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Effect of crude oil pollution on heavy metal content and GST gene expression of *Mucuna pruriens*

Chibuzor Okwudili Okoro, Julian Onyewuonyeoma Osuji and Edache Bernard Ochekwu *

Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

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Abstract

The study investigated the effect of crude oil pollution on the heavy metal content and gene expression of *Mucuna pruriens*. Varying amounts of crude oil (0ml, 200ml, 400ml, 800ml and 1000ml) were used to pollute 10kg bags of loam soil, onto which *Mucuna pruriens* seeds were planted for 8 weeks. Data were collected for heavy metal content of plant tissues and GST gene expression. The highest heavy metal content of the plant tissue was observed at the 800ml treatment (0.0135 g kg^{-1}, 0.074 g kg^{-1}, and 0.0211 g kg^{-1}) and the least was observed for the control treatment (0.0049 g kg^{-1}, 0.034 g kg^{-1} and 0.0142 g kg^{-1}), thus showing percentage increments of 175.51%, 117.65%, and 48.59% for the copper, zinc, and nickel content respectively. This showed that the increment of crude oil pollution in the soil led to a seemingly dose-dependent increment of heavy metal content of the plant tissue. The normalized expression levels of the target gene (GST) in the calibrator (control) sample "A", and that of the treated/polluted (test) sample "B" were established as 0.150726 and 0.145592 respectively; and, it was established that there was no significant change in the normalized expression levels of the target gene (GST) in both the control (calibrator) sample and the treated/polluted (test) sample. Crude oil treatment of *M. pruriens*, despite showing reductions in the morphological parameters and increments in the heavy metal content of the tissues, didn't affect the expression of the target GST gene, as was evident in the absence of significant difference in the normalized expression levels of the target gene (GST) in both the control (calibrator) sample and the treated/polluted (test) sample. Considering the resilience of *M. pruriens* and its ability to adapt to the high amounts of crude oil in the soil, it is advised that it be employed in the possible phyto-extraction of crude oil and/or phyto-remediation in crude oil polluted soils.

Keywords: Crude oil; Heavy metal; GST gene expression; Phytoremediation; Pollution

1. Introduction

Petroleum hydrocarbons are used extensively at the global scale as a source of energy and a fuel. Owing to its global huge and increasing demand as a fuel and energy source, contamination occurs quite often as a result of exploration, production, maintenance, transportation, storage and accidental release, leading to significant ecological impacts. As the modern civilization developed, it created pressure on the energy source, especially on petroleum hydrocarbons (Aggarwal *et al*., 2006).

In recent decades, soil contamination with crude oil and heavy metals has become an environmental crisis due to their long term stability and adverse effect on biological organisms. The general increase of heavy metal content in the soil has been largely caused by crude oil spillage (Anoliefo and Vwioko, 1995). Effects of crude oil and heavy metals pollution are very extreme on biological organisms. Crude oil pollution causes accumulation of metals in soil which adversely affects the physiological and genetic attributes of crop plants. The effect of heavy metals from different polluted sources is very detrimental to both humans and plants.

* Corresponding author: Edache Bernard Ochekwu

Heavy metals are generally referred to as those metals which possess a specific relatively high density (more than 5g cm^{-3}) and adversely affect the environment and living organisms, even at small concentrations. Heavy metal pollution of the soil is caused by various metals especially Copper, Nickel, Cadmium, Chromium, and Lead (Hinojosa *et al*, 2004).

It is a common knowledge that certain types of environmental pollution does not only results in adverse effects on various parameters relating to plant quality and yield, but also causes changes in the size, composition and activity of the microbial activities (Yao *et al,* 2003). In addition, these pollutants make their way into the food chain from soil and plants and pose threat to the ecosystem on a larger perspective and to the human health specifically. Presence of heavy metal has been detected in a large number of leafy vegetables and crops (Ramteke *et al*; 2016, Sharma *et al*, 2016). Eco-friendly biological organisms including plants have been severally utilized in bio-remediation technology for containment of these crude oil and heavy metals pollutants. Therefore, the impact of these pollutants on the genome size and nucleic acid content of plants taking them up is a research-worthy necessity.

