Oxidative Stress Induction in Cassava Plant (*Manihot Esculenta Crantz*) Grown on Soil Contaminated with Diesel

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ABSTRACT

The induction of oxidative stress in plants grown on crude oil-contaminated soils was investigated using a diesel contaminated soils model. Twelve cassava stems were grown in four garden pots containing different amounts of diesel oil as contaminants: 150 ppm, 300 ppm, 600 ppm and control (0 ppm). The growth of the plants was monitored for 12 weeks, after which chlorophyll contents, total proteins, lipid peroxidation and activities of catalase, glutathione, and superoxide dismutase (antioxidant enzymes) were determined from the leaves. Significant decreases ($p<0.05$) were observed in the antioxidant enzymes (67-86%), total proteins (79%) and total chlorophyll content (67%) in the cassava grown on diesel contaminated soil (600 ppm) compared to the control. Consequently, there were significant increase ($p<0.05$) in the leaf ratio and malondialdehyde (a marker for lipid peroxidation) 0.1909 ± 04 and 1.77 ± 0.34, when compared to the control 0.1530 ± 08 sq.cm/g and 0.10 ± 0.01 μmol/mg protein respectively. It was thus concluded that stunted growth of plants and their death in diesel or crude oil contaminated soil could be traced to oxidative stress.

Keywords: oxidative stress, SOD, CAT, MDA, total protein

INTRODUCTION

Accidental spillage and leakages of petroleum have increased the pollution of hydrocarbons in water and soil. The impact of petroleum exploration, distribution and storage are enormous. It involves many alterations of landscapes and marine environments. In most cases, the vegetation is cleared to make way for seismic lines, sites for drilling rigs are built, and drilling mud and oil may reach streams, surface water and land. Vandalism and corrosion of pipes and tanks put together account for many petroleum products' spillages in Nigeria (Umar & Othman, 2017). Interestingly, this occurs mainly in farmlands. In other words, some quantities of petroleum and its products are often released into the environment through operational or accidental spillage and other deliberate human acts (Odukoya *et al.*, 2019). Over 4,919 oil spills have been reported in Nigeria between 2015 and 2021. The consequence of such pollution on soil includes water and oxygen deficits and a shortage of absorbable forms of nutrients such as nitrogen and phosphorus (Wu *et al.*, 2014), which are essential for plant growth.

Diesel oil is one of the most important crude oil products, and it is a significant polluter of the environment. Diesel oil is used extensively in diesel engines of cars, industrial trucks and

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generators. These engines' oil transport and maintenance often result in accidental spillage and pollution of municipal and agricultural lands (Zarinkamar *et al.*, 2013; Mitter *et al.*, 2021). The major constituents of diesel fuel are normal alkanes, branched alkanes and cycloalkanes, isoprenoids, aromatic hydrocarbons and polycyclic aromatic hydrocarbons (Jamhari *et al.*, 2014). While the alkanes and cycloalkanes are readily degradable, the aromatics hydrocarbons and polycyclic aromatic hydrocarbons are usually recalcitrant to biodegradation, thus constituting the priority contaminants of the diesel oil. These pollutants are phytotoxic, cytotoxic, and elicit oxidative stress (Fulekar, 2017).

Oxidative stress occurs when the generation of reactive oxygen species (ROS) in a system exceeds its ability to neutralise and eliminate them. This imbalance often results from lack of antioxidants caused either by disturbance of production, distribution or excessive production of ROS from the environment or behaviour stressor (Noori *et al.*, 2015). In plants, the leaf is constantly exposed to ionic and electromagnetic radiations of the sunlight, being the site of photosynthetic activities. The photosynthetic electron transport system is the primary source of active oxygen in plant tissues (Foyer *et al.*, 2012). The process has the potential to generate singlet oxygen and superoxide dismutase, which can be scavenged by suitable antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT). Despite this fact, most research on contamination of petroleum products has claimed suffocation as the mechanism of phytotoxicity of petroleum products. Recent studies, however, have argued that plants exposed to organic contaminants may experience a combined abiotic and biotic stress (Athar *et al.*, 2015; Odukoya *et al.*, 2019), but there is a dearth of information on the nature of biotic stress. Therefore, it was envisaged that the phytotoxicity of petrochemicals would affect the plant physiology since some of the contaminants can inhibit the activities of certain enzymes in plants generally, and cassava in particular.

