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Effects of Water Soluble Fractions of Crude Oil, Diesel Fuel and Gasoline on *Salvinia nymphellula* (Desv)

J.F. Bamidele[1] G.O. Eshagberi[2*]
1. Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State.
2. Department of Science Laboratory Technology, Delta State Polytechnic, Otefe–Oghara, Delta State.

Abstract
The study investigated the effect of water soluble fractions (WSFs) of crude oil, diesel fuel and gasoline on *Salvinia nymphellula* (Desv). The parameters measured include number of leaves, biomass production, relative growth rate (RGR) and doubling time (DT). The WSF concentrations used were 25, 50 and 100% for crude oil and diesel fuel, 5, 10 and 20% for gasoline. The result showed that leaf production, biomass production and relative growth rate (RGR) were significantly reduced (P<0.01) in S. nymphellula when exposed to all concentrations of the WSFs for four weeks. Doubling time (DT) was also increased. The effects were concentration and medium dependent. WSF of gasoline had the most toxic effect. The macrophyte leaves were chlorotic, withered and disintegrated after four days in concentrations above 25% WSF of gasoline. There was no significant difference (P<0.05) between the effects of WSF of crude oil and WSF of diesel fuel on leaf production even though WSF of diesel had a more toxic effect on biomass production and relative growth rate in S. nymphellula than WSF of crude oil. These findings suggest that WSFs of petroleum hydrocarbons are toxic to aquatic macrophytes and early response to oil spillages is therefore recommended.
Keywords: *Salvinia nymphellula*, water soluble fraction, crude oil, diesel, gasoline.

1.0 Introduction

Oil pollution is a problem in Nigeria delta basin due to exploration and exploitation of petroleum. Crude oil was discovered in commercial quantity in Nigeria in Oloibiri in Rivers State, Nigeria in 1958 by Shell. The discovery, exploitation and exportation of oil led to the oil boom period of the 1970s with oil contributing about 90% of export earnings and 80% of the nation's gross domestic product (GDP). (Adeyanju, 2004; Egberongbe, Nwilo and Badejo, 2006; Ibemesim, 2010).

According to Adeyanju (2004) oil pollution is the introduction by man directly or indirectly any hydrocarbon materials especially crude oil and its refined products into the environment. The most serious oil spillage affects marine and coastal areas including the creeks and water distributaries. The effect of spilled oil on an aquatic ecosystem depend on the type and amount of oil spilled and several natural factors such as weathering, evaporation, oxidation biodegradation and emulsification (Atlas 1995; Njobuenwu, 2004; Egberongbe *et al*, 2006; Abowei and Njobuenwu, 2008).

Oil pollution has negative impact on plant survival. Floating oil covers the water surface and prevents oxygen from dissolving in water. Crude oil and is refined products also contain toxic compounds which interfere with nutrient uptake, photosynthesis and other biochemical processes resulting in poor growth or outright mortality of plants (Agbogidi and Edema, 2003; Bamidele, Agbogidi and Ohenbor, 2007; Lopes, Rosa-Osman and Piedade, 2009).

Water soluble fraction (WSF) of petroleum is that small fraction of oil containing components which are fully or sparingly soluble in water (Kavanu, 1964). Baker (1970) observed that WSF is produced during a long period of oil water contact which may result from delay in cleaning operations after an oil spill. According to Youssef (2002), the uptake of water soluble hydrocarbons by plants is possible through the root system and the foliage. WSF of petroleum have negative effect on growth of aquatic macrophyte (Bamidele and Agbogidi, 2000; Agbogidi and Edema, 2003; Edema, Okoloko and Agbogidi, 2007; Noyo, Okoloko and Agbogidi 2008).

This study seeks to investigate the effect of WSFs of crude oil, diesel fuel and gasoline on *Salvinia nymphellula* Desv.

