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Influence of sub-lethal concentrations of crude oil on tomato yield and quality

Johnson Odukoya[1,2]*, Ronnie Lambert[1] and Ruben Sakrabani[1]

[1]School of Energy, Environment and Agrifood, Vincent Building 52(a), Cranfield University, Bedfordshire, MK43 0AL, United Kingdom.
[2]Chemistry Department, The Federal University of Technology, PMB 704 Akure, Ondo State, Nigeria.
*Email: joodukoya@futa.edu.ng, +2349084486527;

Abstract

Heavy crude oil spills have been recorded to have detrimental effects on the soil properties of agricultural lands and the eventual agricultural produce. However, the influence of sub-lethal concentrations of crude oil on crop quality is not clearly understood. Hence, the present study assessed the response of Micro-Tom tomato fruits to low concentrations of crude oil. Although the range of crude oil concentrations used in this research had no significant effect on most of the nutritional and phytochemical parameters tested, the growth, yield, fruit production and ripening of the Micro-Tom tomato fruits were affected at crude oil remediation intervention value (CRIV) of 5,000 mg/kg TPH.The usual trend of organic acids' distribution in tomatoes was also altered. The experimental results re-affirmed that apart from genetics, environmental factors - such as crude oil contamination – may influence tomato fruit quality and yield. Nonetheless, the research findings suggest that crude oil-contaminated sites at ≤ 3,000 mg/kg TPH presents a similar growing environment to a clean site for the cultivation of sensitive crops with reduced negative impact on crop quality.

Keywords: Agrifood, Crop quality, Crop yield, Crude oil, Micro-Tom, Tomato.

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Introduction

Over the years, crude oil spills have been recorded in crude oil exploration and production sites. The break in pipelines during the transportation of this natural resource, have also been documented to contribute to the introduction of toxic petroleum hydrocarbons in the environment. In response to this, the government at various levels, particularly those in crude oil producing countries, have stipulated a crude oil remediation intervention value (CRIV) in which concentrations of petroleum hydrocarbons in the environment above this level denotes serious contamination (Odukoya, 2015). For instance, several European as well as national regulations have declared soils to be seriously contaminated at an intervention value of 5,000 mg/kg total petroleum hydrocarbons (TPH) (Soil Remediation Circular, 2009; Pinedo et al., 2013). Current clean-up standard at the Nigerian Niger Delta also require soil contamination to be less than this value as impact on plants persists even when remediation to this level is achieved (UNEP, 2011).

On the other hand, Tomato (*Solanum lycopersicum* L.) (Ilahy *et al.,* 2011), one of the world's most important and consumed vegetable crops (Singh *et al.,* 2013)is regarded as a sensitive crop(Kuhn *et al.,* 1998) which could be affected by abiotic factors (Riahi and Hdider, 2013) like petroleum hydrocarbons (Nie *et al.,* 2010). Consequently, this study was aimed at evaluating the impact of sub-lethal concentrations of crude oil /CRIV in the range of 750 – 5,000 mg/kg TPH on the growth, yield and quality of a sensitive crop, tomato (Micro-Tom cultivar). This is required to ascertain if: (1) crude oil contaminated sites at low concentration of crude oil are able to support the

production of one of the world's most important and consumed vegetable crops, as well as(2) sensitive crops, like tomato, from contaminated sites with ≤ 5,000 mg/kg TPH have the desired food quality.

Materials and methods

Tomato culture and mesocosm experimental design

The germinated Micro-Tom (accession WSS1414) tomato seeds were sown in modules filled with Sinclair all-purpose growing medium (William Sinclair Horticulture Ltd., Lincoln, England). The established seedlings in the modules were thereafter transferred into the respective pots containing different crude oil: soil concentrations as shown in Table 1. The randomized complete block design arrangement was adopted involving the use of Optipots.

At the start of the experiment and to achieve the same moisture content for the growing media in the pots, watering of the soil to 50% field capacity was carried out (Coulon *et al.,* 2012). The experiment was done at Cranfield University's research glasshouse facility with an average temperature of 25^{o}C and 55.93% relative humidity.