Considering the adverse effects of crude oil pollution on plants and its attendant implications for food security and environmental integrity in oil rich regions, it has become necessary to screen for plants with strong tolerance to soil crude oil contamination and how the crude oil pollution affects such plants. On the discovery of such plants, they could be possibly employed in the phytoremediation practice in the oil producing areas of the Niger Delta; and, if certified safe for consumption, will help ameliorate the deteriorating the food security situation of the oil rich Niger Delta region of Nigeria. One of such plants that is being probed for its ability to withstand the impact of crude oil is the velvet bean – *Mucuna pruriens* (Ochekwu *et al*., 2012), is a plant of the Fabaceae family (Rajeshwar *et al.*, 2005), known popularly for its plethora of medicinal properties, and grown generally for its green manure and mixed culture.

A plenitude of reports has it that extreme environmental conditions such as heavy metal and/or crude oil pollution affect the morphology and genetic expression of plants. Some reports have shown that the *M. pruriens* planted in crude oil polluted soil have increased presence of heavy metals in the plant tissues (Karangwa *et al*., 2018). This ability of *M. pruriens* to extract heavy metal pollutants into its tissues have been investigated by some researchers (Nwaichi *et al*., 2022). Being a plant that often grows as a weed that thrives in certain extreme environments such as roadsides, bushfallows and wastelands, *M. pruriens* has shown its ability to withstand adverse edaphic and climatic factors; but, there seems to be a dearth of information regarding the effect of crude oil pollution on the plant at the genetic level and relating same to the heavy metal content of the plant tissue.

This study, therefore, aims to investigate the effect of crude oil pollution on the morphology, heavy metal content of the plant tissue, and the variability of genetic expression of the plant.

2. Materials and methods

2.1 Experimental Site

The study was conducted in plastic bags in the Centre for Ecological Studies, Department of Plant Science and Biotechnology, University of Port-Harcourt, Choba, Rivers State, Nigeria.

2.2 Source of Materials Used for the Study

The *Mucuna pruriens* plant materials used for the study were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The loamy soil was obtained from the Agriculture Demonstration Farm of the University of Port Harcourt. The crude oil was obtained from Nigeria National Petroleum Company.(NNPC). PLC.

2.3 Soil Treatment with Crude Oil

Plastic bags filled with 10kg of soil were treated with varying amounts of crude oil – 0ml (control), 200ml, 400ml, 800ml and 1000ml – and allowed to stay and acclimate under environmental conditions for a 2 week period, after which 5 seeds of *Mucuna pruriens* were planted into the crude oil treated soils. The experiment was laid out in a completely randomized design (CRD) with four replicates. The soil was moistened every two days with potable water throughout the 8 weeks duration of the experiment. Every 2 weeks, during the course of the study, data was collected and collated for plant height and number of leaves. At termination (the 8th week), all data was collected and collated, including the data on gene expression, microbial population dynamics and pedological parameters.

2.4 Determination of Heavy Metal Content

Some 5g of air-dried pulverized plant samples were weighed into the extraction bottle, and extracted with 25ml of the respective extraction solutions (Ammonium acetate containing 0.01N EDTA pH 7; 0.1N HCl) was added. The mixture was agitated for 30 minutes using a mechanical shaker. The mixture was filtered using Whatman filter paper No. 1. The value of copper, zinc and nickel in the sample solution was determined from the standard curve using the Atomic Absorption Spectrophotometer (AAS) (Tokaliolu *et al*. 2001).

