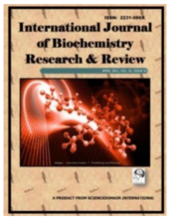

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Petroleum Hydrocarbon-induced Changes in Juice of *Citrus sinensis* following Chronic Exposure

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Authors' contributions

This work was carried out in collaboration between all the authors. Authors GOCO and LAN designed the project. Author LCN performed the experiment and wrote the first draft of the manuscript. Authors GOCO and LAN assisted in manuscript preparation. Author LAN performed the statistical analysis. Authors GOCO and LAN supervised the project. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to investigate the effect of chronic exposure to petroleum hydrocarbon pollution (PHC) on some biochemical parameters of the fruit juice of *Citrus sinensis.*
Place and Duration of study: This study was carried out at Ebocha-Egbema and Uvuru Mbaise in Imo state (Niger Delta Area), Nigeria between October 2008 and October 2011.
Methodology: Acidity (pH), concentrations of ascorbic acid (AA), glutathione (GSH), citric acid, glucose and the activity of lactate dehydrogenase (LDH) in the juice of just-ripe orange fruits (*Citrus sinensis*) from the two environments were investigated by standard methods. The estimated values were analyzed using student t-test and the results expressed as mean ± standard deviation.
Results: The results obtained revealed that there was no significant ($p \geq 0.05$) difference in the mean pH values, ascorbic acid and glucose concentrations of the fruit juice from the two areas studied. Mean concentrations of glutathione and citric acid in the juice from Ebocha (0.44±0.09 and 18.80±1.14mg/l) were significantly ($p \leq 0.05$) lower than the values in the juice from Uvuru (0.66±0.10 and 21.43±2.02 mg/l), respectively. The results also showed that the mean activity of lactate dehydrogenase was significantly higher in the juice from Ebocha (7.033+/-1.73 U/l) than in that from Uvuru (5.344±1.74 U/l).

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Conclusion: The findings of this study are suggestive of a possible alteration in the metabolic activities of *Citrus sinensis* trees evident in its fruit juice due to the PHC pollution in Ebocha in the Niger Delta.

Keywords: Citrus sinensis; petroleum hydrocarbon pollution; glutathione; citric acid; lactate dehyhrogenase; Niger Delta.

1. INTRODUCTION

While petroleum exploration and production is Nigeria's most crucial economic lifeline, the environmental consequences in the Niger Delta Area have been very glaring in terms of their negative impact. Petroleum provides a relatively cheap and convenient source of energy as compared to other fuels such as coal and electricity [1]. However, crude oil occurs with gas in such a way that the gas must be separated out before the oil could be reached. It is expensive to capture and liquefy the gas for transportation, so the oil producing companies in Nigeria chose rather to burn it off into the atmosphere by what is known as gas flaring. The most glaring site in gas production flow station is the ten-meter-high flame that burns continuously from vertical pipes at the many facilities owned by the oil companies. This gas flaring releases huge volumes of greenhouse gases into the atmosphere, while emitted sulphur dioxide returns to the soil as acid rain [2,3]. In addition, accidental spills during transportation of crude oil further contribute to the pollution of the environment. Inhabitants of the region have consistently complained of health problems, mainly respiratory tract diseases as well as damage to wild life and vegetations [2,4]. The environment of Ebocha-Egbema in the Niger Delta has been shown to be polluted as a result of the oil activities (gas flaring and oil spillage), which have gone on there for over five decades [5]. It has also been shown that the pollution in Ebocha has had adverse effects on some biochemical parameters of the native fowl *(Gallus domesticus)* native to that environment [6]. However, there is no information on whether the effect is the same in plants. The present study was therefore designed to investigate the effect of chronic exposure to petroleum hydrocarbon (PHC) pollution on some biochemical parameters of the fruit juice of the citrus plant *(Citrus sinensis)* native to the Ebocha–Egbema environment in the Niger Delta Area.

2. MATERIALS AND METHODS

2.1 Sample Collection and Extraction of Fruit Juice

Ten fresh and apparently healthy ripe orange fruits each were selected by radom and distance distribution selection from five (5) different trees that have existed for many years in Ebocha. The same was also done from Uvuru Mbaise. The trees from the two environments were of the same age bracket. The fruits were spread on a paper on the floor to reduce the rate of deterioration of the biochemical components of the fruits. The fruit juice was extracted using manual juice press method.

2.2 Determination of Biochemical Parameters

The pH of juice was measured using digital pH meter standardized with a buffer solution as described by Walter [7]. Ascorbic acid concentration was determined using the method of Roe and Kuether [8]. Ascorbic acid was converted to dehydroascorbic acid by shaking with Norit and this was coupled to 2,4-dinitrophenyl hydrazine in the presence of thiourea (a mild reducing agent).This was determined according to the method described by Raja et al. [9]. Citric acid concentration was determined by titimetric method as was described by Haleblian et al. [10]. Glucose concentration was estimated based on glucose oxidase method as described by Trinder [11]. Assay of lactate dehydrogenase activity was carried out using lactate dehydrogenase (LDH) liquid reagent kit supplied by Teco Diagnostics, U.S.A.