Cassava is a common food crop in western and south-eastern Nigeria and the oil-producing states of the Niger delta. Besides its preference for food security, cassava has tremendous industrial applications such as alcoholic beverages, industrial starch production, biofuel production and animal feed production (Li *et al.*, 2017). In addition, raw or boiled forms of the tuber and leaves have been reported to have medicinal properties (Nwose *et al.*, 2017). Cassava, therefore, provides income for small farmers and food for the nation. The plant tolerates drought, low soil fertility, and its production requires minimum external inputs (Mupakati & Tanyanyiwa, 2017).

We presumed that the growth of cassava under petroleum stress could elicit some oxidative stress and damage vital biomolecules in plants such as lipids, proteins and chlorophyll. In this study, the effects of diesel oil on cassava plants were studied at the molecular level, the possibility of eliciting oxidative stress was also monitored using prominent markers.

MATERIALS AND METHODS

Trichloroacetic Acid (TCA), 5,5-dithiobisnitro benzoic acid (DTNB) and 1-Chloro-2,4-dinitrobenzene (CDNB) were supplied by BDH, England. The diesel oil was obtained from a filling station at Oju-Elegba, Lagos. The Cassava stems (15 cm each) were obtained from Lagos State Polytechnics (LASPOTECH) farm in Lagos, Nigeria. Other reagents were of analytical grade and the purest quality available.

Garden topsoil with no previous history of crude oil contamination was collected, air-dried and sieved using 6 mm mesh. Twelve (12) stems were randomly distributed into four groups of three each and were planted in 500 g of soil contaminated with 75, 150 and 300 ml of diesel; that is, 150, 300 and 600 ppm, respectively. No diesel was added to the control. The

leaves of the plants were harvested after twelve weeks, weighed and analysed. Plunged leaves were washed in 1.15% KCl solution, blotted and weighed. Cell-free extracts for the various antioxidant enzymes were prepared by macerating leaves in chilled ice-cold extraction phosphate buffer pH 7.8 containing 0.1 mM EDTA, 1% w/v PVP, 0.5% Triton X-100 and 20% glycerol. The homogenate was centrifuged at 2,500 rpm for 15 min at 4°C (Sun *et al.*, 2021). The clear supernatant was used to estimate the enzymes and lipid profile parameters.

The activity of SOD was assayed by applying the method of Sun and Zigma (1978), by monitoring the inhibition of auto-oxidation of epinephrine as an increase in absorbance at 480 nm. Catalase activity was assayed colourimetrically at 620 nm and expressed as μmoles of H_2O_2 consumed/min/mg protein at 25°C according to the method of Singha (1972). The reduced glutathione content of the leaf was estimated as the non-protein sulphydryls according to the method described by Sedlak and Lindsay (1968), using 5-5' dithiobis2-nitrobenzoic acid (DTNB). The extend of lipid peroxidation level in leaf was estimated by measuring the pink coloured chromophore formed by the reaction of thiobarbituric acid with malondialdehyde (MDA) according to the method of Buege and Aust (1978). For the total protein, the leaf's homogenate was extracted using 0.05M HEPES buffer pH 7.5. Total soluble protein was determined by Biuret reaction as described by Gornall *et al.* (1949), using bovine serum albumin as standard.

The impacts of the contaminant on growth were determined in terms of leaf area, leaf weight and chlorophyll content. The leaf area ratio was calculated according to Kang and Van Lersel (2004) as the ratio of leaf area and its dry weight. Leaf area was measured as 0.5LB, where L and B were the length and breadth of the leaf, respectively (Pearcy *et al.*, 1989). The dry weight was determined after oven drying the leaves at 60°C for 24 h (Merkl *et al.*, 2004). Chlorophyll content was determined according to Arnon (1949). Briefly, leaves samples were homogenised in 80% acetone (40 ml/g leaf). The homogenate was filtered and centrifuged at 2500 rpm for 10 min. The absorbances of the supernatant were measured at 645 and 663 nm. Chlorophyll content was calculated as:

$$\text{Total chlorophyll (mg/g)} = 100(0.0202 \text{ O.D.}_{645} + 0.0082 \text{ O.D.}_{663})$$