2.5 Protocol for Extraction of Plant DNA

For optimal performance add beta-marcaptoethanol (user supplied) to the Genomic Lysis Buffer to a final dilution of 0.5% (v/v) i.e 250ul per 50ml ulor 500ul per 100ml. 150mg of finely cut plant or seed sample[1] were added to a ZR Bashing Bead™ Lysis Tube (2.0mm). 750 ul BashingBead™ Buffer was added to the tube and capped tightly. This is secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for ≥5 minutes. The ZR BashingBead™Lysis Tube (2.0) was centrifuged in a microcentrifuge at ≥ 10,000xg for 1minute. 400ul Supernatant was transferred to a Zymo-Spin™ IIIF Filter in a Collection Tube and centrifuged at 8,000 xg for 1minute; and, the Zymo-Spin Tm III-F Filter was discarded. Add 1,200uI of Genomic Lysis Buffer in the filtrate in the collection Tube from Step 4 mix well. 800ul of the mixture was transferred to a Zymo-Spin™ IIC colum[2] in a collection Tube and centrifuged at 10.000 x g for 1minute. 200ul DNA Pre-wash Buffer was added to the Zymo-Spin™ IIC colum[2] in a new collection Tube and centrifuged at 10,000xg for 1 minute. 500ul g-DNA Wash Buffer was added to the Zymo-Spin™ IIC colum and centrifuged at 10,000xg for 1 minute. The Zymo-Spin™ IIC colum was transferred to a clean 1.5ml microcentrifuge tube and 100 ul (50ul minimum) DNA Elution Buffer added directly to the column matrix and centrifuged at 10,000xg for 30 seconds to elute the DNA. A Zymo-Spin™ III-HRC Filter was placed in a clean collection tube and 600 ul PrepSolution was added, and centrifuged at 8,000 xg for 3 minutes. The eluted DNA was transferred to a prepared Zymo-Spin™ III-HRC Spin Filter in a clean 1.5ml microcentrifuge tube and centrifuged at exactly 16,000 x g for 3 minutes.

2.6 Protocol for Quantifying GST Gene Expression

Following the method adopted by Badawy *et al*. (2018), Gene Expression Analysis through Quantitative Real-Time (qRT-PCR) was done. According to the manufacturer's instructions, total RNA was extracted using Quick-RNA Miniprep Kit. The cDNA was synthesized. The ubiquitin (UB10) gene was used as a housekeeping internal reference gene for normalization, while the GST gene was the targeted gene. The relative GST gene expression level was calculated by the Livak ($2^{-\Delta\Delta C_T}$) technique.

2.7 RNA extraction

RNA was extracted using the Quick-RNA Miniprep Kit (Zymo Research, Catalogue No. D3024). 400 µl of Genomic Lysis Buffer was added to 100 µl of the crushed sample was mixed completely by vortexing 4-6 seconds, then let stand 5-10 minutes at room temperature. The mixture was transferred to a Zymo-Spin™ IICR Column in a Collection Tube and centrifuged at ≥ 10,000 x g for one minute. Discard the Collection Tube with the flow through.**.** The Zymo-Spin™ IICR Column was transferred to a new Collection Tube, and 200µl of DNA Pre-Wash Buffer to the spin column. Centrifuge at ≥ 10,000 x gfor one minute. 500 µl of g-DNA Wash Buffer was added to the spin column and centrifuged at ≥ 10,000 x g for one minute. The spin column was transferred to a clean microcentrifuge tube and 100 µl DNA Elution Buffer added to the spin column. The column was incubated for 2-5 minutes at room temperature and then centrifuged at ≥ 10,000 x g for 30 seconds to elute the DNA.

2.8 DNA Quantification

The quality and quantity of the extracted RNA was measured using a NanoDrop (Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer). The system was blanked using 1ul of DNA Elution Buffer. Afterwards, 1ul of the DNA was placed on the pedestal and measured.

2.9 qPCR Protocol

RT-qPCR reactions were performed using SYBRLuna® Universal qPCR Master Mix (New England Biolabs, USA) on a CFX-96 Real-Time PCR (Bio-Rad,USA) according to the manufacturer's instructions. Component: 20 µl REACTION; Luna Universal One-Step Reaction Mix (2X): 10 µl; Luna WarmStart® RT Enzyme Mix (20X): 1 µl; Forward primer (10 µM): 0.8 µl; Reverse primer (10 µM): 0.8 µl; Template RNA: 1 ul; Nuclease-free Water: 20 µl. Cycling conditions: Reverse Transcription, 55°C, 10 minutes, 1 cycle; Initial Denaturation, 95°C, 1 minute, 1 cycle; Denaturation, 95°C, 10 seconds, 40 cycles; Extension, 60°C, 30 seconds, 40 cycles; Melt Curve, 60-95°C, varied time, 1 cycle.

2.10 Experimental design

The experimental design for this study followed a completely randomized design (CRD) and was replicated three times.