Growth parameters and post-harvest measurements

The growth parameters of the Micro-Tom tomato cultivar such as the average plant height, average number of leaves ≥ 2.0 cm and average number of fruits produced prior to harvesting were recorded.The Instron Bluehill® 2 (System ID Number 5542K3233; assembled in USA) equipment was employed for the firmness determination while the Chroma meter CR - 400 (Konica Minolta Sensing, Inc., Japan) calibrated on a white plate was used in evaluating the chroma (C*) (saturation), hue angle (h^{o}) (colour) and lightness (luminescence) of the harvested samples.

Samples preparation and analysis

At the end of the experiment, the harvested fresh tomato fruit samples were transferred to the laboratory. Meanwhile, in order to overcome the possible 'concentration effect', as plants under some stress conditions have been found to have lower water content (Wang and Frei, 2011), and to effectively compare the nutritional/phytochemical content as well as active accumulation of these components (Krauss *et al.,* 2006) in the harvested agricultural produce, the quantitative chemical assessments were carried out on a dry weight basis. Snap-freezing of the fruit samples was carried out after which they were freeze-dried, ground into powder and kept at -40^{o}C prior to chemical analyses.

Determination of nutritional composition

Determination of non-structural carbohydrate (sugars) distribution: The modified methods of O'Donoghue *et al.* (2004) and Chope *et al.,* (2007) were used for the extraction as well as quantification of sugars using the Evaporative Light Scattering Detector (ELSD). For these, 3 ml of High Performance Liquid Chromatography (HPLC) grade methanol: water (62.5: 37.5; v/v) mixture was added to 150 mg of the powdered freeze-dried Micro-Tom tomato samples in a 7-ml vial. The obtained extract filtrate (20 µl) from the extraction and filtration processes was injected into the Agilent Technologies 1260 Infinity HPLC system and ELSD (Agilent Technologies, Germany) for the analysis which involved the use of GRACE Prevail carbohydrate ES 5µ column. The mobile phase was HPLC grade water and acetonitrile at a gradient elution from 20:80 (%) to 50:50 (%) and run at a flow rate of 1.0 ml/min as well as column temperature of 30^{o}C.

Determination of crude protein contents: The Dumas method was used in evaluating the total nitrogen content (g/100g) of the powdered tomato fruit samples. According to Simonne *et al.,* (1997), the Dumas (combustion) method gives a true reflection of total nitrogen. Appropriate modification of the Dumas nitrogen (DN) content was done to express the nitrogen content in the tomato samples as a measure of protein content.

Determination of minerals contents: Dry mass (0.5 g) of the powdered tomato fruit samples was used for the initial digestion with nitric acid and hydrogen peroxide solution. Empty liner without the addition of sample was used as the blank. Final digestion of the samples was carried out using the Microwave Accelerated Reaction System (Model MARS®, CEM Corporation, USA) while the National Institute of Standards and Technology (Gaithersburg, MD, USA) Standard Reference Material (SRM) was also similarly treated to assess the suitability as well as accuracy of the method used.

The Perkin-Elmer Elan 9000 Inductively Coupled Plasma - Mass Spectrometer (ICP-MS; USA) was used to

analyse the filtrate obtained while the PerkinElmer Atomic Absorption Spectrometer (AAS; AAnalyst 800) was also used where appropriate.

Determination of phytochemicals composition

Determination of total phenolics and total flavonoids contents: The Howard Davies extraction method was used for assessing the total phenolics and total flavonoids in the powdered Micro-Tom tomato fruit samples. In this, 150 mg of the freeze-dried powdered sample was weighed in a 7-ml vial with the addition of 3 ml of ethanol (HPLC grade; Fisher Scientific, UK): water (80:20, v/v) for the extraction and filtration processes. The quantification involved the use of20 µl of the gallic acid (Sigma-Aldrich, USA) standard, 3.2 ml of deionised water, 200 µl of Folin and Ciocalteu's phenol reagent (Fisher Scientific, UK), 600 µl of prepared sodium carbonate solution and incubation in the dark for 2 h at room temperature. Other calibration standard concentrations, sample filtrate and sample blank were similarly treated with the measurement of the absorbance at 765 nm using the Helios UV/Visible spectrophotometer (Unicam Limited, UK). The concentration of the total phenolics in the powdered tomato samples was then calibrated against gallic acid. The total phenolics content was expressed as mg of gallic acid equivalents (GAE) per g of dry weight extract

The determination of the total flavonoids content followed a similar procedure but involved the use of 3 ml of sodium hydroxide (40 mg/ml), 100 µl of the calibration standard of quercetin (Sigma-Aldrich, USA) and measurement of absorbance at 420 nm. The total flavonoids content was expressed as mg of quercetin equivalents (QE) per g of dry weight extract.