All values have been expressed as mean±standard error of the mean (SEM) of triplicate observations. Data were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's post-test to analyse biochemical data. Statistical analyses were performed using SPSS statistical software package version 10. Values were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Selected oxidative stress markers and growth parameters were used to assess the oxidative stress, lipid peroxidation and changes in leaf veracity in cassava plants after twelve weeks' growth on diesel contaminated soil. The effect of diesel on selected oxidative stress markers showed that the enzymes SOD and CAT's activities were reduced significantly compared to the control. The reduction proceeds as the pollutant concentration increases (figure 1). The figure shows a significant ($p < 0.05$) decrease in SOD activity from 24.03±0.83 U/g leaf in control to 8.40±0.28, 6.76±0.43, 3.47±0.8 U/g leaf in plants grown on 150, 300 and 600 ppm diesel contaminated soil, respectively. Similar trends were observed for CAT with 27.56±0.20, 18.10±0.32, 16.43±0.73 and 9.11±0.16 μmol/min/g for 0, 150, 300 and 600 ppm contamination, respectively. The GSH level also depreciated from 10.24 ± 0.62 to 1.55±0.22 mg/g.
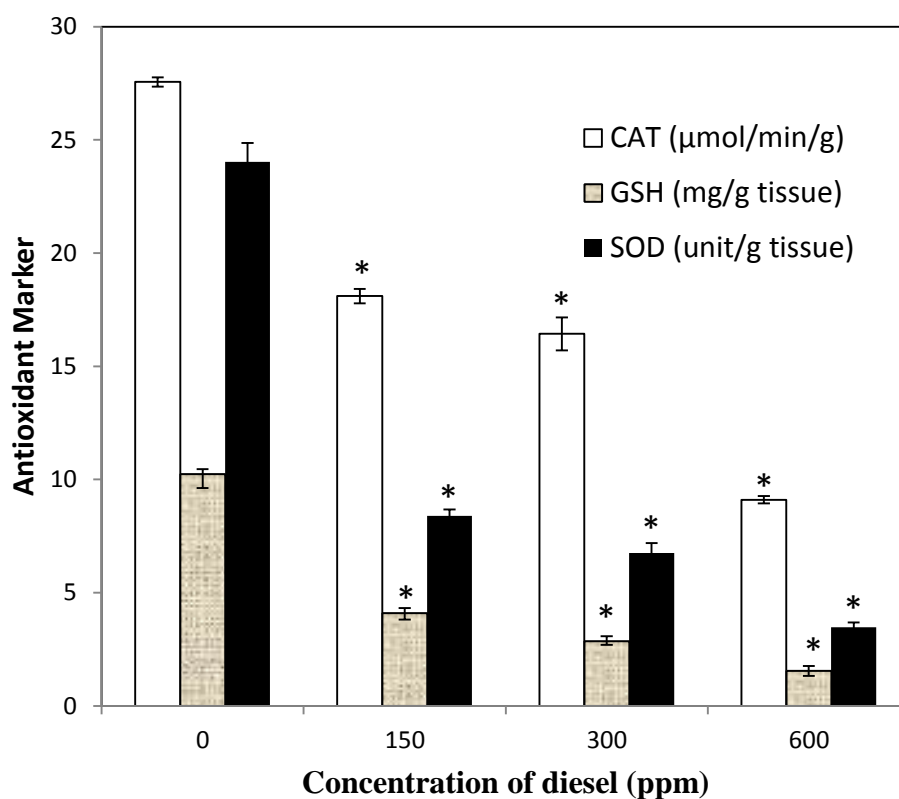
Fig 1: Effect of diesel on cassava oxidative stress markers: catalase (CAT) and superoxide dismutase (SOD) and reduced glutathione (GSH). Error bars showed the standard error of the mean of triplicate readings; * Significant difference at $p < 0.05$ compared with control.