2.11 Data analysis

Data collated was analyzed with Analysis of Variance (ANOVA), and represented on graphical plots using MS Excel.

3. Results

3.1 Results for Effect of Crude Oil on Plant Heavy Metals Content

At the eighth week after planting (8 WAP), the concentration of heavy metal in the plants increased with increase in the amount of crude oil in the soil, except for Lead and Cadmium, which were not observed.

The highest plant copper content was 0.0135 g kg-1, which was observed for the 800 ml treatment; and, the lowest copper content was 0.0049 g kg-1, which was observed for the 0 ml (control) treatment. Compared to the control treatment, this showed a 175.51% increment for the 800 ml treatment. This showed a dose-dependent increment in plant copper content as the amount of crude oil in the soil increased.

The highest plant zinc content was 0.074 g kg-1, which was observed for the 800 ml treatment; and, the lowest copper content was 0.034 g kg-1, which was observed for the 0 ml (control) treatment. Compared to the control treatment, this showed a 117.65% increment for the 800 ml treatment. This showed a dose-dependent increment in plant zinc content as the amount of crude oil in the soil increased.

The highest plant nickel content was 0.0211 g kg-1, which was observed for the 800 ml treatment; and, the lowest copper content was 0.0142 g kg-1, which was observed for the 0 ml (control) treatment. Compared to the control treatment, this showed a 48.59% increment for the 800 ml treatment. This showed a dose-dependent increment in plant nickel content as the amount of crude oil in the soil increased.

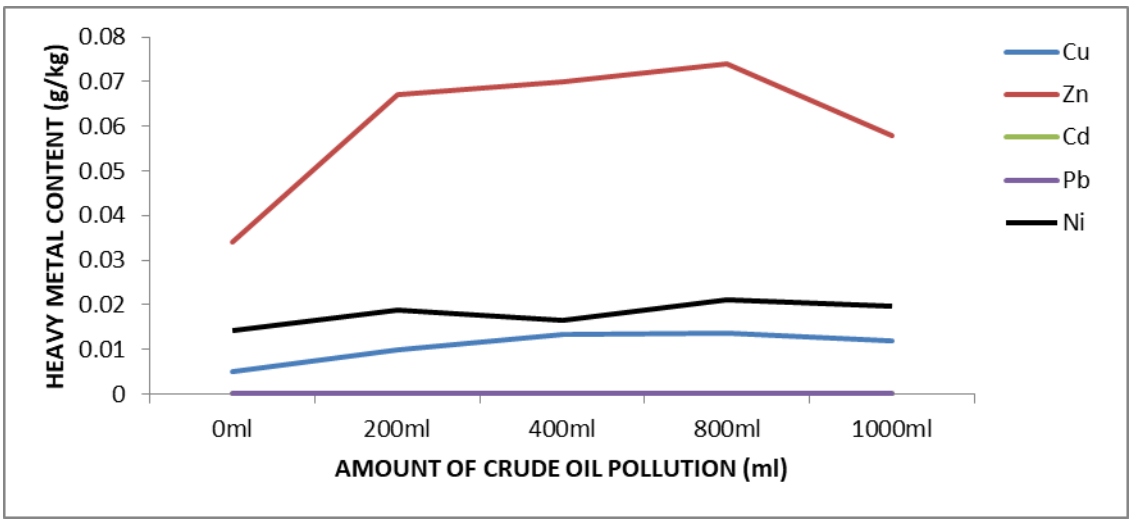

Figure 1 Plot of effect of crude oil on the tissue heavy metal content of M. pruriens

This showed that the increment of crude oil pollution in the soil led to the increment of heavy metal content of the plant tissue. These heavy metals were possibly taken up by the phyto-extraction mechanism, an approach to phyto-remediation. These could be some source of stress for the plants, resulting to most of the decrements in the growth parameters studied.