Determination of lycopene and β-carotene contents: The extraction method of Nagata and Yamashita (1992) was employed for the determination of β-carotene and lycopene contents of the tomato samples with their modified equations. The pigments in 1 g of the tomato fruit samples were extracted with 20 ml of acetone-hexane mixture (4:6, v/v) by vigorous shaking for 60 s followed by filtration. The absorbance of the filtrate obtained was then measured at four different wavelengths: 453 nm, 505 nm, 645 nm and 663 nm using glass cuvettes on the Helios Gamma UV/Visible spectrophotometer. The pigments content were calculated using the following equations:

β-carotene (mg/100ml) = $0.216 \times A_{663} - 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$;

Lycopene (mg/100ml) = $-0.0458 \times A_{663} + 0.204 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$

The obtained pigments content were then further expressed in mg per 100 g of sample with A_{453}, A_{505}, A_{645} and A_{663} being the absorbance at the corresponding wavelength respectively.

Determination of non-volatile organic acids distribution: The modified method of Terry *et al.,* (2007) was used in the extraction and quantification of organic acids present in the tomato powdered samples. In this, 300 mg of the sample was weighed into a 7-ml vial with the addition of 3 ml of HPLC grade water. The vegetable extract (20 µl) obtained from the extraction and filtration processes was then injected into Alltima HP C18 AQ 5µ (GRACE) 250 mm × 4.6 mm column with OPTI-GUARD® 1 mm guard column. Analytical grade of 25 mM KH_2PO_4 (Fisher Scientific, UK) in HPLC grade water was used as the mobile phase while the separation was achieved using Agilent Technologies 1200 series HPLC system (Germany) at isocratic conditions for 10 min, flow rate of 1.5 ml/min and column temperature of 35°C.The desired non-volatile organic acids concentrations in the samples' extracts were then quantified against the calibration standards.

Statistical analyses

The IBM SPSS Statistical package (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0) was used in analysing the changes in the tomatoes' growth, postharvest measurements and chemical composition arising from the crude oil contamination. One-way ANOVA was used to compare the means involving Tukey HSD *post-hoc* analysis when the difference is statistically significant ($p < 0.05$). Sigma Plot for Windows Version 11.0 (Systat Software, Inc., 2008) was also employed where applicable.

Results and Discussion

Growth parameters and postharvest measurements

Effect on Micro-Tom tomato growth and yield: The experimental results as shown in Figure 1revealed that CRIV of 5,000 mg/kg TPH had the most reducing effect on the Micro-Tom tomato growth, fruit production and fruit ripening. Also, only the crude oil contamination

at 5,000 mg/kg TPH, compared with the control treatment, significantly reduced ($p < 0.05$) both the fruit and root yield of the Micro-Tom tomato cultivar (Table 2).

The decrease in the Micro-Tom tomato growth from the 5,000 mg/kg TPH treatment can be attributed to the: (i) toxic compounds involved in petroleum hydrocarbons contamination (Merkl *et al.,* 2004), (ii) limitation caused by the oil on transpiration and photosynthesis (Baruah *et al.,* 2014), (iii) effect of petroleum hydrocarbons on soil physico-chemistry leading to poor wetting and aeration of the soil (Nwazue, 2011), as well as (iv) the ability of hydrocarbons to coat plant roots which affect water and nutrient absorption (Xu and Johnson, 1995). Following the report of Inckot *et al.,* (2011), the decrease in shoot development as recorded in the Micro-Tom tomato fruits arising from the use of low TPH concentration such as 5,000 mg/kg TPH in this experiment, is as a result of physicochemical alterations in the soil but not directly linked to petroleum effect. In line with Inckot *et al.,* (2011), this decrease in shoot development of the Micro-Tom tomato cultivar at this CRIV (i.e. 5,000 mg/kg TPH), suggests a survival strategy of the plant in reducing the rate of water use and delaying the onset of more severe stress.

