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Comparative assessment of the effect of produced water (PW) and water soluble fraction (WSF) of crude oil on the growth and catalase activity of *Allium cepa* L.

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ABSTRACT
Objective: To comparatively assess the effect of produced water (PW) and the water soluble fraction (WSF) of Ughelli east crude oil on the growth and catalase activity of *Allium cepa*.
Methodology and results: The levels of treatments used were 0 for control (deionized water), 25, 50 and 100% (PW and WSF) of crude oil, each having three replicates. The plants were exposed to the treatments for 12 days. The results showed a reduction in root lengths and the number of roots of *A. cepa* with increase in concentrations of the two treatments, except at 50% WSF treatment, where the result had significantly (P<0.005) lower values for root length (2.96cm ±0.47) as compared to 100% WSF (3.69cm± 6.71). At the same level of treatment of 50% WSF there was significant (P<0.005) increase in the number of roots when compared with 25% WSF treatment. Initiation of growth at 50 and 100% WSF were recorded on the first and second days of exposure. Leaf bud formation was observed only at 25% WSF treatment level. Increase in catalase activity was observed with increase in percent WSF. Catalase activity could not be measured for PW because there was inhibition (zero growth) at 100% treatment. While 25 and 50% (PW) the fresh weights produced from the three replicates were not sufficient for the analysis Significant difference of P<0.005 was recorded for root lengths for the three treatment levels. The pHs of the media (WSF and PW) at all levels were slightly acidic before exposure to *A. cepa.* After exposure of media to the plant the pH values increased from 6.67±0.06, 6.80±0.03, 6.86±0.03 to 7.30±30, 6.81±0.06 and 7.70±0.00 at 25, 50 and 100% WSF respectively. Produced water showed increase in values from 6.67±0.03 and 6.89±0.06 to 7.90±0.06 and 7.50±0.00 at 50 and 100% PW respectively, while exposure to 25% PW reduced pH from 6.66 to 6.40
Conclusion and application of findings: Based on the results, it is concluded that the effects of WSF and PW on the number and length of roots of onion plants were dependent on their concentrations, the higher the concentration the greater their effects on the plants. The results showed that PW of Ughelli East crude oil was more toxic than its WSF. These results show that there is the need for the government to implement measures to safeguard and reduce the effects of oil contaminated water on the environment.
Keywords: Produced water (PW), Water soluble fraction (WSF), crude oil, growth, catalase activity and *Allium cepa.*

INTRODUCTION
Onion plant belongs to the division Magnoliophyta, the class liliopsida and the family Liliaceae (Dutta, 1964; Stern, 2000). There are about 400 species in the genus *Allium,* including some magnificent

ornamentals. and Onions grow best in a loose, well-drained soil of high fertility and plenty organic matter. A constant supply of adequate moisture is necessary for best result; onion is sensitive to high acidic soil and grows best when the pH is between 6.2 and 6.8. Consumption of onions can be protective against the development of many health problems, in addition to adding flavor to food. Onion is easy to cultivate, grows very fast.

Crude oil, which is a mixture of hydrocarbons and inorganic compounds, is drilled through the rocks. Baker (1970) observed that water soluble fraction (WSF) is produced during a long period of oil - water contact. When there is delay in clean up after spillage has occurred, the water soluble components of the crude oil seep into the aquatic ecosystem (Edema, 2006). The components of crude oil that go into solution make up the WSF. Edema and Okoloko (1997) reported that at higher concentrations of 25, 50 and 100% WSF reduced cell size and inhibit root elongation of plants, while at 12.5% WSF growth promotion was recorded.

Almost all offshore oil produces large quantities of contaminated water that can have significant environmental effects if not handled appropriately (Wills, 2000). Oil and gas reservoirs have a natural water layer (called formation water) that, being denser, lies under the hydrocarbons. Oil reservoirs frequently contain large volumes of water. To achieve maximum oil recovery, additional water is usually injected into surface. Both formation and injected water are eventually produced along with the hydrocarbons.