3.2 Results for Effect of Crude Oil on the Gene Expression

Table 1 Gene Quantification Data

Well	Fluor	Target	Content	Sample	Cq	Cq Mean	Cq Std. Dev
A10	SYBR	GST	Unkn-13	A	29.54	29.64	0.143
A11	SYBR	GST	Unkn-13	A	29.75	29.64	0.143
B10	SYBR	GST	Unkn-14	B	29.60	29.54	0.087
B11	SYBR	GST	Unkn-14	B	29.48	29.54	0.087
C10	SYBR	GST	NTC		N/A	0.00	0.00
F10	SYBR	UB10	Unkn-15	A	26.88	26.91	0.054
F11	SYBR	UB10	Unkn-15	A	26.95	26.91	0.054
F12	SYBR	UB10	NTC		N/A	0.00	0.00
G11	SYBR	UB10	Unkn-16	B	26.69	26.76	0.095
G12	SYBR	UB10	Unkn-16	B	26.83	26.76	0.095

Table 2 Normalized expressions using the ΔCq Using a Reference Gene

Sample	Cq Target (GST)	Cq Reference (UB10)	Normalized Expression
Control (calibrator) A	29.64	26.91	0.150726
Polluted (Test) B	29.54	26.76	0.145592

Table 3 Gene Expression - Scatter Plot Data

Display	Target	A Normalized Expression	B Normalized Expression	B Regulation	Compared To Regulation Threshold
True	GST	0.15065	0.14592	-1.03238	No change
True	UB10	N/A	N/A	N/A	No change

4. Discussion

4.1 Effect of Crude Oil on Plant Tissue Heavy Metal Content

Evidence from the study (Figure 1) showed that, at the eighth week after planting (8 WAP), the concentration of heavy metal in the plants increased with increase in the amount of crude oil in the soil, except for Lead and Cadmium, which were not observed.

The highest plant copper content was 0.0135 g kg^{-1}, which was observed for the 800 ml treatment; and, the lowest copper content was 0.0049 g kg^{-1}, which was observed for the 0 ml (control) treatment. Compared to the control treatment, this showed a 175.51% increment for the 800 ml treatment. The highest plant zinc content was 0.074 g kg^{-1}, which was observed for the 800 ml treatment; and, the lowest copper content was 0.034 g kg^{-1}, which was observed for the 0 ml (control) treatment. Compared to the control treatment, this showed a 117.65% increment for the 800 ml treatment. The highest plant nickel content was 0.0211 g kg^{-1}, which was observed for the 800 ml treatment; and, the lowest copper content was 0.0142 g kg^{-1}, which was observed for the 0 ml (control) treatment. Compared to the control treatment, this showed a 48.59% increment for the 800 ml treatment. These values, though not up to the toxic level of

100 ppm (Charman and Murphy, 1992), they could accumulate (in the plant or in the body of the human consumer) to that level and become too localized for human safety.

This showed that the increment of crude oil pollution in the soil led to a seemingly dose-dependent increment of heavy metal content of the plant tissue. These heavy metals were possibly taken up by the phyto-extraction mechanism, one of the approaches to phyto-remediation, and then translocating the heavy metals to the other parts of the plant. These could be some source of stress for the plants, resulting to most of the decrements in the growth parameters studied.

This is in line with the findings of some researchers (Odiyi *et al*., 2020) who investigated the effect of crude oil pollution on the heavy metal content of maize (*Zea mays*). The researchers observed that, of the plant parts studied, the root had the highest content of Chromium, Nickel, Lead and Cadmium, while the leaves had the highest concentration of Copper. The researchers showed that the heavy metal concentration of the plant parts significantly increased with increase in the volume of crude oil pollution in the soil. The researchers explained that the higher concentration of the heavy metals in the roots than in the leaves of the treated *Zea mays* plants is as a result of the roots absorbing the heavy metals present in the treated soil, and translocating them to other parts of the maize plant.

In corroboration with the results of this study, another group of researchers (Bada and Olarinre, 2012) have shown that crude oil pollution of the soil has an incremental impact on the heavy metal concentration of the soil and the plants growing some varying distances (1m, 10m, 20m and 30m) from the crude oil pipeline. The researchers identified the plants growing in the impacted sites as *Calapogonium mucunoides*, *Axonopus compressus* and *Sida acuta*. The authors reported that the heavy metal content of the leaves of the plants were higher than those of the stems and the roots, thus, once again, indicating that the crude oil and its concomitant heavy metal content was translocated to the leaves of the plants.