2.0 Materials and Methods

2.1 Plant Collection

Salvinia nymphellula (Desv) was collected from a slow flowing freshwater stream in Aghalokpe Community in Okpe Local Government Area of Delta State, Nigeria. *S. nymphellula* is a free floating aquatic fern with slender stems, floating leaves and a root-like structure. It belongs to the family salviniaceae and the order salviniales. It is a pteridophyte (Akobundu and Agyakwa, 1998).

2.2 Study site

The study was carried out in the Botany Screen House of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State.

2.3 Source of crude oil

The crude oil used in this study was collected from Forcados Tank Farm owned by Shell Petroleum Development Company (SPDC) of Nigeria.

2.4 Source of diesel fuel and gasoline

Diesel fuel and gasoline were obtained from Nigerian National Petroleum Corporation (NNPC) filling station along Sapele/Warri Road, Amukpe, Sapele.

2.5 Preparation of Water Soluble Fractions (WSFs) of crude oil, diesel fuel and gasoline

Water soluble fractions of crude oil, diesel fuel and gasoline were prepared following the procedure of Afolabi *et al.* (1985). 500cm^3 of crude oil, was mixed with 1500cm^3 of borehole water and the mixture was stirred using Hallenkamp orbital magnetic stirrer for 24 hours at room temperature. After 24 hours of shaking, the mixture was allowed to stand for a minimum of 3hours to obtain clear interphase between oil and water. The oil was decanted and the mixture was then poured into a glass stopper separating funnel and allowed to stand overnight. Pure and clear WSF obtained at the lower part of the funnel was siphoned into capped bottles to make the stock (100% WSF). The procedure was repeated using diesel fuel and gasoline respectively until sufficient quantities of WSFs of crude oil, diesel fuel and gasoline were obtained. Each stock was diluted with pond water to give 50% and 25% strength WSF.

The experiment was designed with four treatments (0% - control, 25%, 50% and 100% WSFs). 200cm^3 of each diluted WSF including the 100%WSF and the 0% (pond water) were poured into containers into which the macrophytes were introduced. There were 4 replicates per treatment. The set up was observed for 4 weeks and solutions continuously replenished.

2.6 Data recording

The following data were collected during the experimentation period.

2.6.1 Leaf production (number of leaves)

The leaves of the macrophyte were counted daily.

2.6.2 Fresh weight measurement

The fresh weights of *Salvinia nymphellula* was determined after 2 weeks and at the 4th week.

2.6.3 Dry weight measurement

The test plants for biomass production were harvested after 28 days and oven dried at 65^{0}C to constant weight (Lin *et al.* 2002).

2.6.4 Growth measurements

The relative growth rate was calculated using the equation described in Jackson (1980).

$$RGR = \frac{(\ln m_2 - \ln m_1)}{T_2 - T_1}$$

Where RGR = relative growth rate
ln = natural log
T = Time (days)
m_1 = dry mass of shoot at time T_1
m_2 = dry mass of shoot at time T_2

The doubling time was calculated using the formula described by Mitchell (1974).

$$DT = \frac{\ln(2)}{RGR}$$

Where DT = doubling time

2.6.5 Statistical analysis

The data was statistically analysed using the method described by Ogbeibu (2005).

3.0 Results

There was significant decrease (P<0.01) in leaf production when *Salvinia nymphellula* was exposed to 20, 50 and 100% concentrations of WSF of crude oil. Leaf production was highest in the control with progressive decrease in leaf number as the WSF concentration increased (figure 1). There was also significant decrease (P<0.01) in leaf number when *S. nymphellula* was exposed to 25, 50 and 100% concentrations of WSF of diesel fuel. Leaf production decreased as the concentration increased (figure 2). *S. nymphellula* did not survive in 50 and 100% concentration of WSF of gasoline for more than four days. The concentration was therefore adjusted downwards to 5, 10 and 20%. The decrease in leaf production was significant (P<0.01) in all concentrations of WSF of gasoline. (Figure 3).

