PRELIMINARY STUDY ON THE IMPACTS OF PRODUCED WATER TO THE SEEDLING OF TERRESTRIAL PLANT

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ABSTRACT

This research study used the oil-field brines (produced water) from West Java as a test material and aimed to discover the short term and long term effects on the test plant. The chemical properties of such oil-field brines were also examined and classified.

Young (15 days old) stage of soy bean (Glycine max, (Merr), L.) was used as the test plant. Experiment was carried out in a small green house using several pots. A completely randomized design was applied to 6 treatments (concentrations) and 4 replicates for the period of 12 days under the DC50 (Damage Concentration) value. Each replicate consists of 3 pots, and every pot contains 3 test plants. The observation was carried out until day 10 after 12 days of treatment.

The following were observed: the height of the plants, the width of the canopy and the number of leaves. Chlorophyl contents were also examined as an additional observation at the end of experiment. Data was analyzed by Anova oneway using the Minitab package. The results of the experiment indicate that almost all treatments were significantly different from the control treatment.

I. INTRODUCTION

A. *Background Information*

Exploration and production activities of petroleum industries in Indonesia have intensified recently. Such activities will of course bring positive impacts in such form as the improvement of foreign exchange for national development.

Consequently, output of produced water or oil-field brines is increasing. If such effluent is discarded in any place, it could lead to environmental pollution problems, not only damaging the agriculture, but may also pollute lakes and rivers which provide drinking water as well as water for irrigation purpose (API, 1962; Kinghorn, 1984). This means that either the community structure of aquatic organisms or the terrestrial plants with their carrying capacity will suffer from deterioration and degradation.

Field observation in North Sumatra, in 1986, showed that several species of plants, i.e. cassava (*Manihot utilisima*), corn (*Zea mays*), banana (*Musa paradisiaca*) which were grown in the allotment near the effluent water-pond, died. It was suspected that this might be due to the overflow of the pond during rain fall. Some trees in the wood nearby the oil-field which received the overflow of the pond were observed to be also suffering from growth disturbances, though the oil content in the effluent was relatively low (10-30 ppm).

The impacts of oil pollution in soil on rice and soy bean plants have been studied by Partodidjojo, et al., (1974). But there is lack of information on the impacts of produced water on terrestrial plants. Impacts on vegetative growth of soy bean are presented in this report.

B. *Estimates of the Amount of Effluents*

Since the amount of effluent varied annually and there are also some variation on water contents in crude oil among oil reservoirs. Several factors determine the amount of effluent:

- water contents of the oil which is usually between 40-80 %
- draining frequency of effluent
- daily oil production capacity
- total production active wells.

The annual average production of oil producers in Indonesia from 1984 to 1990 is 448,352,756 barrel.yr^{-1} respectively. If we take the assumption about 60% (the median value between 40-80%) water contents in the oil-fluid, then the average volume of effluent (produced water) to be discarded into the environment will be 672,529,134 barrel.yr^{-1} or 56,044,094 barrel.month^{-1} more or less.

Unfortunately, very little information is available about the amount of produced water which is injected back to the well for increasing oil production by water-lifting method.

Skeie (1989) predicted that discharges of produced water in the North Sea were at a rate of 110.106 tonnes.yr^{-1} in 1990. Using the above assumptions, it seems, that the amount of discharges in Indonesia in the same year (± 701,214,115 barrel.yr^{-1}) is less than the above rate in the North Sea.

C. *The Aim of the Study*

The aim of the present study was to determine :

a. Level of concentration of produced water which starts to damage 50 % on the test plant (DC50).

b. Long term biological effects of produced water on the test plant.

II. LITERATURE REVIEW

A. *General Chemical Properties of Produced Water*

Ostrof (1979) and Kinghorn (1984) stated that the dissolved solids are commonly found in the range of 0 to more than 300,000 mg/l. The main ions are Ca^{2+}, Na^+, Mg^{2+}, Cl^-, $SO_4^{=}$ and HCO_3^-. Cations consist of mostly metals and NH_4^+. Ammonia is in the form of dissolved gas and it appears to depend on temperature and pressure. Another ion which solubility is affected by temperature is Ca^{2+}. Furthermore if there is a considerable amount of dissolved CO_2, it will produce Ca-bicarbonate salts, following the reaction:

$$CaCO_3 + H_2O + CO_2 \rightleftharpoons Ca(HCO_3)_2$$

Table 1

Oil production from oil producers in Indonesia

unit : barrel

Year	Pertamina	PPT Migas	Producer		
			Prod. Shar.	Oil Contr.	Total
1984	31421141	203081	397309300	5766985	434700507
1985	29990472	169792	401432029	6450978	438043271
1986	29228101	192816	425768894	7276073	462465884
1987	26705789	210251	418901360	9422321	455239721
1988	24721128	48200	391812724	14640175	431222227
1989	25403162	*	408808395	15110047	449321604
1990	24299044	*	430774159	12402874	467476077