Effect on tomato fruits' firmness and colour parameters: With the exception of the Micro-Tom tomato fruit samples from the 3,000 mg/kg TPH treatment, crude oil contamination in the range of 750 - 5,000 mg/kg TPH had no significant effect on the firmness of the harvested tomatoes as shown in Table 3.

On the other hand, although external colour measured in terms of hue angle is considered the most vital indicator of tomato quality level (Radzevicius *et al.,* 2009), assessment of the effect of crude oil contamination on the colour of the harvested tomato fruits revealed that CRIV in the range of 750 – 5,000 mg/kg TPH as used in this experiment, had no significant effect ($p > 0.05$) on the tomato fruits' colour. The overall means of the lightness, chroma and hue angle of the Micro-Tom tomato fruits from all the treatments were: 40.17, 39.65 and 41.69, respectively.

The significant difference ($p < 0.05$) in the firmness of Micro-Tom tomato fruits from the 3,000 mg/kg TPH relative to the control treatment (no crude oil contamination) samples is as a result of delayed fruit production of samples from the crude oil-containing treatments (Fig. 1) as tomatoes lose their firmness as they ripen (Nunes, 2008). On the other hand, with firmness (N/mm) defined as average slope of the force/deformation curve (Batu, 2004), the firmness of the tomato samples being > 1.46 N/mm indicated that all the harvested tomato fruits are very firm (Batu, 2004).

The results of the tomato fruits colour parameters agree with the outcome of the levels of lycopene, the carotenoid pigment responsible for the red colour of tomatoes (Ronen *et al.,* 1999), in the analysed tomato samples which although could be altered by environmental growing conditions (Sahlin *et al.,* 2004), were statistically unaffected ($p > 0.05$) by the presence of petroleum hydrocarbons (between 750 - 5,000 mg/kg TPH) in the growing medium.

Nutritional composition analysis

The HPLC analysis carried out showed that crude oil contamination in the experimental range had no significant effect on the sugars' (total sugar, fructose, glucose and sucrose) concentration in the Micro-Tom tomato fruits when compared with the amount found in the control treatment samples. The investigation on the crude protein content and major dietary minerals (Na, Mg, Ca, K and P) also gave a similar result. The overall means of these nutrients in the tomato fruits are provided in Table 4.

Practically, results of the sugars distribution in the harvested tomato samples agree with the view noted in Velterop and Vos (2001) that most of the sugars in tomato fruits are in the form of fructose and glucose while sucrose may be present in low quantities as shown in Table 4. Whereas the outcome of the crude protein content of the tomato cultivars' samples involving environmental stress with no significant effect obtained in this study is uncommon with respect to published literature (Wang and Frei, 2011), the experimental results suggest that the intensity of the crude oil contamination (at 750 - 5,000 mg/kg

Table 1: Composition of the different treatments

Pot Treatments	Crude oil: soil concentrations
Treatment 1	0 mg/kg TPH (Control)
Treatment 2	750 mg/kg TPH
Treatment 3	1,500 mg/kg TPH
Treatment 4	3,000 mg/kg TPH
Treatment 5	5,000 mg/kg TPH

Table 2: Effect of different CRIV on Micro-Tom tomato yield

Treatments	Yield	
	Total fruit mass per plant (g/plant)	Root fresh weight (g/plant)
Treatment 1 (0 mg/kg TPH, Control)	159.63 ± 3.71[a]	5.20 ± 0.59[a]
Treatment 2 (750 mg/kg TPH)	154.50 ± 1.83[a]	5.01 ± 0.49[a,b]
Treatment 3 (1,500 mg/kg TPH)	142.63 ± 9.98[a]	4.94 ± 0.54[a,b]
Treatment 4 (3,000 mg/kg TPH)	133.60 ± 6.72[a]	4.59 ± 0.36[a,b]
Treatment 5 (5,000 mg/kg TPH)	59.70 ± 3.97[b]	3.04 ± 0.19[b]

Values are the means of three replicates ± SE. Means followed by different letters are significantly different ($p < 0.05$) according to Tukey *post-hoc* test.

