

Petroleum Hydrocarbon-Induced Changes in Juice of Citrus sinensis following Chronic Exposure.

ABSTRACT

The effect of chronic exposure to petroleum hydrocarbon (PHC) pollution on some biochemical parameters of the fruit juice of the citrus plant native to the Ebocha-Egbema environment in the Niger Delta Area (NDA) was investigated. *Citrus sinensis* fruit samples from Uvuru Mbaise in Imo State, with no history of PHC pollution, served as the control. 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 ascertained. Results obtained revealed that there was no significant ($p \leq 0.05$) difference in the mean pH values (acidity) of the juice from the two environments. Mean concentration of ascorbic acid in the juice from Ebocha did not significantly ($p \leq 0.05$) deviate from the value obtained for ascorbic acid in the juice of oranges from Uvuru. Mean concentrations of glutathione and citric acid in the juice from Ebocha (0.44 ± 0.09 and 18.80 ± 1.14 mg/l) respectively were significantly ($p \geq 0.05$) lower than the values in the juice from Uvuru (0.66 ± 0.10 and 21.43 ± 2.02 mg/l) respectively. Mean concentrations of glucose in the juice from the two environments were essentially similar. The results also showed that the mean activity of lactate dehydrogenase was higher in the juice from Ebocha (7.033 ± 1.73 U/l) than in that from Uvuru (5.344 ± 1.74 U/l). These findings might be indicative of a possible alteration in the metabolic activities of the orange fruit due to the PHC pollution in Ebocha in the the Niger Delta.

Keywords: *Citrus sinensis*, petroleum hydrocarbon pollution, 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 (Ake, 1979). 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 (Ikoro, 2003; Campbell, 2001). 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 (Ikoro, 2003; Dung *et al.*, 2008). 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 (Nwaogu and Onyeze, 2010). 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 (Nwaogu et al., 2008). 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:

Fresh and apparently healthy ripe orange fruits were harvested from trees that have existed for many years in Ebocha. The same was also done from Uvuru Mbaise. The trees 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.

2.2 Extraction of Juice:

The fruits were peeled and the juice squeezed through a funnel into a conical flask, then filtered before use.

The pH of juice was measured using digital pH meter standardized with a buffer solution as described by Walter (1981).

Ascorbic acid concentration was determined using the method of Roe and Kuether (1961). Ascorbic acid is 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* (2007).

Citric acid concentration was determined by titimetric method as was described by Haleblan *et al*. (2008).

Glucose concentration was estimated based on glucose oxidase method as described by Trinder (1969).

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 $\pm$ standard deviation and analysed for statistical significance using students't-test. Values were considered significant at $p \leq 0.05$ (Packer, 1979).

3. RESULTS AND DISCUSSION:

The results obtained are presented in Figures 1-6 below. Values obtained revealed no significant ($p \leq 0.05$) difference between the mean pH of the juice from Ebocha and that of the juice from Uvuru. (Figure 1). This could be attributed to the metabolic flexibility of plants by which they are able to adapt to stress (Lincoln and Eduardo, 2006). Gehl and Colman (1985) stated that plants use energy to maintain their pH. This energy in stressed plants is acquired by anaerobic metabolism (Kennedy *et al*, 1992). The increased production of lactate in anaerobic metabolism which should result in marked decrease in pH is counterbalanced by the conversion of lactate to glucose by gluconeogenesis to sustain glycolysis and energy production (Miligan and Wood, 1986). 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 (Kumar, 2011, Lincoln and Eduardo, 2006).