* = *no data*

Quoted from : Data dan informasi migas dan panas bumi. Ditjen Migas, DPE, Des. 1991

Magnesium salts appear to be more soluble than calcium salts, while elemental potassium which may produce stable minerals such as feldspar and mica, is rarely found in oil-field brines. Alkali metals such as lithium, rubidium and caecium can also be found in a small amount. Other metals such as iron, aluminium, manganese, zinc, copper, lead, mercury, and boron are found in very small amounts.

A lot of silicate is common in the sediments, so this ion is expected to appear in oil-field brines, usually in the form of unionized colloidal liquid. If there is no calcium, then fluoride will appear. Iodine can also be found in produced water and this is presumably considered as a substance derived from marine flora origin.

Organic compounds that contain nitrogen can be detected though in a small amount and in the form of amino acids such as serine, glycine, alanine, arginine since these compounds are easy to dissolve in water.

Fatty acids which contain hydrophilic and acidic groups tend to dissolve with the colloidal formation. The solubility will increase with the increasing temperature.

Naphtenic acid may also be present in produced water. It means that if the wells produce naphtenic crudes, the naphtenic acid content will be very high compared to those that produce paraffinic or intermediate crudes. Rittenhouse (in Ostrof, 1979) concluded that elements and chemical compounds in produced water were: primary compounds (Na^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Cl^-, $CO_3^=$, HCO_3^-, $SO_4^=$), other compounds (H_2S, oil contents, amino acids, fatty acids, iodine and NH_4^+) and micro elements (Cr, Cu, Mn, Ni, Sn, Ti, Zr, Be, Cd, Pb, Co, Al, B, Zn, Li, Rb, Ce and Hg).

B. *Some Biological Properties of Soy Bean*

Soy bean as a second crop is a well known plant. This plant belongs to the family of legumes or *Papilionoidae*, genus: *Glycine* and species: *Glycine max* (Merril), L.

As a small shrub, this plant stands upright, has a short period of life and dense leaves with a variety of morphology. The height of the plant ranges between 10-200 cm with a few or many branches depends on its cultivar and environmental condition. The young sprout appears in 4-5 days after the seeds are innoculated into the substrate.

Vegetative growth is the stage of the growth starting from the existence of the sprout (VE) then continue to the cotiledon stage (Vc), first node stage (V1), V2, V3, ...Vn. The growth period from VE to Vn usually needs 5 to 6 weeks respectively.

There is evidence that during the vegetative growth, this plant is influenced by several factors such as :

- temperature
- photoperiodicity
- pH of substrates
- nutrients.

III. METHODOLOGY

A. *Test Container*

Several pots with the size of 20 cm high and 24 cm in diameter were used as test containers. These containers were arranged at random in 4 series (4 replicates). Each replicate consists of 3 pots and every pot contains 3 test plants. All pots were filled with ± 6.5 kg of soil mixed with sand and manure in a certain ratio (2 : 1 : 1 parts by vol.) as a substrate. Inorganic fertilizers were also added with 330 mg urea, 560 mg TSP and 380 mg KCl. Urea was given twice, first at the beginning of inoculation of the seeds and secondly at the time where the plants were 30 days of age. While TSP and KCl were given once at the same time that was at the inoculation of the seeds.

The experiment was carried out in a small green house.

B. *Test Plant*

The young (15 days old) stage of soy bean *(Glycine max*, (Merril), L) was used as the test plant. This species was chosen because of its short period of life, so the impacts could be determined promptly and easily. The reason of using seedling is that the young stage is usually vulnerable against the change of environmental factors.

The seeds of soy bean were obtained from Bogor Horticultural Research Institute.

C. *Test Material*

Produced water, kindly supplied by Pertamina Exploration and Production Unit III (UEP III), was used as test material.

The produced water was sampled at the outlet point after the effluent flew through the oil catcher at 10.00 a.m. At the time of sampling, pH and temperature were measured on site. Carbonate and bicarbonate ions were examined by titration in the field after the temperature decreased to the ambient temperature more or less. The water sample was preserved by 2 ml/l of concentrated HNO_3 for metals examination, and the other container filled with water sample was preserved by some amount (± 2 g/l) of $HgCl_2$ for hydrocarbon examination. $HgCl_2$ was used to kill all microbes by oligodynamic mode of action so that hopefully no hydrocarbons were metabolized by them.

The chemical properties of such oil-field brines were examined in LEMIGAS laboratory and then classified according to Palmer (1911). They are shown in Table 2.