Table 3: Effect of different CRIV on Micro-Tom tomato firmness (N/mm)

Treatments	Tomato firmness (N/mm)
Treatment 1 (0 mg/kg TPH, Control)	2.87 ± 0.11[b]
Treatment 2 (750 mg/kg TPH)	3.12 ± 0.04[a,b]
Treatment 3 (1,500 mg/kg TPH)	3.32 ± 0.10[a,b]
Treatment 4 (3,000 mg/kg TPH)	3.55 ± 0.03[a]
Treatment 5 (5,000 mg/kg TPH)	3.16 ± 0.20[a,b]

Values are the means of three replicates ± SE. Means followed by different letters are significantly different ($p < 0.05$) according to Tukey *post-hoc* test.

Table 4: Overall means of nutrients in Micro- Tom tomato fruits

Nutrients	Overall Means
Non-structural carbohydrate (sugars, mg/g DW)	
Total sugar	432.69
Fructose	239.80
Glucose	161.74
Sucrose	31.16
Crude Protein (g/100g DW)	
Crude Protein	7.14
Major Dietary Minerals (mg/kg DW)	
Na	318.00
Mg	1,694.56
Ca	1,323.47
K	32,596.00
P	4,328.07

Table 6: Malic acid/Citric acid ratios in the Micro-Tom tomato fruits

Treatments	Malic acid/Citric acid
0 mg/kg TPH (Control)	0.08 ± 0.01a
750 mg/kg TPH	0.05 ± 0.01b
1,500 mg/kg TPH	0.03 ± 0.00b
3,000 mg/kg TPH	0.04 ± 0.00b
5,000 mg/kg TPH	0.04 ± 0.00b

Values are the means of three replicates ± SE. Means followed by different letters are significantly different ($p < 0.05$) according to Tukey post-hoc test.