Despite always being mislabeled with the other heavy metals as being “toxic”, some of the heavy metals such as copper (Cu), molybdenum (Mo), zinc (Zn) etc are considered as either trace elements or ultra-trace elements because their presence is essentially needed in the soil environment in very small amounts: (10mg/Kg or mg/L in soil/aquatic medium) for trace elements and/or (1µg/Kg or µg/L in soil / aquatic medium) for ultra-trace elements.

These essential heavy metals for plants (Fe, Cu, Mo, and Zn) have two key functions in plant cells, which are: involvement in redox reactions, and being an integral part of important enzymes. Heavy metals such as Cu, Mo, Zn, etc. serve as cofactor and activator of various enzyme reactions and play a vital role in the formation of enzymes / substrate metal complexes or as a catalytic property as a prothesis group in metalloenzymes participate in electron transport and structural functions in nucleic acid metabolism (Nagajyoti *et al.,* 2010).

The other group of heavy metals, considered the “Class B” metals, are the non-essential heavy metals which include mercury (Hg), silver (Ag), lead (Pb), nickel (Ni) etc. these heavy metals are very toxic to plant life.

These heavy metal are reported to interfere with ionic homeostasis and enzyme activity in such a way that affects single-organ physiological processes (such as root nutrient uptake) followed by multiple processes such as germination, photosynthesis, respiration, plant water balance, metabolism, and reproduction. These leads to visible symptoms of toxicity to heavy metals, some of which include chlorosis, necrosis, senescence, and wilting, stunted growth, low production of biomass, limited seed numbers, and eventually death. There are reports that plants that grow under heavy metal stress spend more energy on their survival, which would otherwise have been available for their other process. This deficiency in the amount of energy required may result in the overall decrease in the growth of the plant in such hostile metal-stressed environment (Kumar and Aery, 2016). Some of the heavy metals are also reported to hamper the growth and activities of the soil microbes, which also indirectly affect the plant growth of plants (Kumar *et al.,* 2016).

At higher or increasing amounts, there are reports that zinc causes physiological alteration and inhibition of growth (Cakmak, 2000). High zinc exposure in growing medium has also been shown to inhibit several metabolic functions of plants, leads to stunted growth, and causes senescence. Zn toxicity has been reported to limit root and shoot growth (Fontes and Cox, 1998). It also causes chlorosis in premature leaves at high concentration, which may extend on prolonged high exposure to older leaves. The high concentration of Ni in growing medium have been reported to cause physiological process alteration and various symptoms of toxicity such as chlorosis, necrosis, and wilting. Plants growing in excess of Ni medium have negative effects on photosynthesis, mineral nutrients, transport of sugar, and balance of water (Gajewska *et al.,* 2006; Sethy and Ghosh, 2013).

4.2 Effect of Crude Oil on Gene Expression

The GST gene is a gene that encodes for a family of enzymes called glutathione S-transferases (GSTs). These enzymes are involved in the detoxification of various compounds, including reactive oxygen species, heavy metals, and many types of drugs and environmental toxins. GSTs catalyze the conjugation of glutathione, a tripeptide composed of glutamate, cysteine and glycine, with these compounds, making them more water-soluble and easier to excrete from the body. In this study, the GST was used as a 'target gene" – a gene of interest the expression levels of which are typically compared to the expression levels of the reference gene(s) in order to determine whether the target gene(s) are differentially expressed in different samples or conditions (Hossain *et al*., 2012).

The UB10 gene is a gene that encodes the ubiquitin protein. Ubiquitin is a small protein that is found in almost all eukaryotic cells and is involved in a wide variety of cellular processes, including protein degradation, DNA repair, and regulation of the cell cycle. It acts as a post-translational modifier by covalently binding to lysine residues of target proteins thus altering their activity, stability, or localization. Ubiquitin is synthesized as a precursor protein, and then is modified by a series of enzymatic reactions. The UB10 gene encodes for the ubiquitin precursor, which is then modified by a cascade of enzymes to form the mature ubiquitin protein. The UB10 gene acts as a "reference gene" – a gene the expression level of which is assumed to be stable across different samples or conditions, and is used to normalize the expression levels of the target gene(s) of interest, to determine whether the expression of the target gene(s) is significantly different from the reference gene (Yao *et al*., 2022).