There is more in produced water than water and oil. Neff (1981) described produced water for ocean discharge as containing up to 48ppm of petroleum. This is because it had usually been in contact with crude oil in the reservoir rock. There were also elevated concentrations of barium, beryllium, cadmium, chromium, copper, Iron, lead, nickel, silver and zinc and “small amount of the neutral radionucleids, radium 226 and radium 228, and non-volatile dissolved organic material of unknown composition. Due to rapid mixing with seawater, most physical-chemical features of produced water (low dissolved oxygen and pH, elevated salinity and metals) do not pose any hazard to water, elevated concentration of hydrocarbons may be detected in surface sediments up to about 1,000m from the discharge, that contains aromatic hydrocarbons and metals. These aromatic hydrocarbons and metals in produced water were reported by Neff (1981) to be toxic to organisms. This study aimed at assessing the impact of the water soluble fraction (WSF) and produced water (PW) on the biological properties of plants, using onion as the experimental plant.

MATERIALS AND METHODS

Study site: The study was carried out in the Botany Laboratory of the Delta State University, Abraka, Nigeria. The experimental plant used was onion (*Allium cepa* L.) of which bulbs were bought from the local market. The crude oil used was collected from Ughelli East flow station owned by Shell Petroleum Development Company (SPDC) of Nigeria. These areas are located at latitude 5.30^{0}N and 5.58^{0}E.

Produced water: The produced water was the water that came with the crude oil. The crude oil used has been standing in the Botany Laboratory since 2004. The mixture of crude oil and produced water was allowed to stand in a separating funnel for 24 hours, after which the lower phase was collected and used as the stock or 100% produced water.

Water soluble fraction: The water soluble fraction was prepared according to the method of Anderson *et al.* (1974). The stock solutions of both the produced water and water soluble fraction were diluted with deionized water serially to give 50 and 25% strength and were stored in screw-cap bottles prior to use. Deionized water was used as the control and three levels of treatments (25, 50 and 100%) were used. Each treatment, including the control had three replicates.

Experiment set up and data recording: The plants were exposed to the treatments for 12 days. Twenty one onion bulbs of equal size were used for the experiment. The outer scales of the onion bulbs were removed and the primordial cells were exposed using a razor blade. The onion bulbs with the outer scales removed were introduced into different concentrations of WSF and PW. The numbers of roots were counted visually after 24 hours and the length of the roots were determined with the aid of a calibrated ruler (cm) after 5 days. Catalase activity was assessed by measuring the volume of gas/volume of oxygen released at the end of the exposure period of 12 days. The apparatus used for assessing the catalase activity was modified after Moore (1974).

The data recorded were subjected to 3x2x3 factorial (2-way ANOVA) means compared by the Bonferroni test using statistical package version 5.0.

RESULTS AND DISCUSSION

The results obtained in the present study showed reduction in values of root lengths (Table I) and the number of roots (Table II a & b) with increase in concentrations for both types of water samples. At 50% WSF there was significant ($P<0.005$) reduction in root length being 2.96cm ± 0.47 as compared to 3.61cm±6.71 for 100% WSF (Table I). At the same level of 50% WSF treatment, there was slight increase in the number of roots as compared to 25% WSF treatment (Table II b). Initiation of root growth was recorded at 50 and 100% WSF for the first and second days of exposure as compared to 25% WSF treatment, while leaf bud formation was observed only at 25% WSF treatment level.

These results show that WSF treatment can trigger root initiation at 50 and 100% WSF. Total inhibition (zero growth) of root was also recorded for PW at 100%, which shows that PW of Ughelli East crude oil was more stressful to the plant. Significant difference of $P<0.005$ was recorded for all the treatments. Neff (1981) reported that the aromatic hydrocarbons and metals in produced water are toxic to organisms, but however "the toxicity of soluble organic fraction of produced water to is not known".

The pH values for WSF and PW samples were 6.67±0.06, 6.80±0.03, 6.86±0.03 to 7.30±30, 6.81±0.06 and 7.70±0.00 at 25, 50 and 100% WSF respectively. Produced water showed increase in values from 6.67±0.03 and 6.89±0.06 to 7.90±0.06 and 7.50±0.00 at 50 and 100% PW respectively, before exposure to *A. cepa*. Some species of plants have been found to produce catalase when grown in an environment with low pH (Boom, *et al*; 2006). The values before exposure were within the ideal soil pH values of 6.2 – 6.8 for the cultivation of onion (Dutta, 1964), except at 100% PW treatment samples which had slightly higher pH value of 6.89. After exposure of onion plants to WSF and PW the pH values increased from 6.86 and 6.89 to 7.50 and 7.70 at 100% WSF and PW respectively. At 25% PW there was reduction in pH value from 6.66 to 6.40. Statistical analysis shows significant difference ($P<0.001$) for both WSF and PW treatments.