The data from Table 2 show that the produced water has no sulfide contents. It might be due to the chemical composition of its sedimentary rocks and the crude oil that has very low in sulphur contents. Cl^- and Na^+ are

Table 2
Chemical properties of oil-field brines

No.	Parameter	Unit	Methods	1st	2nd	Mean
1.	pH	–	pH-meter	7.2	7.5	7.36
2.	HCO_3^-	mg/l	Titrimetry	454.9	344.7	399.8
3.	$CO_3^=$	mg/l	Titrimetry	nil	59.07	29.54
4.	Cl^-	mg/l	Titrimetry	15597.12	15636.0	15616.56
5.	$SO_4^=$	mg/l	Gravimetry	57.0	nil	28.5
6.	$S^=$	mg/l	Potentiometry	nil	nil	nil
7.	J^-	mg/l	Vis-spectro	2.51	3.13	2.82
8.	Oil cont.	ppm	IR-spectro	4.97	2.15	3.56
9.	Na^+	ppm	Calc	10105.18	10130.38	10117.78
10.	Ca^{2+}	ppm	AAS	560	702.2	631.10
11.	Mg^{2+}	ppm	AAS	0.5	7.04	3.77
12.	Ba^{2+}	ppm	AAS	nil	3.24	1.62
13.	Sr	ppm	AAS	89.03	*	–
14.	Cd	ppm	AAS	< 0.01	0.03	< 0.02
15.	Cu	ppm	AAS	0.014	0.03	0.02
16.	Ni	ppm	AAS	< 0.01	0.08	< 0.05
17.	Fe	ppm	ASS	0.77	1.18	0.98
18.	Pb	ppm	AAS	nil	0.18	0.09
19.	Mn	ppm	AAS	nil	0.55	0.28
20.	Co	ppm	AAS	nil	0.15	0.08

**) lamp was out of order*

the main ions of produced water as in the marine water, this gives it the saline taste. Based on this property, produced water was considered as connate water from ancient sea water followed with deposition process and series of reactions for a long time. This was considered as an 'old fashion' theory since the composition of sea water is not identical with connate water. Connate in geological terminology is any water trapped in sedimentary rock during its deposition. Kinghorn (1984) stated that produced water is any water that occured naturally and have existed in sedimentary rock before drilling, without considering its origin.

The pH is more than 7 so the impacts on seedling can be eliminated. Barium and strontium are very important determinants because these two elements may produce the insoluble sulphate and carbonate salts which tend to close the pores of the soil, resulting in the decrease of its permeability.

It is evident that the oil content is very low (less than 5 ppm) in the produced water, so that the impacts of oil on the test plant could also be eliminated.

D. *Classification Method*

The first step in classifying produced water is to calculate the reaction value (R.V.) of the primary compounds. The R.V. is a result of multiplication of concentration and reaction coefficient (R.C.). The RC is a figure obtained from the valency of the element devided by its atomic weight. The calculation of reaction value is shown in Table 3.

The next step is to classify the produced water according to Palmer (1911) by using the percentage of reaction value.

If a = the percentage for alkaline metals (Na) = 47.81 %

b = the percentage for alkaline earth (Ca, Mg, Ba) = 3.47 %

d = the percentage for radicals of strong acids = 47.9 %

it seems that :

d > a; d < (a + b), so : 2a = Primary salinity = 95.6 %

2.(d - a)= Secondary salinity = 0.18 %

2.(a + b - d) = Secondary alkalinity = 6.76 %

The produced water is classified as Class III or S1S2A2.

E. *Bio-assay*

Though the implementation of the test in this study followed the general principle of assessment as recommended by APPHA (1990) and OECD (1984) there was

Table 3
Calculation of reaction value

Ion	R.C.	(meq/mg)	Concentration (mg/l)	R.V.(meq/l)	%
Na^+	1/23	0.0435	10117.78	440.123	47.81
Ba^{2+}	2/137.3	0.0146	1.62	0.024	0.003
Ca^{2+}	2/40	0.05	631.1	31.555	3.43
Mg^{2+}	2/24.305	0.0823	3.77	0.31	0.034
$SO_4^=$	2/96	0.0208	28.5	0.593	0.064
Cl^-	1/35.5	0.0282	15616.561	440.387	47.84
$CO_3^=$	2/60	0.0333	29.54	0.984	0.107
HCO_3^-	1/61	0.0164	399.8	6.557	0.712
				920.533	100.0

a little modification since treatments were not applied on population level but on individual level. So the output was not the LC 50 but the DC 50 (damage concentration) within a certain period of time which was determined arbitrarily.