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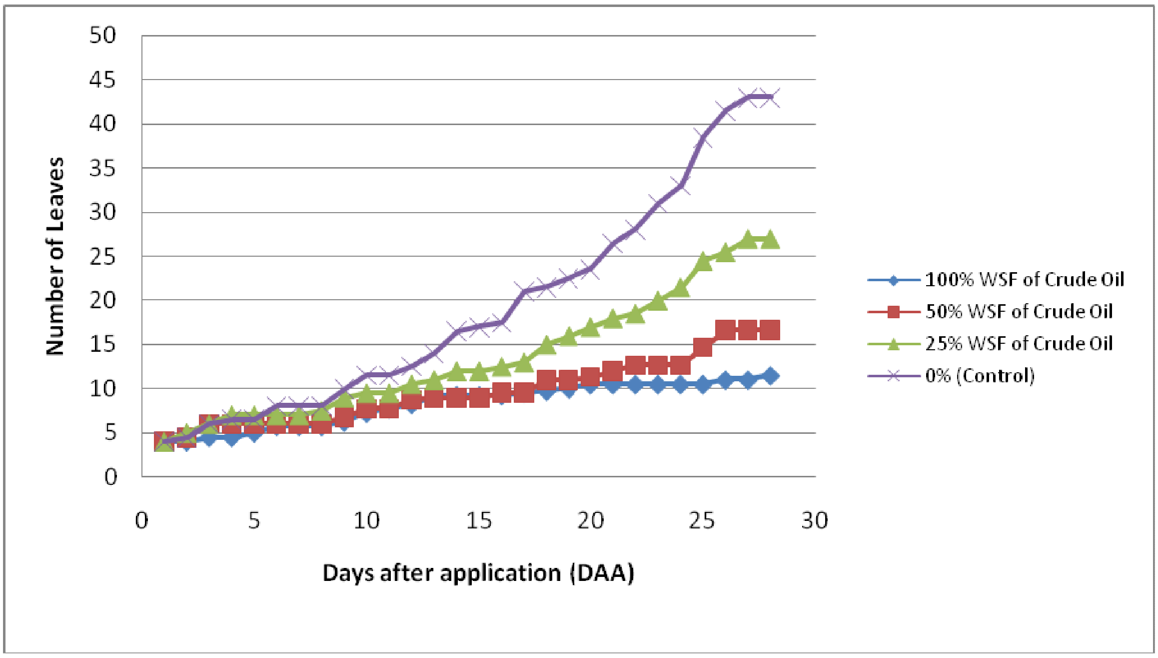

Fig. 1: Effect of Water Soluble Fraction (WSF) of crude oil on leaf production in *Salvinia nymphellula*.