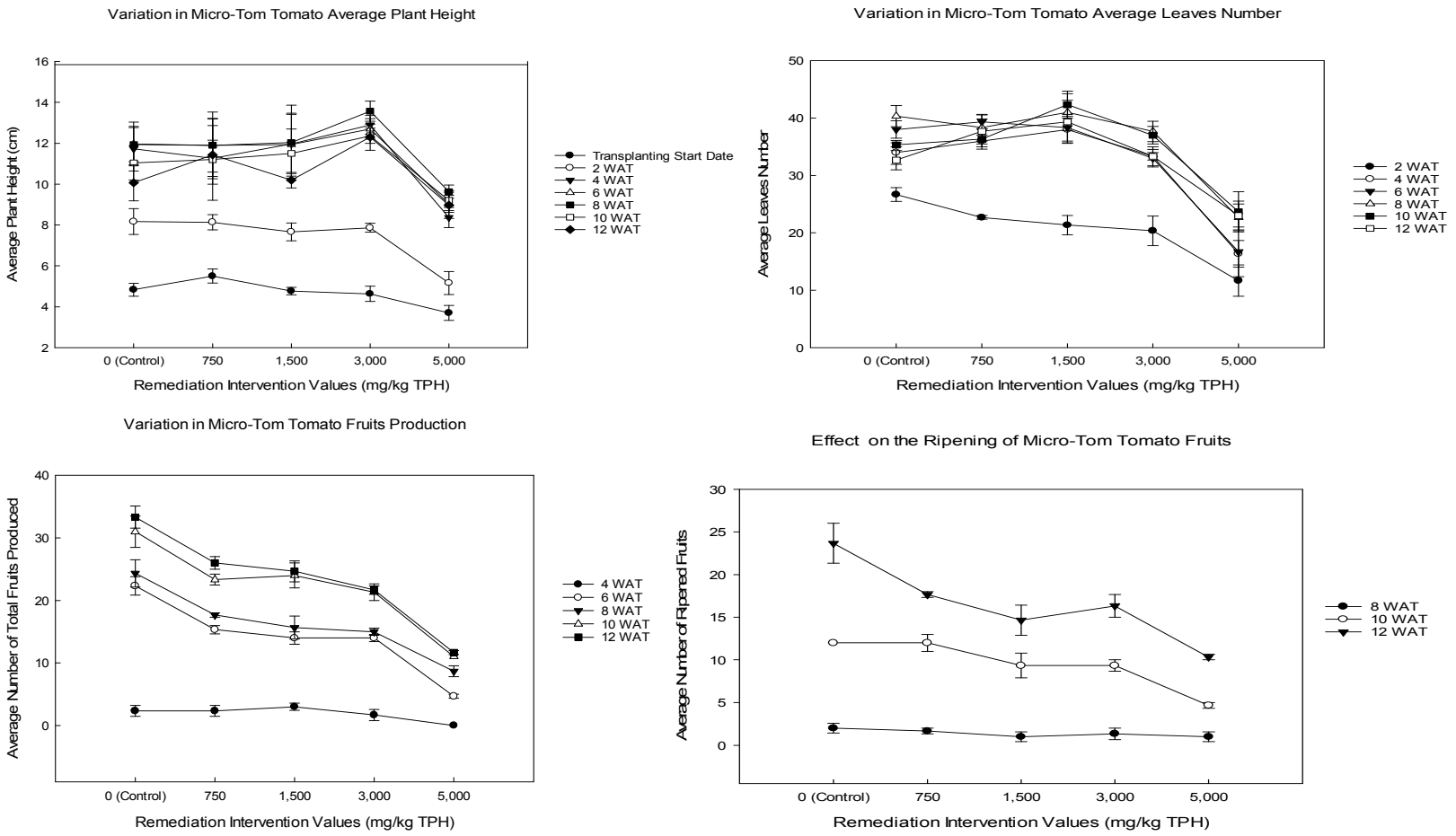

Fig. 1: Effect of different CRIV on Micro-Tom tomato growth parameters and fruits production.

Values are the means of three replicates ± SE. WAT = Weeks after transplanting.

Table 5: Distribution of minor dietary minerals and mineral contaminants (mg/kg) in Micro-Tom tomato fruits

Treatments	Minor Dietary Minerals						Mineral Contaminants			
	Cu	Zn	Mo	Fe	V	Ni	Sr	Ba	Cd	Pb
Treatment 1 (0 mg/kg TPH, Control)	4.42 ± 0.35[a]	12.08 ± 0.22[a]	6.01 ± 0.95[a]	25.60 ± 0.50[a]	0.063 ± 0.01[a]	0.31 ± 0.08[a]	1.52 ± 0.09[b]	0.59 ± 0.07[b]	0.049 ± 0.00[a]	0.071 ± 0.01[a]
Treatment 2 (750 mg/kg TPH)	3.90 ± 0.12[a]	10.33 ± 0.30[a]	3.44 ± 0.85[a,b]	24.47 ± 0.93[a]	0.042 ± 0.01[a]	0.24 ± 0.09[a]	1.27 ± 0.07[a,b]	0.33 ± 0.01[a]	0.052 ± 0.00[a]	0.044 ± 0.01[a]
Treatment 3 (1,500 mg/kg TPH)	3.98 ± 0.16[a]	11.45 ± 0.82[a]	2.30 ± 0.29[b]	25.87 ± 0.35[a]	0.048 ± 0.00[a]	0.40 ± 0.06[a]	1.09 ± 0.06[a,b]	0.33 ± 0.09[a]	0.046 ± 0.00[a]	0.041 ± 0.01[a]
Treatment 4 (3,000 mg/kg TPH)	4.59 ± 0.07[a]	13.41 ± 0.27[a]	2.08 ± 0.28[b]	26.60 ± 1.78[a]	0.049 ± 0.01[a]	0.46 ± 0.19[a]	1.07 ± 0.02[a,b]	0.35 ± 0.01[a,b]	0.046 ± 0.00[a]	0.039 ± 0.00[a]
Treatment 5 (5,000 mg/kg TPH)	4.71 ± 0.04[a]	13.49 ± 1.21[a]	1.87 ± 0.12[b]	27.13 ± 0.79[a]	0.065 ± 0.01[a]	0.61 ± 0.14[a]	1.01 ± 0.18[a]	0.48 ± 0.03[a,b]	0.039 ± 0.00[a]	0.038 ± 0.01[a]

Values are the means of three replicates ± SE. Means followed by different letters are significantly different ($p < 0.05$) according to Tukey *post-hoc* test.

