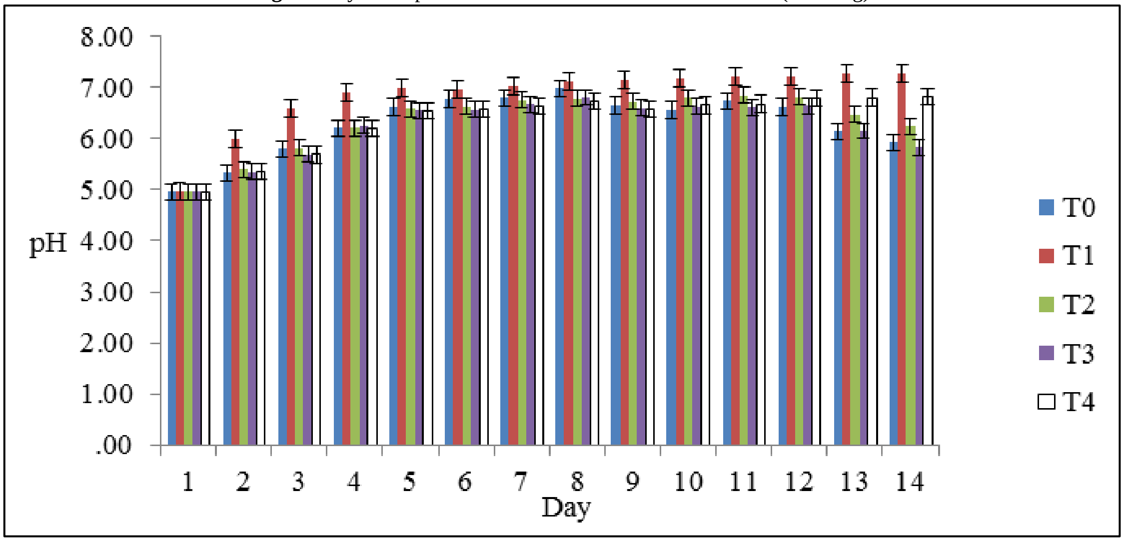


Table-1. Mean pH values of treated acidic borehole water (Morning; 14 days)

Treatment	pH				Standard
	Minimum	Maximum	Range	Overall Mean ± SEM	
T0	4.96	6.98	2.02	6.29±0.35	6.5-9.0 [13];6.0-9.0 UNESCO/WHO/UNEP [12]
T1	4.96	7.28	2.32	6.85±0.37	6.5-9.0 [13];6.0-9.0 UNESCO/WHO/UNEP [12]
T2	4.96	6.84	1.88	6.36±0.34	6.5-9.0 [13];6.0-9.0 UNESCO/WHO/UNEP [12]
T3	4.96	6.80	1.84	6.24±0.33	6.5-9.0 [13];6.0-9.0 UNESCO/WHO/UNEP [12]
T4	4.96	6.83	1.87	6.36±0.34	6.5-9.0 [13];6.0-9.0 UNESCO/WHO/UNEP [12]

Fig-2. Daily mean pH values of treated acidic borehole water (Evening)

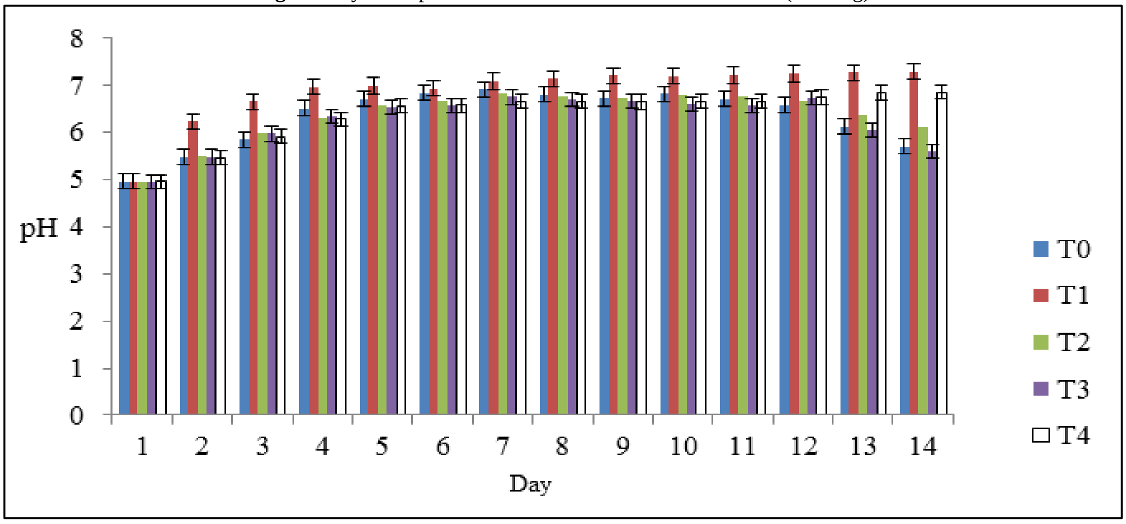


Table-2. Mean pH values of treated acidic borehole water (Whole Method, evening, 14 days)