2.3 Statistical Analysis

Each reading was taken in triplicate. All data were expressed as mean ± standard deviation and analysed for statistical significance using students't-test. Values were considered significant at $p \leq 0.05$ [12].

3. RESULTS AND DISCUSSION

The results obtained are presented in Figs. 1-6 below. Values obtained revealed no significant ($p \geq 0.05$) difference between the mean pH of the juice from Ebocha and that of the juice from Uvuru. (Fig. 1). This could be attributed to the metabolic flexibility of plants by which they are able to adapt to stress [13]. Gehl and Colman [14] stated that plants use energy to maintain their pH. This energy in stressed plants is acquired by anaerobic metabolism [15]. The increased production of lactate in anaerobic metabolism which should result in marked decrease in pH is counter balanced by the conversion of lactate to glucose by gluconeogenesis to sustain glycolysis and energy production [16]. However, there might be a slight decrease in pH, but the low concentration of citrate as a result of reduction in tricarboxylic acid cycle (TCA) and electron transfer chain (ETC) due to reactive intermediates from PHC pollution tends to balance this decrease [17,13].
The results revealed that there was no significant ($p \geq 0.05$) difference between the mean concentrations of ascorbic acid in the juice from Ebocha and Uvuru (Fig. 2). There is no information in literature to explain this finding. However, it could be a peculiarity with citrus fruits. This is because organisms exposed to situations such as environmental pollution, produce a lot of free radicals which cause chain reactions of oxidations in living organisms [18]. As a water-soluble antioxidant, ascorbic acid in conjunction with vitamin E, a fat-soluble antioxidant and the enzyme glutathione peroxidase, help to quench free radical chain reactions that lead to oxidative stress [19]. Since ascorbic acid is a product of citrus plants, it could be that it was being replaced as fast as it was used to scavenge free radicals; thus, resulting in no significant ($p \geq 0.05$) different, (Fig. 2).

The mean concentration of glutathione in the juice from Ebocha was found to be significantly ($p \geq 0.05$) lower than the value obtained for the juice from Uvuru (Fig. 3). This could also be as a result of the antioxidant function of glutathione by which it scavenges free radicals induced by the pollution in the environment. This is in accordance with the report of Foyer et al. [19], that glutathione is associated with stress resistance owing to its redox-thiol group. Nwaogu et al. [5] also reported a reduction in the mean concentration of glutathione in the native fowl (*Gallus domesticus*) following chronic exposure to petroleum hydrocarbon pollution, although, the organisms used were not the same.