Variations in levels of GSH and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) have been proposed as indicators of pollutant mediated oxidative stress (Mitra *et al.*, 2012; Xie *et al.*, 2019). The survival of living things under stressful conditions depends on the balance between the free radical generation and the free radical scavengers. Plants possess very efficient scavenging systems: antioxidant enzymes such as CAT and SOD (Pandey *et al.*, 2017). In this study, the activities of these enzymes reduced significantly in cassava plants planted in diesel contaminated soil compared to control. For instance, the CAT decreased in a dose-dependent manner by 34.31, 59.96 and 65.05% in plants planted on 150, 300 and 600 ppm, respectively. Similar but higher decreases of 59.96, 72.07 and 84.86% were observed for GSH, while the percentage decreases were 65.04, 71.87 and 85.56% for SOD. Therefore, it was inferred that the high level of free radical produced caused oxidative damage to membrane lipids, proteins, and other macromolecules in the leaves of the plants. The high level of MDA further substantiates that the decreased antioxidant enzymes activities observed in the leaf of plants grown on contaminated soil resulted from oxidative damage.

In the leaves of the plants grown on diesel contaminated soil, the levels of lipid peroxidation product – MDA were found to be elevated significantly ($p<0.01$). At the same time, the total protein decreased significantly ($p<0.05$) when compared to that of the control plants. At the end of the study, there was about a 79% reduction in total protein levels in cassava leaves grown on 600 ppm diesel contaminated soil; and well over 2000% elevation of MDA in the leaves of the same plants compared to control (Table 1).

Table 1: Effect of diesel on total chlorophyll, total protein and MDA level in the leaf tissues of cassava

Concentration of diesel (ppm)	Total Chlorophyll (mg/L)	Total Protein (mg/g fresh leaf)	MDA (µmol/mg protein)
Control (0)	0.025±0.002	1.064±0.454	0.10±0.01
150	0.016±0.001 **(36)***	0.312±0.014 **(71)***	1.77±0.34**(-1670)****
300	0.010±0.001 **(60)***	0.262±0.011 **(75)***	2.34±0.63 **(-2240)****
600	0.0083±0.001 **(67)***	0.219±0.005 **(79)***	2.72±0.07 **(-2620)****

Percentage decrease in parentheses, values are significant at $p<0.05$ * and $p<0.001$ ** when compared with control

The significantly high level of MDA level and the increase experienced as the concentration of the diesel increased, with the cassava plant grown on 600 ppm diesel having the highest level of MDA (figure 2), showed that cassava plants grown on soils contaminated with diesel are more susceptible to lipid peroxidation than the control. Malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids, has been utilised as a biomarker for lipid peroxidation (Khoubnasabjafari *et al.*, 2015). A high level of MDA is an indicator of increased oxidative processes in the cell. Lucas *et al.* (2019) have associated the high level of MDA to air pollution stress in Madrid compared to the same plant in Ciudad Real. During inevitable stress, MDA accumulates rapidly at the expense of the plasma membrane. The significant reduction of the total protein content of the leaves as the concentration of the diesel oil increased in the soil also suggested that diesel oil elicit oxidative damage to DNA and RNA, which are essential biomolecules in protein biosynthesis. Oxidative damage of protein is attributed to the presence of free radicals leading to the formation of carbonyl compounds (Tola *et al.*, 2021). Thus, as seen in this study, the total protein depletion is evidence of stress.
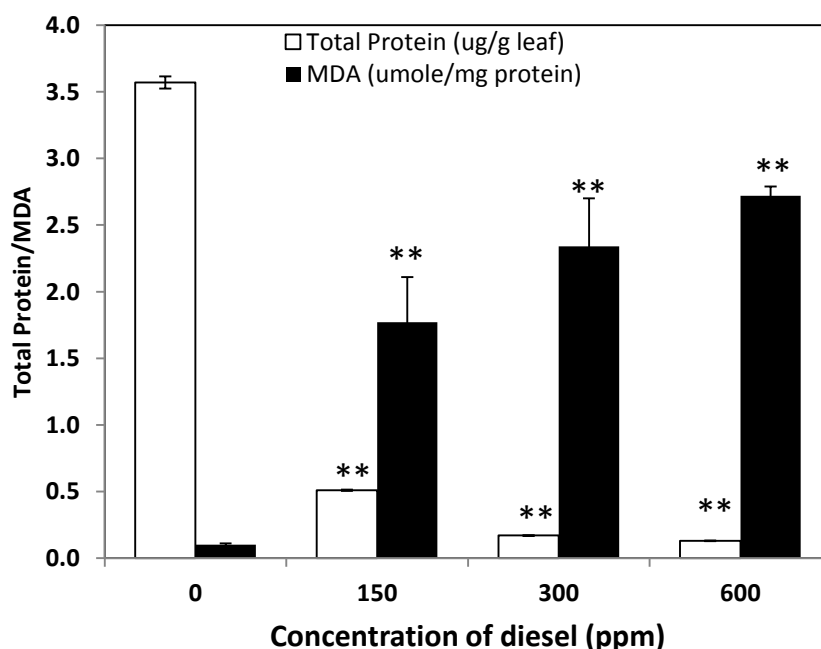