Treatment	pH				Standard
	Minimum	Maximum	Range	Overall Mean ± SEM	
T0	4.96	6.91	1.95	6.34±0.35	6.5-9.0 [13];6.0-9.0 UNESCO/WHO/UNEP [12]
T1	4.96	7.29	2.33	6.89±0.36	6.5-9.0 [13];6.0-9.0 UNESCO/WHO/UNEP [12]
T2	4.96	6.82	1.86	6.36±0.32	6.5-9.0 [13];6.0-9.0 UNESCO/WHO/UNEP [12]
T3	4.96	6.75	1.79	6.25±0.32	6.5-9.0 [13];6.0-9.0 UNESCO/WHO/UNEP [12]
T4	4.96	6.85	1.89	6.39±0.33	6.5-9.0 [13];6.0-9.0 UNESCO/WHO/UNEP [12]

Table-3. ANOVA Table during the 14 Days Morning and Evening (pH, Oxidative Reduction Potential, Total Alkalinity, TDS, Electric Conductivity and Temperature)

Significance										
Day	**pH**		**ORP (mV)**		**TDS**		**EC**		**Temperature**	
	M	E	M	E	M	E	M	E	M	E
2	.000	.000	.000	.000	.000	.000	.000	.000	.013	.032
3	.000	.000	.000	.000	.000	.000	.000	.000	.012	.000
4	.000	.000	.000	.000	.000	.000	.000	.000	.013	.000
5	.000	.000	.000	.000	.000	.000	.000	.000	0..1	.001
6	.000	.000	.000	.000	.000	.000	.000	.000	.001	.021
7	.004	.001	.000	.000	.000	.000	.000	.000	.024	.001
8	.000	.000	.000	.000	.000	.000	.000	.000	.002	.086
9	.000	.000	.000	.000	.000	.000	.000	.000	.135	.212
10	.004	.001	.000	.000	.000	.000	.000	.000	.015	.002
11	.019	.014	.000	.000	.000	.000	.000	.000	.134	.001
12	.087	.190	.000	.007	.000	.000	.000	.000	.001	.009
13	.077	.095	.097	.067	.000	.000	.000	.000	.001	.000
14	.044	.020	.021	.075	.000	.000	.000	.000	.000	.000

M-Morning; E-Evening, ORP-Oxidative Reduction Potential, TDS-Total Dissolved Solids; EC-Electric Conductivity

Table-4. Minimum and Maximum Values for Calcium and Turbidity (weekly) and other measured Water Quality Parameters across the Evening of the14 Days (Morning)

Parameter	**Treatment**					**Standard**
	T0	**T1**	**T2**	**T3**	**T4**	
ORP (mV)	131.33-224	45-224	133-224	165.67-254.33	163-269.33	650mV World Health Organization [18]
Total Alkalinity (mg/L)	17-17	17-51	17-17	17-17	17-17	30-500 UNESCO/WHO/UNEP [12]
TDS (mg/L)	57-74.33	57-144	57-79.33	57-75	57-78	<1000 McNeely, *et al.* [19]; ≤500 World Health Organization [18]
E. C. (µS/cm)	122-160	122-243	122-168.67	122-159.67	122-165.67	340-700(µs/cm) World Health Organization [18]
Temperature (°C)	26.27-30.17	26.6-29.83	27.4-29.9	27.2-30.23	26.53-30.3	0 ºC (under ice cover) - 40 ºC (in hot springs) McNeely, *et al.* [19]
Calcium (mg/L)	2.1-2.1	2.1-4.1	2.1-3.1	2.1-2.1	2.1-2.1	>20 mg/L Tucker [20]
Turbidity (NTU)	0.2-0.21	0.2-0.37	0.2-0.31	0.2-0.3	0.2-0.39	20-40 NTU Rhode Island Rivers Council [21]; 5 NTU World Health Organization [18]

Table-5. Minimum and Maximum Values for Calcium and Turbidity (weekly) and other measured Water Quality Parameters across the Evening of the14 Days (Evening)