TPH) / induced abiotic stresses were not up to the level that could affect the crude protein content of the harvested tomato fruits.

With the exception of Mo and Sr content, the concentration of the minor dietary minerals and mineral contaminants tested in the tomato fruits were also unaffected as illustrated in Table 5. The results of the minerals' distribution in the Micro-Tom tomato fruits indicated that there was no direct interaction between crude oil contaminations in the range of 750 - 5,000 mg/kg TPH on the concentration of most of the minerals in the Micro-Tom tomato fruits, including the major dietary minerals. The results of the major dietary minerals distribution in the harvested tomato samples support the findings in literature that fruits and vegetables are poor sources of Na (Halevy *et al.,* 1957) while the high K content in these cultivars (Table 4) indicates that they may be used with advantage in potassium-repletion diets (Halevy *et al.,* 1957).

Furthermore, the mineral analysis results revealed that there was an antagonistic effect (Jarrell and Beverly, 1981) of crude oil contamination in the experimental range on the concentration of Mo in the harvested Micro-Tom tomato fruits (Table 5) from these treatment pots. The antagonistic effect of crude oil application at 5,000 mg/kg TPH and 750 – 1,500 mg/kg TPH also led to reduced Sr and Ba contents respectively in the Micro-Tom tomato fruits. In this latter case, however, the reduction in the Sr and Ba concentrations in the Micro-Tom tomato fruits from these treatments is beneficial as the two minerals are regarded as contaminants which have harmful effect on human health when in excess (Nieman *et al.,* 1992). The outcome of the minerals analysis also revealed that among all the harvested tomatoes, only the Micro-Tom tomato fruits from the control treatment (no crude oil contamination), had a Pb content (0.071 mg/kg) (Table 5) above the FAO/WHO Codex maximum level (ML) of 0.05 mg/kg for fruiting vegetables (Codex Alimentarius, 2015). However, this Pb concentration in the Micro-Tom tomato control samples, as noted in Samara (1992), is below the maximum permissible concentration of the element (2.0 – 2.5 mg/kg DW) in vegetables for human consumption.

Phytochemicals composition analysis

Similar to some of the results obtained in the nutritional analysis, crude oil contamination at CRIV between 750-5,000 mg/kg TPH had no significant effect ($p > 0.05$) on the total phenolics, total flavonoids, lycopene and β-carotene contents of the Micro-Tom tomato fruits with mean values of: 8.69 mg GAE/g DW, 10.78 mg QE/g DW, 7.71 mg/100g DW and 7.34 mg/100g DW, respectively.

Investigation on the organic acids distribution in the harvested tomato samples revealed that crude oil contamination at 1,500 and 3,000 mg/kg TPH led to a reduction in the malic acid content. Meanwhile, contrary to the view noted in Gaur and Sharma (2014) that vitamin C loss is the most sensitive indicator of stress exposure, there was a significant increase ($p = 0.018$) in the concentration of ascorbic acid found in the Micro-Tom tomato samples from the 3,000 mg/kg TPH treatment (Fig. 2).

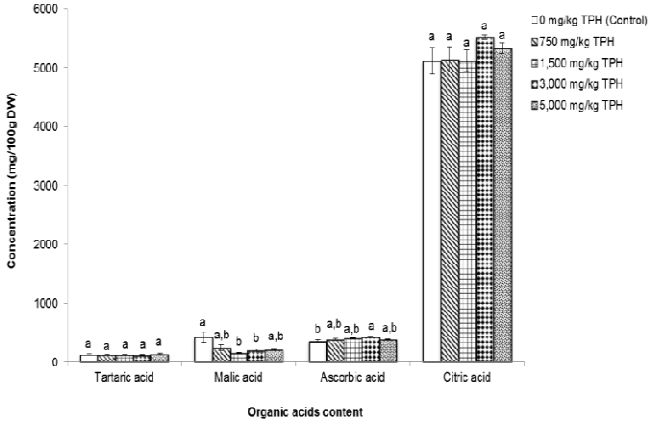

Fig. 2: Organic acids distribution in Micro-Tom tomatoes.