Fig 2: Effect of varied concentration of diesel on total protein and MDA level in cassava leaf. Error bars showed the standard error of the mean of triplicate readings.
** Significant difference at $p < 0.01$ when compared with control.

Figure 3 shows the mean leaf area, leaf weight, and leaf area ratio of cassava plants grown

in diesel oil-contaminated soil at concentrations of 0, 150, 300 and 600 ppm g. The highest values were obtained for the leaf area and leaf ratio with 0 mL (control) diesel oil contamination, while the least was obtained for 300 mL diesel oil contamination. The difference in treatment and control was significant ($P<0.05$) across the concentration drift. Conversely, there was a significant increase in the leaf weight across the concentration drift (from 0 to 300 mL/500 g); the increase was, however, not sustained from concentration to concentration. The significant reductions in the leaf area ratio observed in this study suggested retarded growth in the cassava plant grown on diesel contaminated soil. Studies have shown that a high concentration of pollutants can restrain the synthesis of chlorophyll enzyme, thereby reducing the plants' chlorophyll content, photosynthesis and inhibiting the growth of plants (Feng, 2006; Odukoya *et al.*, 2019).
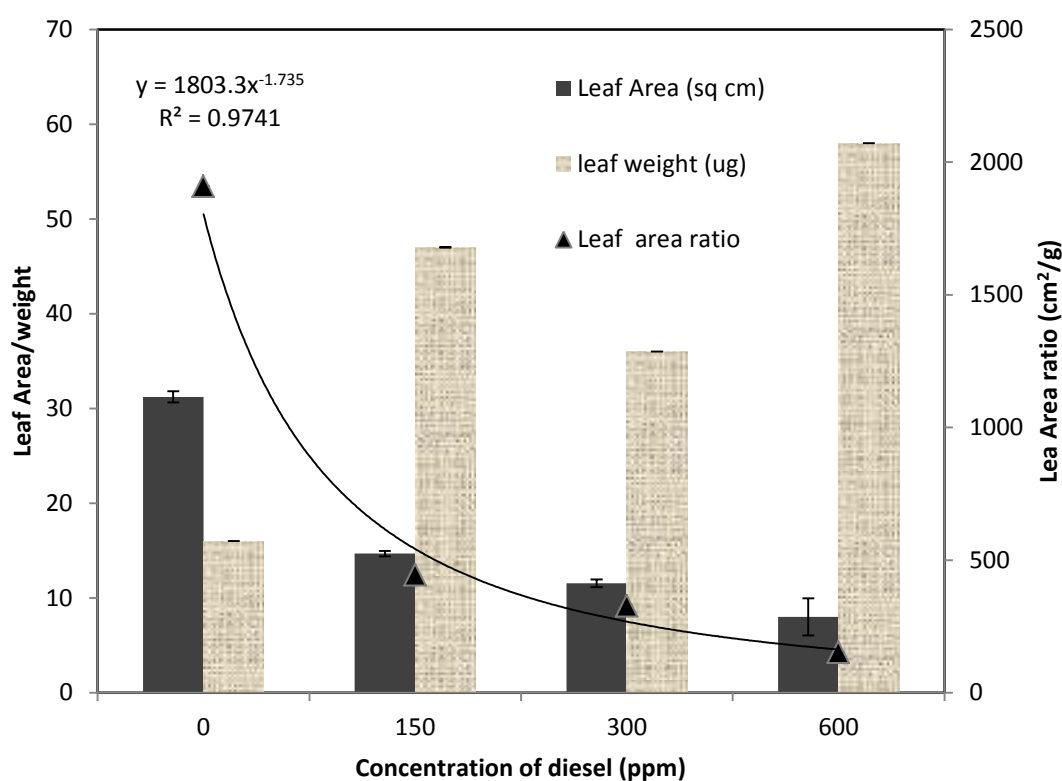
Fig 3: Variation in leaf area, leaf weight and leaf area ratio of cassava plant grown on soil contaminated with different diesel amounts. Error bars showed the standard error of the mean of triplicate readings.