Parameter	**Treatment**					**Standard**
	T0	**T1**	**T2**	**T3**	**T4**	
ORP (mV)	131.33-224	45-224	133-224	165.67-254.33	163-269.33	650mV World Health Organization [18]
Total Alkalinity (mg/L)	17-17	17-51	17-17	17-17	17-17	30-500 UNESCO/WHO/UNEP [12]
TDS (mg/L)	57-74.33	57-144	57-79.33	57-75	57-78	< McNeely, *et al.* [19]; ≤500 World Health Organization [18]
E. C. (µS/cm)	122-160	122-243	122-168.67	122-159.67	122-165.67	340-700(µs/cm) World Health Organization [18]
Temperature (°C)	26.27-30.17	26.6-29.83	27.4-29.9	27.2-30.23	26.53-30.3	0 ºC (under ice cover) - 40 ºC (in hot springs) McNeely, *et al.* [19]
Calcium (mg/L)	2.1-2.1	2.1-4.1	2.1-3.1	2.1-2.1	2.1-2.1	>20 mg/L Tucker [20]
Turbidity (NTU)	0.2-0.21	0.2-0.37	0.2-0.31	0.2-0.3	0.2-0.39	20-40 NTU Rhode Island Rivers Council [21]; 5 NTU World Health Organization [18]

alkalinity and pH. There was no significant difference between treatment T1 and United Nations Educational, Scientific and CulturalOrganization/World Health Organization/United Nations Environment Program

UNESCO/WHO/UNEP [12] which ranged between 30-500 mg/L. The total dissolved solids also increased on a daily basis. Treatment T1 had the highest mean values of Total dissolved solids after the 14 days as 114 mg/L (Evening, T1). This is comparable to McNeely, *et al.* [19]. The increase in the total dissolved solids may be as a result of minerals contained in the bitter-leaf and its ash content. Electric Conductivity Steadily increased on Daily Basis with T1 having the highest values over other treatments having its mean value range as 122µS/cm to 243µS/cm (evening). The result showed that the Electric Conductivity increased with increase in the water pH. This result specifically agreed to that of Nwoye, *et al.* [22] on the predictability of Electrical Conductivity as a function of pH.The mean daily temperature varied on daily basis. The temperature was not controlled as it was affected by the temperature of the environment at the Aquaculture center in Rivers State University. The temperature reported for the 14 Days of Treatment compares in agreement with the report of Boyd [13]. The highest daily mean temperature during the course of study was observed on Day 13 (31.3°C in T0, Sac method morning) and on Day 4 (30.6°C in T2, Whole Method morning).The calcium level increased with increase with the pH for treatment for T1 and T2 on the second week of study with final mean values of 3.1mg/L (Maximum, T1) and 2.5mg/L (Minimum, T2). The Calcium values are similar to the Standards of Tucker [20] which is >20 mg/L.Turbidity level was increased on weekly purpose having its highest overall mean values (0.29±0.07 NTU) at T1 and its lowest overall mean values (0.21±0.00 NTU) at T0. The turbidity values are comparable to the recommended values of 20-40 NTU Rhode Island Rivers Council [21]; 5 NTU World Health Organization [18].During this period of Study Tanks T1 had a colour change to greenish brown which may be as a result of its concentration of Chlorophyl-A on the water as it lost its moisture content before introducing it to the water which made it react fast with the water.

4. Conclusion and Recommendations

The bitter-leaf parts (dried leaves, fresh leaves, dried stems and fresh stems) of *V.corolata* buffered the water successfully for the 4 treatments (T1, T2, T3 & T4). Also the control (T0) buffered as a result of water been kept over time. This implies that when acidic bore hole water is been kept open to aeration and natural factors and prevented from rain water entering it, it buffers naturally. The Treatment tank T1 (dried leaves) buffered faster than every other treatment tanks to a pH range of 6 to 6.5 within 2 to 3 days respectively for the culture of fish. The pH of all treatment tanks had positive relationship with the Total alkalinity, Total Dissolved Solids. It had an inverse relationship with the Oxidative reduction potential while all other factors including the pH was affected by the temperature which is the master parameter.

Based on the results, the study recommends the use of organic buffers like bitter-leaf parts to synthetic buffers as it is cheaper to afford and it contains anti-oxidative properties that could help in the survival rate of the fishes, Use of dried bitter-leaf parts (to obtain faster results) to buffer the pH of waters for fish culture as it gets a suitable pH level for fish culture (6-9 or 6.5-9) within 2 to 3 days with a quantity of 3.8g/litre. The quantity could be doubled with the view that it may fasten the buffering efficiency on the water.

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