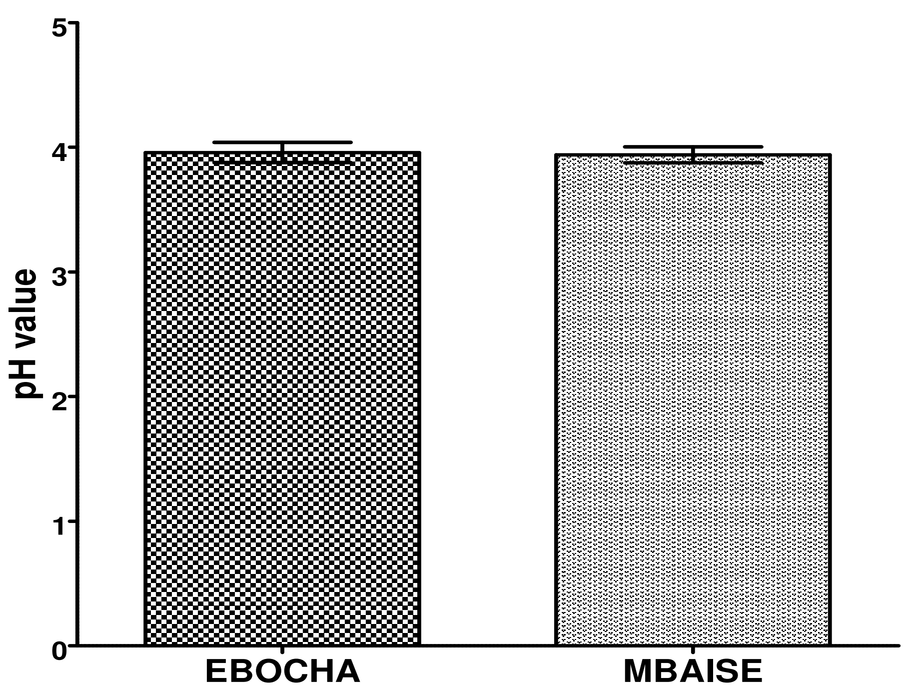

Fig. 1. Mean values of pH of orange fruits from Ebocha and Mbaise

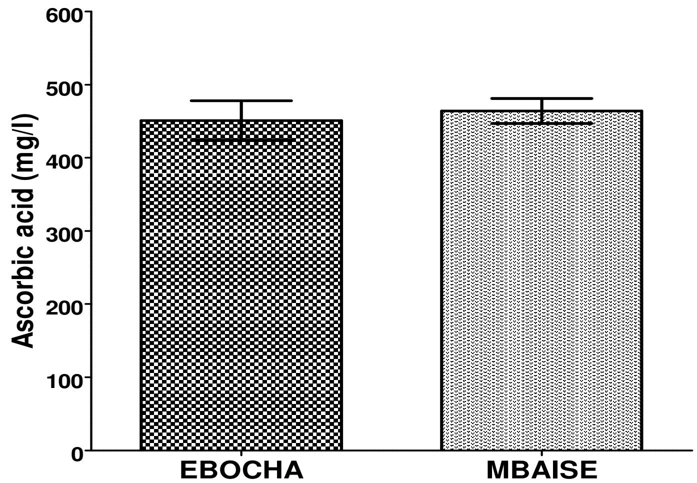

Fig. 2. Mean values of ascorbic acid concentrations of orange fruits from Ebocha and Mbaise

The present study revealed that the mean concentration of citric acid in the juice from Ebocha was significantly ($p \leq 0.05$) lower than the mean concentration of citric acid in the juice from Uvuru (Fig. 4). This could be because the plants from Ebocha, which is a polluted environment, were carrying out the citric acid stage of respiration at a slower rate than the plants from Uvuru. Free radicals due to PHC pollution inhibit the ETC and prevent oxygen from being reduced to water, thereby limiting the TCA cycle as well, since it does not proceed in the absence of oxygen [17]. Consequently, the concentration of citrate also is decreased. In addition, oxaloacetate, which condenses with acetyl Co-A to form citric acid, is directly gluconeogenic. This further reduces the concentration of citric acid because the plant (in this study) was utilising oxaloacetate in gluconeogenesis to maintain glucose availability for glycolysis. Citric acid has antioxidant properties by which it helps to preserve the flavour of fruit juices [20]. This might also account for the lower value obtained for citric acid in the juice from Eboch because the chronic exposure to PHC pollution would have had some effect on the flavour of the juice. Results obtained did not show any significant ($p \geq 0.05$) difference in the mean glucose concentrations of juice from the two environments (Fig. 5). According to Murray et al. [21], the glucose is metabolized in both aerobic and anaerobic situations. However, due to the blocking of oxidative phosphorylation by free radicals, the plant shifted to anaerobic respiration, but glucose metabolism in anaerobic respiration yields only little energy [13]. In order for plants to meet up with its energy demands, glycolysis had to proceed at a much faster pace leading to increased availability of lactate and depletion of glucose [17]. The plant subsequently resorted to gluconeogenesis to refurnish its depleting store of glucose so as to sustain glycolysis and energy production, and thereby using up the accumulating lactate [16]. This could explain why there was no significant ($p \geq 0.05$) difference in the mean glucose concentrations in the juice from both environments.

The mean activity of lactate dehydrogenase was found to be markedly higher in the juice from Ebocha than in the juice from Uvuru (Fig. 6) This could be because Ebocha is a polluted environment [6], and plants tend to shift to anaerobic metabolism under free radical-producing stress conditions, which is regarded as an adaptive phenomenon to maintain the capacity for ATP synthesis [13,15,22,23,24]. During anaerobic respiration, pyruvate is reduced to lactate. This results in the production of high concentrations of lactate and an increase in the activity of lactate dehydrogenase. This result agrees with the finding of Jian et al. [25] that during water logging, the LDH activity in adventitious-root- retained seedlings was higher than that in the control. It also corroborates the findings of Hoffman et al. [26] that LDH activity in barley gradually increased under hypoxic stress. Achuba [27] showed that maize and cowpea seedlings had marked increase in LDH activity following exposure to refined petroleum products (kerosene, diesel, gasoline). Stephen Onodjede

[28] also noted an increase in LDH activity of fowls native to Warri in Niger Delta with chronic PHC pollution when compared to that of fowls from Ughara, an unpolluted environment.