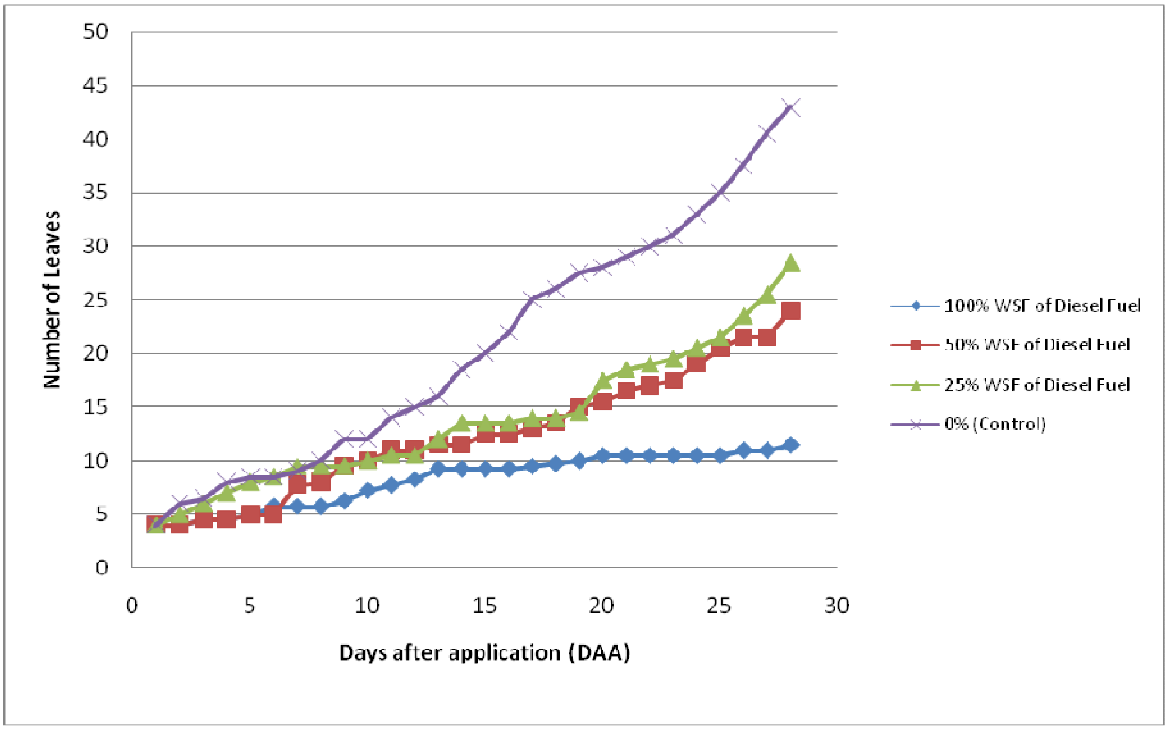

Fig. 2: Effect of Water Soluble Fraction (WSF) of diesel fuel on leaf production in *Salvinia nymphellula*.

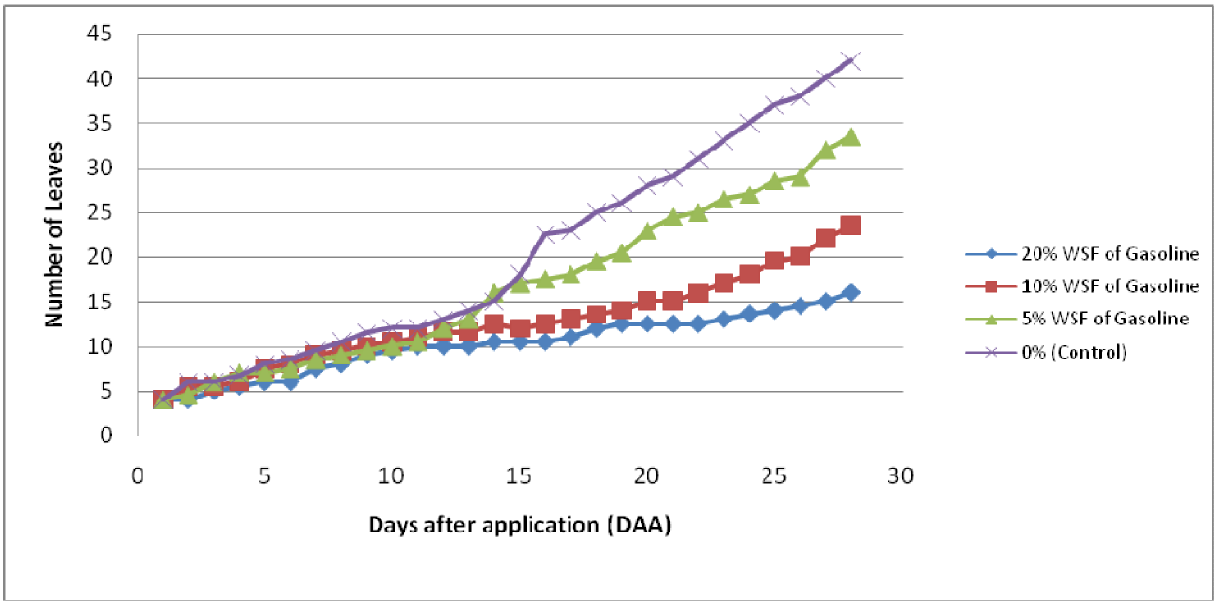

Fig. 3: Effect of Water Soluble Fraction (WSF) of gasoline on leaf production in *Salvinia nymphellula*.