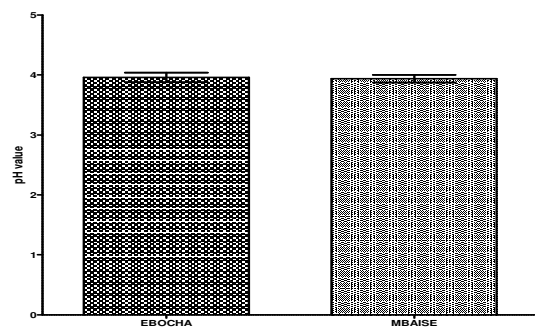


Fig.1. Mean values of pH

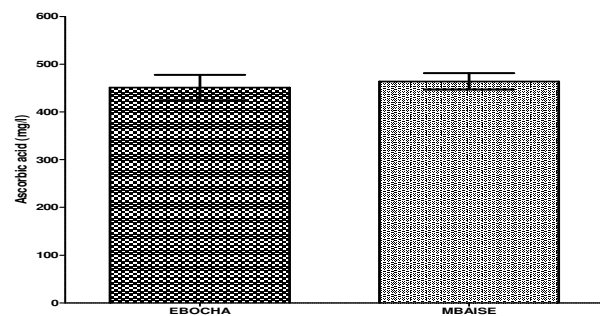


Fig.2. Mean values of ascorbic acid concentration

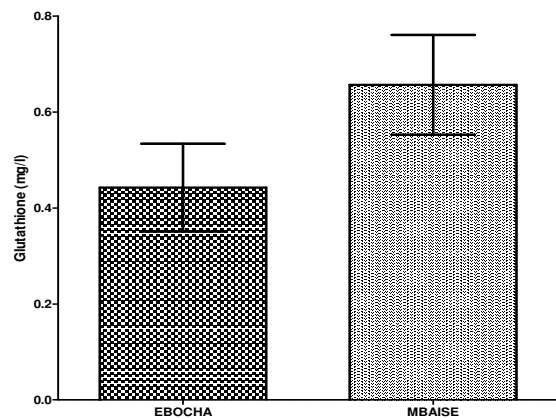


Fig.3. Mean values of glutathione concentration

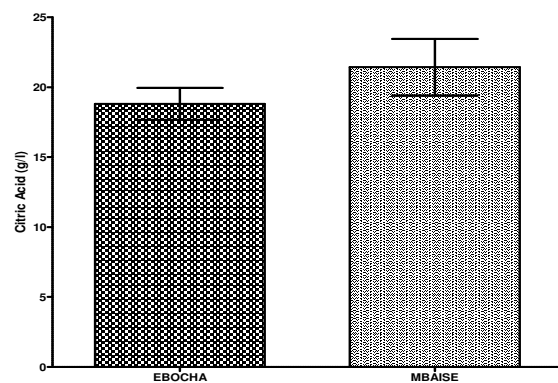


Fig.4. Mean values of citric acid concentration.



Fig.5. Mean values of glucose concentration

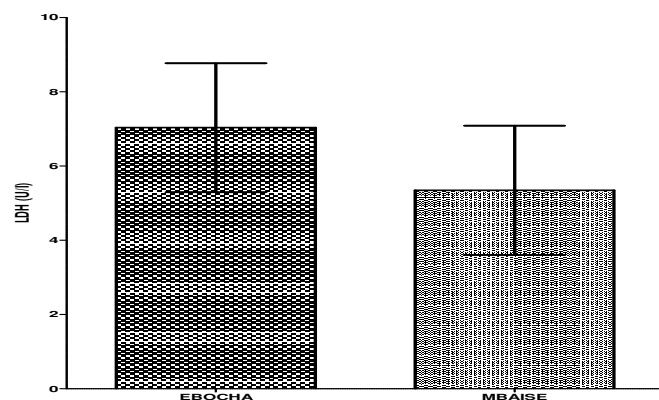


Fig.6. Mean values of LDH activity

The results revealed that there was no significant ($p \leq 0.05$) 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 (Gossette *et al*, 1994). As a water-soluble antioxidant, ascorbic acid in conjunction with with vitamin E, a fat-soluble antioxidant and the enzyme glutathione peroxidase, help to quench free radical chain reactions that lead to oxidative stress (Foyer *et al*, 1994). 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 significance ($p \leq 0.05$), (Figure 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 (Figure 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* (2001), that glutathione is associated with stress resistance owing to its redox-thiol group. Nwaogu *et al* (2008) 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.

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 (Kumar, 2011). Consequently, the concentration of citrate also is decreased. In addition, oxaloacetate, which condenses with acetyl CoA 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 (Nelson and Cox, 2005). This might also account for the lower value obtained for citric acid in the juice from Ebocha 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 ($p \leq 0.05$) difference in the mean glucose concentrations of the juice from the two environments (Fig 5). According to Murray *et al* (2003), glucose is

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231 metabolised in both aerobic and anaerobic situations. However, due
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233 to the blocking of oxidative phosphorylation by free radicals, the
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235 plant shifted to anaerobic respiration, but glucose metabolism in
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237 anaerobic respiration yields only little energy (Lincoln and Eduardo,
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239 2006). In order for the plant to meet up with its energy demands,
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241 glycolysis had to proceed at a much faster pace leading to increased
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243 availability of lactate and depletion of glucose (Kumar, 2011). The
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245 plant subsequently resorted to gluconeogenesis to refurnish its
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247 depleting store of glucose so as to sustain glycolysis and energy
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249 production, and thereby using up the accumulating lactate (Miligan
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251 and Wood, 1986). This could explain why there was no significant
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253 ($p \leq 0.05$) difference in the mean glucose concentrations of the juice
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255 from both environments.

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257 The mean activity of lactate dehydrogenase was found to be
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259 markedly higher in the juice from Ebocha than in the juice fro
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261 Uvuru (Figure 6) This could be because Ebocha is a polluted
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263 environment (Nwaogu and Onyeze, 2010), and plants tend to shift to
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265 anaerobic metabolism under free radical-producing stress conditions,
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267 which is regarded as an adaptive phenomenon to maintain the
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269 capacity for ATP synthesis (Kennedy *et al*, 1992; Hochachka and

Somero, 2002; Good and Muench, 1993; Mandal, 2006; Lincoln and Eduardo, 2006). 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* (2012) 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* (1986) that LDH activity in barley gradually increased under hypoxic stress. Achuba (2011) showed that maize and cowpea seedlings had marked increase in LDH activity following exposure to refined petroleum products (kerosene, diesel, gasoline). Stephen Onodjede (2013) 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.

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 ($p \geq 0.05$) 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. This knowledge is relevant to the ongoing efforts of agricultural biotechnologists to produce transgenic crops having improved resistance to environmental extremes via the process of metabolic engineering.

ACKNOWLEDGEMENT

Authors wish to acknowledge the technical assistance received from Mr. Uche Arukwe of the Department of Biochemistry, Abia State University, Uturu, Nigeria and Dr. C. U. Igwe of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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