The results (Table III) showed an increase in the amount of oxygen released with increase in concentrations of WSF sample. The release of oxygen is brought about by the breakdown of hydrogen peroxide by catalase into water and oxygen (harmless substances) (Taylor *et al*., 1998). The increase in antioxidant enzyme could be metabolism mediated and an indication of toxic challenge or suicide reactions (Maduka, 2008)

Hydrogen peroxide is produced as a by-product of many normal cellular reactions. If the cells do not breakdown the hydrogen peroxide, they would be poisoned. The increased level of catalase activity is an indication of increased production of free radicals occasioned by exposure of plants to crude oil. Increase in the activity of enzyme may be due to activation of the catalase. Activation of enzymes could occur through increased synthesis of enzyme (Timbrell, 1991). Catalase activity ensures the maintenance of cell integrity and functional performances (Eruster, 1993; Aitken, 1995). Increased activity of antioxidant enzymes could contribute to better cell protection from chemical toxins (Sevenian and Hochstein, 1985; Saltman, 1989), thereby improving the ATP production during photosynthesis.

The results in Table III show total inhibition of catalase activity with produced water, since there was no oxygen released. It has been suggested that a decrease in activity of the detoxification mechanisms of hydrogen peroxide can generate severe cell damage due to increased production of toxic oxygen radicals.

In conclusion, the effects of WSF and PW on the number and length of roots of onion plants were dependent on their concentrations, the higher the concentration the greater their effects on the plants. The results obtained indicate that plant exposure to WSF and PW altered the activity of the catalase. The results showed that PW of Ughelli East crude oil was more toxic than its WSF. These results show that there is the need for the government to implement measures to safeguard and reduce the effects of oil contaminated water on the environment.

Table 1: The mean root length (cm) per onion bulb after exposure

Treatment (%)	Exposure Periods															
	DAY 5		**DAY 6**		**DAY 7**		**DAY 8**		**DAY 9**		**DAY 10**		**DAY 11**		**DAY 12**	
	PW	**WSF**	**PW**	**WSF**	**PW**	**WSF**	**PW**	**WSF**	**PW**	**WSF**	**PW**	**WSF**	**PW**	**WSF**	**PW**	**WSF**
Control0	3.09±0.25	3.09±0.25	4.20±0.27	4.20±0.27	4.87±0.33	4.87±0.33	5.43±0.39	5.43±0.39	6.01±0.48	6.01±0.48	6.83±0.71	6.83±0.71	7.64±0.91	7.64±0.91	8.63±1.28	8.63±1.28
25	0.77±0.41^{H}	0.84±0.38^{H}	0.50±0.08^{H}	1.22±0.54^{H}	0.59±0.10^{H}	1.44±0.64^{H}	0.66±0.13^{H}	1.57±0.69^{H}	0.79±0.16^{H}	1.78±0.74^{H}	1.28±0.23^{H}	2.60±0.74^{H}	1.37±0.23^{H}	3.00±0.71^{H}	1.46±0.24^{H}	3.61±0.74^{H}
50	0.21±0.06^{H}	0.57±0.16^{H}	0.26±0.06^{H}	0..82±0.21^{H}	0.32±0.06^{H}	0.97±0.23^{H}	0.38±0.08^{*H}	1.08±0.23^{*H}	0.44±0.08^{*H}	1.21±0.26^{*H}	0.60±0.13^{*H}	1.64±0.26^{*H}	0.98±0.18^{*H}	2.48±0.40^{*H}	1.04±0.17^{*H}	2.96±0.47^{*H}
100	0.00±0.00*	0.92±0.09^{*H}	0.00±0.00^{*H}	1.24±0.00^{*H}	0.00±0.00^{*H}	1.43±0.11^{*H}	0.00±0.00^{*H}	1.77±0.14^{*H}	0.00±0.00^{*H}	1.94±0.15^{*H}	0.00±0.00^{*H}	2.31±0.16^{*H}	0.00±0.00^{*H}	2.79±0.17^{*H}	0.00±0.00^{*H}	0.98±0.20^{*H}