The effects of water soluble fraction of crude oil, diesel fuel and gasoline on the dry weight of *S. nymphellula* are shown in tables 1, 2 and 3. Maximum values in dry weight were obtained in the control plants. *S. nymphellula* exposed to the WSFs showed progressive decline in biomass production. The decrease was significant ($P<0.05$) for 25% and ($P<0.01$) for 50 and 100% crude oil (Table 1). For all concentrations of WSF of diesel fuel and gasoline there was significant decrease ($P<0.01$) in dry weight (tables 2 and 3). The decrease was concentration dependent.

Table 1: Effect of Water Soluble Fraction (WSF) of crude oil on the biomass of *Salvinia nymphellula*. Results are mean of 4 replicates ±SE

CONCENTRATION OF WATER SOLUBLE FRACTION (%)	DRY WEIGHT (g)
(Control) 0	0.055±0.003
25	0.050±0.001*
50	0.036±0.001**
100	0.017±0.001**

Key: *P<0.05 and **P<0.01 significant compared to control.

Table 2: Effect of Water Soluble Fraction (WSF) of diesel fuel on the biomass of *Salvinia nymphellula*. Results are mean of 4 replicates ±SE

CONCENTRATION OF WATER SOLUBLE FRACTION (%)	DRY WEIGHT (g)
(Control) 0	0.055±0.003
25	0.043±0.002**
50	0.032±0.001**
100	0.016±0.001**

Key: **P<0.01 significant compared to control.

Table 3: Effect of Water Soluble Fraction (WSF) of gasoline on the biomass of *Salvinia nymphellula*. Results are mean of 4 replicates ±SE

CONCENTRATION OF WATER SOLUBLE FRACTION (%)	DRY WEIGHT (g)
(Control) 0	0.065±0.004
25	0.042±0.003**
50	0.032±0.001**
100	0.021±0.002**

Key: **P<0.01 significant compared to control.

The data in tables 4, 5, and 6 shows the effect of WSFs of crude oil, diesel fuel and gasoline on the relative growth rate (RGR) and doubling time (DT) of *S. nymphellula*. The control plants recorded the highest relative growth rate and lowest doubling time. When exposed to WSFs of crude oil, diesel fuel and gasoline *S. nymphellula* showed a significant decrease ($P<0.01$) in relative growth rate and increase in doubling time (Tables 4, 5 and 6). The decrease in relative growth rate and increase in doubling time was concentration dependent.

Table 4: Effect of Water Soluble Fraction (WSF) of crude oil on the Relative Growth Rate ± SE. and Doubling Time of *Salvinia nymphellula*.

CONCENTRATION (%) OF WATER SOLUBLE FRACTION	RELATIVE GROWTH RATE (RGR) (g g^{-1} day^{-1})	DOUBLING TIME (DT) (days)
(Control) 0	0.079±0.004	8.7
25	0.061±0.004*	11
50	0.051±0.003**	13.5
100	0.028±0.001**	24.7

Key: * $P<0.05$ and ** $P<0.01$ significant.

Table 5: Effect of Water Soluble Fraction (WSF) of diesel fuel on the Relative Growth Rate ± SE. and Doubling Time of *Salvinia nymphellula*.

CONCENTRATION (%) OF WATER SOLUBLE FRACTION	RELATIVE GROWTH RATE (RGR) (g g^{-1} day^{-1})	DOUBLING TIME (DT) (days)
(Control) 0	0.086±0.003	8
25	0.069±0.002**	10
50	0.057±0.004**	12
100	0.026±0.002**	26.6

Key: ** $P<0.01$ significant compared to control.