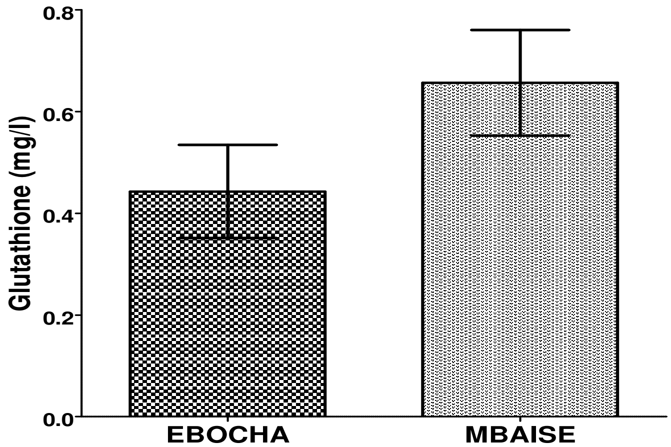

Fig. 3. Mean values of glutathione concentrations of orange fruits from Ebocha and Mbaise

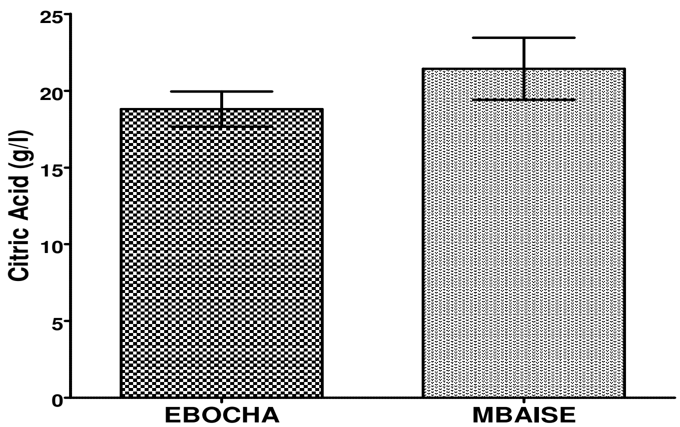

Fig. 4. Mean values of citric acid concentrations of orange fruits from Ebocha and Mbaise

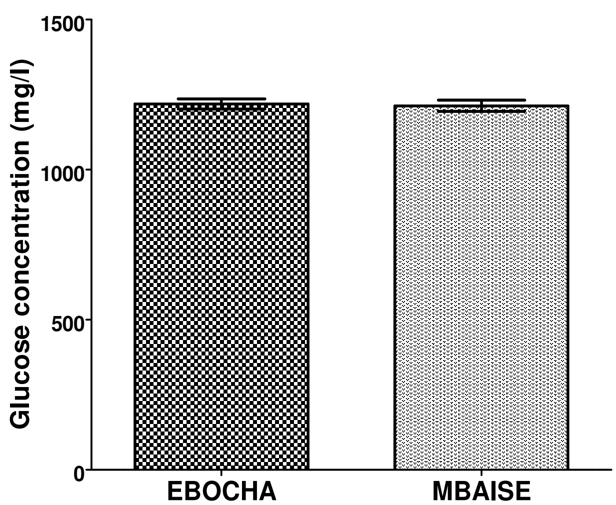

Fig. 5. Mean values of glucose concentrations of orange fruits from Ebocha and Mbaise

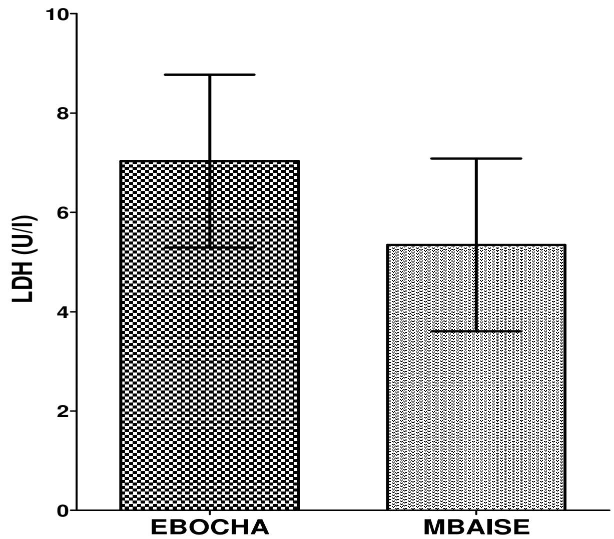

Fig. 6. Mean values of LDH activities of orange fruits from Ebocha and Mbaise

4. CONCLUSION

The present study revealed that the chronic exposure to PHC in the Niger Delta did not affect the concentration of ascorbic acid in the juice of *Citrus sinensis* native to that environment. However, the concentrations of glutathione and citric acid were markedly reduced. The activity of LDH was significantly ($p \leq 0.05$) increased. Based on these findings, it was concluded that the chronic exposure to PHC pollution in the Niger Delta has induced some changes in the metabolic activities of *Citrus sinensis* fruit native to that area.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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