* - Significant difference ($P<0.05$) between the two media
H – Significantly different ($P<0.05$) compared to the control

NB as the concentration increased the values decreased (WSF and PW)

Table IIa: Mean number of roots after exposure

Treatment (%)	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6	
	WSF	PW	WSF	PW	WSF	PW	WSF	PW	WSF	PW	WSF	PW
Control, 0	6.67±4.01	6.67±4.05	12.00±3.79	12.00±3.79	14.67±5.55	14.67±5.55	15.67±5.73	15.67±5.73	16.00±6.24	16.00±6.24	17.00±6.66	17.00±6.66
25	0.00±0.00	0.33±0.33	4.67±2.60	3.33±2.85	8.00±1.73	7.67±4.41	9.33±0.88	8.00±4.16	9.33±0.88	8.33±4.49	10.67±0.33	8.33±4.49
50	* 5.00±3.21	* 0.33±0.33	* 7.00±4.04	* 1.33±1.33	* 9.33±6.17	* 2.00±1.53	* 10.33±7.13	* 3.67±1.20	* 10.67±6.89	* 5.67±1.45	* 13.00±7.00	* 8.00±2.31
100	* 4.33±1.67	* 0.00±0.00	* 6.00±1.52	* 0.00±0.00	* 7.33±1.20	* 0.00±0.00	* 7.33±1.20	* 0.00±0.00	* 7.33±1.20	* 0.00±0.00	* 10.00±2.00	H* 0.00±0.00

Table IIb: Mean number of roots after exposure

Treatment (%)	DAY 7		DAY 8		DAY 9		DAY 10		DAY 11		DAY 12	
	WSF	PW	WSF	PW	WSF	PW	WSF	PW	WSF	PW	WSF	PW
Control, 0	17.33±6.98	17.33±6.98	17.33±6.98	17.33±6.98	17.67±7.31	17.67±7.31	20.33±8.35	20.33±8.35	20.33±8.35	20.33±8.35	20.33±8.35	20.33±8.35
25	11.00±0.00	8.33±4.49	11.00±0.00	8.67±4.41	11.67±0.67	9.67±4.06	12.67±0.33	11.00±3.79	12.67±3.79	11.00±3.79	12.67±0.33	11.00±3.79
50	13.00±7.00	8.67±2.33	13.00±7.00	8.67±2.33	13.00±7.00	10.00±1.53	13.33±6.74	9.33±2.60	13.33±6.74	9.33±2.60	13.33±6.74	9.33±2.60
100	* 10.67±1.86	H* 0.00±0.00	* 10.67±1.86	H* 0.00±0.00	* 12.33±3.18	H* 0.00±0.00	* 12.33±3.18	H* 0.00±0.00	* 12.67±2.91	H* 0.00±0.00	* 12.67±2.91	H* 0.00±0.00

P >0.05 (Insignificant different – Bonferoni test) - WSF

* - Significant difference (P<0.05) between the two media
H – Significantly different (P<0.05) compared to the control

Table III: Mean catalase activity of *Allium cepa* as affected by water soluble fraction and the produced water of oil.

Treatment %	mg/fresh weight				
	WSF	PW	% control of WSF	% control of PW	% difference between WSF & PW
Control, 0	2.67± 0.96	2.67±0.96	100	100	0.00
25	5.00±0.00	Nd	187.26	Nd	187.26
50	5.17±0.45	Nd	193.63	Nd	193.63
100	6.67±0.04	Nd	249.81	Nd	249.81

* Nd – Not determined

Table IV: The Mean pH before and after exposure to % WSF and PW

Treatment	Mean pH			
%	WSF		PW	
	BE	AE	BE	AE
25	6.67±0.06	7.30±0.00	6.67±0.00	6.40±0.06
50	6.80±0.03	6.81±0.06	6.67±0.03	7.90±0.06
100	6.86±0.03	7.70±0.00	6.89±0.03	7.50±0.05

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