Table 6: Effect of Water Soluble Fraction (WSF) of gasoline on the Relative Growth Rate ± SE. and Doubling Time of *Salvinia nymphellula*.

CONCENTRATION (%) OF WATER SOLUBLE FRACTION	RELATIVE GROWTH RATE (RGR) (g g^{-1} day^{-1})	DOUBLING TIME (DT) (days)
(Control) 0	0.092±0.003	7.5
3	0.081±0.001**	8.5
6	0.073±0.002**	9.4
12	0.047±0.001**	14.7

Key: ** $P<0.01$ significant compared to control.

4.0 Discussion

The result of this study shows that water soluble fractions of crude oil, diesel fuel and gasoline have effects on *Salvinia nymphellula*. The introduction of water soluble fraction (WSF) of petroleum into aquatic system can change the characteristics of the aquatic ecosystem because it contains metallic ions and other toxic components (Kauss and Hutchinson, 1975; Edema, Okoloko and Agbogidi, 2007; Noyo, Okoloko and Agbogidi, 2008).

When exposed to various concentrations of water soluble fraction (WSFs) of crude oil, diesel fuel and gasoline, *S. nymphellula* showed a significant decrease in leaf production. This is consistent with the findings of Edema *et al* (2007), who observed a reduction in the number of leaves of *Pistia stratiotes* after exposure to WSF of crude oil. Noyo *et al* (2008) also observed a decrease in population of *Azolla africana* after exposure to WSF of crude oil. High concentration of crude oil caused reduction in leaf numbers of the free floating water hyacinth *Eichhornia crassipies* and the semi-aquatic grass *Eichinochloa polystachya* (Lopes, Rosa-Osman and Piedade, 2009).

The exposure of *salvinia nymphellua* to WSFs of crude oil, diesel and gasoline also resulted in significant decrease in biomass production. This agree with the findings of Bamidele and Agbogidi (2000), that the dry weights of three aquatic macrophytes *Panicum repens, Ludwigia stolonifera and Acroceras zizoniodes* showed a significant decrease with increasing concentrations of crude oil and its WSF. Silva and Camargo (2007) reviewed several studies which reported significant reduction in biomass production of the following aquatic macrophytes, *Spartina patens, Spartina alterniflora* and *Eichhornia crassipes* when exposed to various concentration of crude oil.

The WSFs also significantly reduced the relative growth rate while increasing the doubling time of *S. nymphellula*. Studies have confirmed the negative effect of petroleum and its WSF on plant growth (Chinda, 2007).

The toxicity of the WSF may be due to the presence of toxic components like xylene, naphthalene, benzene and toluene (Overton, 1994) and the presence of harmful metallic ions which may inhibit root growth

(Winter, O'Donnell Batterton and Becalen, 1976; Edema et al 2007). Oil pollution cause oxygen deprivation in plants root due to exhaustion of oxygen by hydrocarbon degrading micro-organism. Such anaerobic conditions lead to microbial generation of phytotoxic components such as hydrogen sulphide. Oil degrading organisms also competes with plants for mineral nutrients (Vartepetian, 1978).

When the effects of the three WSFs were compared, the WSF of gasoline had the most negative effect on *S. nymphellula* followed by WSF of diesel fuel. The difference in effect between diesel fuel and crude oil was not significant. This shows that the effect of oil pollution depends on the type and quantity of petroleum hydrocarbon. Lighter refined products of crude oil such as diesel and gasoline are more readily dissolved in water and are more toxic even though they do not persist long in the environment (Egberongbe *et al* 2006; Collins, 2007 and Ibemesim, 2010).

Conclusion

Oil pollution adversely affects aquatic macrophytes which play very important role in aquatic habitats. Apart from providing cover for plants and substrate for invertebrates, they provide food for aquatic animals. Their presence or absences are excellent indicators of water quality. When there is delay in cleaning operations after an oil spillage, the water soluble components that dissolve in the water remain toxic to aquatic life even after the cleaning exercise.

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