The variations in total chlorophyll content of leaf of cassava plants grown in soils contaminated with different volumes of diesel oil are shown in Table 1. Decreasing trends, which are significant, were noticed in the chlorophyll contents of the plants from control to soils with the highest level of diesel content. The mean value of the total chlorophyll for the control was 2.48±0.20 mg/g, and the lowest mean value of 0.82±0.10 mg/g was recorded in leaves of plants grown on 300 mL diesel. However, the leaf area and ratio pose as good parameters for measuring the effect of pollutants on plant growth. Omosun *et al.* (2008) have linked the progressive depression in height, numbers of leaves and leaf area of *A. hybridus* grown in different crude oil concentrations to changes in soil condition, which imposed stressful conditions. The reduced chlorophyll content of the leaves on cassava plants grown

on diesel contaminated soil further explained the poor growth. In recent work, Wang *et al.* (2011) observed an initial increase in the total chlorophyll content in reeds grown on diesel contaminated soils (up to 1000 mg/kg). This increase in the total chlorophyll content is contrary to the findings in this study, in which the total chlorophyll decreases with increased diesel concentration. The discrepancies in the two studies suggested that different plants might react differently to initial low diesel concentrations. It is important to note that Reed has phytoremediation ability (Wang *et al.*, 2011); this ability has not been reported in cassava.

Generally, the effects of pollutants on plants are assessed by changes in the plants' morphology since growth is measured as an increase in size over a given period. The results on leaf physiology showed that the leaf weight might not be a suitable parameter to assess the extent of pollutant since the increase in weight was not consistent across the concentration drift. The study, however, shows a steady and significant decline in the living area as the concentration of diesel increased. A similar trend was observed with leaf area ratio but in a parabolic trend. This suggested that while leaf weight might not be a good index for measuring growth, the leaf area and leaf ratio are good indices from plant growth measurements. Several mechanisms have been proposed for these adverse effects of petroleum and petroleum products on plants. The toxic effects of these products at a concentration as low as 20 mL/kg on seed germination and growth of alfalfa, barley, clover and wheat, have been reported (Houshmanfar & Asli, 2011; Wyszkowski, 2020). It was also argued that the most potent plant growth inhibitors among petroleum products are long-chain alkanes and aromatic compounds. Wyszokowski and Ziolkowska (2008) have also reported that the addition of diesel oil to the soil led to a significant reduction of the organic carbon content of the soil.

CONCLUSION

Exposure of cassava plants to soil contaminated with diesel oil had adverse effects on their antioxidant activities, lipid peroxidation and growth performance, thereby altering the physiological and biochemical processes of the plant. These alterations in both physiological and biological processes are dose-dependent, as observed when the contaminant concentration was increased from 150 ppm to 300 and then 600 ppm. The impact of the contaminant was more in the reduction of GSH (85%) and SOD (86%) than it was to CAT (65%) at the contaminant concentration of 600 ppm. There were also damages to proteins (79%) and lipids (in the form of elevated MDA, >99%). This research thus concluded that diesel induced antioxidant stress in cassava plants grown on soil contaminated by it and that the generated reactive oxygen species contributed to stunted growth and death often experienced in plants grown on oil-contaminated soils.

GRANT SUPPORT DETAILS

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. In addition, the authors have wholly observed the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and falsification, double publication or submission, and redundancy.

LIFE SCIENCE REPORTING

No life science threat was practised in this research.

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