Values are the means of three replicates ± SE. Means followed by different letters are Significantly different ($p < 0.05$) according to Tukey *post-hoc* test.

Nonetheless, crude oil contamination in the experimental range altered the usual trend of tomatoes' organic acids distribution in the harvested Micro-Tom tomato fruits. In line with Emin (2001), citric and malic acids are the main organic acids in tomatoes but this was only true for Micro-Tom tomato fruit samples from the control treatment (i.e. with citric acid > malic acid > ascorbic acid > tartaric acid).

Assessment of the total phenolic and total flavonoids content of the tomato fruits revealed that crude oil contamination in the range used in this experiment had no significant effect on the metabolic activity of the plant. Although some studies involving the effect of environmental stresses on lycopene content in tomato fruits reported by Wang and Frei (2011) indicated an increase in lycopene concentration, such increment according to Krauss et al. (2006) as well as Wang and Frei (2011) could partly be as a result of concentration effect arising from reduced water content in the stressed crops. This increase in carotenoid content of crops has also been linked with enhanced biosynthesis (Krauss *et al.,* 2006; Wang and Frei, 2011). The results of the lycopene and β-carotene distributions in the tomato fruits as obtained in this experiment indicated that crude oil contamination within the experimental range had no significant effect on their biosynthesis.

Table 6 showed that Micro-Tom tomato fruits from the control (no crude oil) treatment had significantly ($p < 0.05$) higher malic acid/citric acid ratio than those from the crude oil-contaminated treatments. Generally, the results of the malic acid/citric acid ratio of the harvested tomato cultivar (Table 6) support the view of Emin (2001) that the malic acid/citric acid ratio in red ripe tomato is ≤ 0.5.

The significant increase in the L-ascorbic acid content of the Micro-Tom tomato fruits from the 3,000 mg/kg TPH treatment (419.54 mg/100g DW) is as a result of the induced water stress on the plants via the presence of crude oil in the growing medium. In line with the report of Akinci and Losel (2012), this led to the accumulation of this organic acid which contributes to osmotic adjustment. To some extent, results from previous experiments on the effect of environmental stress on ascorbate (vitamin C) content in harvested products have, however, been controversial (Wang and Frei, 2011). Following the report of Meredith *et al.,* (1989), the significantly higher malic acid/citric acid ratio ($p < 0.05$) in the Micro-Tom tomato fruits from the control treatment compared with those obtained in this cultivar's fruits from the crude oil-contaminated treatments (Table 6), indicates that the Micro-Tom tomato fruits from the control treatment have increased maturity. This agrees with the data presented in Figure 1 in which Micro-Tom tomato fruits from the control treatment had the highest average number of ripened fruits.

Conclusion

In contrast to the finding of Alexander and Webb (1987) in which Libyan crude oil of up to 5 mg/g did not affect the growth of *S. alterniflora*, the experimental results in this study revealed that crude oil contamination at 5,000 mg/kg TPH affected the growth, fruit production and ripening of tomato, a sensitive crop. The yield of the Micro-Tom tomatoes was also significantly ($p < 0.05$) affected by crude oil contamination at this CRIV (i.e. 5,000 mg/kg TPH). Aside from the organic acids and some minerals distribution in the Micro-Tom tomatoes from the crude oil-contaminated treatments, most of the nutritional and phytochemical contents of the harvested tomato samples were unaffected (on dry weight basis) by the different CRIV considered in this experiment. The elimination of 'concentration effects' and observed changes in some of the chemical compositions (e.g. minerals and organic acids) tested further re-affirm that apart from genetics, environmental factors (such as crude oil contamination) may affect tomato fruit quality as well as yield (Baxter *et al.,* 2005). The research findings suggest the ability of CRIV ≤ 3,000 mg/kg TPH to support the cultivation of sensitive crops with reduced negative impact on crop quality.

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