From the results of the study (Table 2 and 3), the mean Cq (cycle threshold) for the target gene (GST) for both the calibrator sample (control) and the polluted (test) sample were 29.64 and 29.54 respectively; while, the mean Cq (cycle threshold) for the target gene (GST) for both the calibrator sample (control) and the polluted (test) sample were 26.91 and 26.76 respectively. The gene expression levels of the samples were quantified using the ΔCq with a Reference Gene method (Badawy *et al*., 2018)), and the normalized expression levels of the target gene (GST) in the calibrator (control) sample "A", and that of the treated/polluted (test) sample "B" were established as 0.150726 and 0.145592 respectively (Table 4.3 and 4.4); and, it was established that there was no significant change in the normalized expression levels of the target gene (GST) in both the control (calibrator) sample and the treated/polluted (test) sample. This therefore interprets to mean that the application of crude oil and its concomitant increment in the heavy metal content of the plant tissues did not affect the expression of the pollution marker GST gene. This could be possibly due to the amount of crude oil introduced into the soil being too small to cause enough increment in plant tissue heavy metal content to such extent as to influence or impact the expression of the target GST gene. This is slightly explained by the work of Charman and Murphy (1992) who explained that the amount of heavy metal content that can impact the health of a living plant or human body would be a minimum of 100 ppm. This goes against the findings of several scientific reports some of which have reported that heavy metals such as copper (Cu) and zinc (Zn) influenced the expression of 450 proteins including the defense-related proteins such as GST in *Populus* sp. (Lingua *et al*., 2012),

Helaoui *et al*. (2020) have shown that the presence of Nickel (Ni) in the roots and shoots tissues of *Medicago sativa* plants in the amounts of 50,150,250 and 500 mg/kg increased the expression of GST over a 60 days period. Another author (Christou *et al*., 2020) have shown that presence of Cr in the leaves of *Medicago sativa* plants in the quantities of 0.05, 0.5, 1, 5 and 10 mg/L over a 59 days period increased the expression of the GST7 and GST17 genes.

While the lack of change in the GST gene expression due to increased heavy metal content of the plant tissue can be attributed to the small concentration of the heavy metals in the plant tissue, it is also possible that the heavy metal content of the plant tissue did not cause any change in the expression of the GST gene in the plant because the plant lacks the necessary genetic machinery to express the GST gene or that the GST gene is not directly influenced by heavy metal pollution but rather by other environmental or endogenous factors.

5. Conclusion

Increasing the crude oil concentration of the soil had a generally incremental effect on the heavy metal (Cu, Zn, Ni) content of the plant tissues. These heavy metals have certain toxic and detrimental effects on the growth and general performance of the plant, thus amounting to reductions in all the growth parameters of the plant, as the crude oil pollution in the soil increased. Findings from this study showed that the plant's contact with increasing treatments of crude oil and concomitant increment of the heavy metal content of the plant tissue had no significant change in the normalized expression levels of the target gene (GST) in both the control (calibrator) sample and the treated/polluted (test) sample. This therefore interprets to mean that the application of crude oil and its concomitant increment in the heavy metal content of the plant tissues did not affect the expression of the pollution marker GST gene. This could be possibly due to the amount of crude oil introduced into the soil being too small to cause enough increment in plant

tissue heavy metal content to such extent as to influence or impact the expression of the target GST gene. While the lack of change in the GST gene expression due to increased heavy metal content of the plant tissue can be attributed to the small concentration of the heavy metals in the plant tissue, it is also possible that the heavy metal content of the plant tissue did not cause any change in the expression of the GST gene in the plant because the plant lacks the necessary genetic machinery to express the GST gene or that the GST gene is not directly influenced by heavy metal pollution but rather by other environmental or endogenous factors. Considering the resilience of *M. pruriens* and its ability to adapt to the high amounts of crude oil in the soil and accumulate heavy metals in the plant tissues, it is advised that it be employed in the possible phyto-extraction of crude oil and/or phyto-remediation in crude oil and heavy metal polluted soils.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest exists among the Authors.

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