1. Germination phase

The seeds were germinated and acclimated for at least 18-20 days from the time of inoculation or 15 days after the seeds started to sprout. During this phase the seedlings were sprinkled with artesian water.

2. Preliminary test

This was to determine the range of critical concentration between the upper threshold (N) and the lower threshold (n) concentrations, where:

N = the lower concentration at which most of test plants are damaged at the level of more than 50% within 7 days of treatments.

n = the highest concentration at which most of test plants are still in good condition or less than 50% level of damage within 14 days of treatments.

A gradual increase in concentrations of produced water were made by mixing it with artesian water as follows: 10, 50, 100, 500, 1000 ml/l and Controls, sprinkled to the seedlings, twice daily. For the accuracy of the test, OECD (1984) recommended that at least 80 % of test plants in Control treatments should be in good and healthy conditions from sprout stage until the end of the experiment. From this phase, the n and N value were obtained: n = 100 ml/l and N = 500 ml/l as shown in Appendix 1.

3. The DC 50 test

At this phase the interval treatments between n and N were arranged and calculated logarithmically as shown in the formula :

$$\frac{a}{n} = \frac{b}{a} = \frac{c}{b} = \frac{d}{c} = \frac{x}{d} \quad \ldots\ldots\ldots \quad \frac{N}{x} \quad (1)$$

$$\log \frac{N}{n} = k \log \frac{a}{n} \quad \ldots\ldots\ldots (2)$$

where a = the smaller concentration following n

k = the amount of treatments to be desired

Any concentration that produces 50% level of damage on the test plant during 14 days of treatments can be determined by plotting the results on a semilog graph paper. The abscis represents log concentration and ordinate represents the percentage of damage level. Determination on damage level was also carried out by scoring according to IRRI (1983) as a supporting method. The results of the DC 50 test are shown in Appendix 2 and the DC 50 value was obtained from the graph which shows as much as 426.58 ml/l.

4. Long term test

Some concentrations were arranged as follows :

20 % DC 50 = 20 % X 426.58 ml/l = 85.32 ml/l.

40 % DC 50 = 40 % X 426.58 ml/l = 170.63 ml/l.

60 % DC 50 = 60 % X 426.58 ml/l = 255.95 ml/l.

80 % DC 50 = 80 % X 426.58 ml/l = 341.26 ml/l.

100% DC 50 = 426.58 ml/l

and Control treatments.

These concentrations above were given for 12 days of treatment and then stopped, followed by watering from artesian well only up to day 10 of observation.

F. *Parameters to be Observed*

The observation was carried out until the plants were 38 days old which is the maximum age of vegetative growth phase.

The following were observed :

a. The height of the plants, measured once every 3 days.

b. The width of the canopy, measured once every 3 days.

c. The amount of leaves, calculated every 3 days.

d. Chlorophyll contents were examined at the end of the experiment.

Some leaves were taken from the green house of each treatment, wrapped by aluminium foil, and then kept in an ice-box after labelling. Chlorophyll was extracted in the laboratory from 2 g of leaves of each treatment using 80% aceton solution as solvent. The gross extract was filtered with no.42 Whatman paper filter and poured into 125 ml dark glass bottle and closed with glass stopper. All bottles were wrapped by black plastic sheets to protect them from any light which may destruct chlorophyll. They are then placed and kept in an ice-box at the temperature of ± 5°C. Spectrophotometer was used to examine the chlorophyll contents at the wave length of 663 nm and 645 nm.

G. *Experimental Design*

A completely randomized design was applied in this study with 6 concentrations under the DC 50 value and 4 replicates for the period of 12 days. The data was analyzed by Anova one way using the Minitab package with a P.C.

IV. RESULTS AND DISCUSSIONS

A. *The Height of the Plants*

When the seedlings were 15 days old, the mean of height was 13.85 ± 0.52 cm. At day 3 to day 6 of treatments, the height was ranging between 15-17 cm. It seems that the difference of height occured at day 9 where the plants in Controls grow higher rather than in treatments. The significant differences amongst treatments were clearly seen at day 12 to day 10 after treatments.

The results of observation on height of the plants at day 10 after treatments as shown in Table 4.

The statistical (Table 4a)shows that F value at the level of 0.05 with degrees of freedom (df) = 5 is 2.77 and at the level of 0.01 the value is 4.25. anova shows that F value is as much as 13.26 which is higher than those given by 95% reliability. It means that all treatments were significantly different from the Controls. The significant difference on height was presumably due to the disturbances on cell metabolism particularly at the point of growth affecting the growth hormone, e.g. auxin. This process might have started from the decrease of permeability of the soil caused by the production of insoluble sulphate and carbonate salts of Ba and Sr as mentioned earlier. At higher concentration of treatments, the permeability decreases rapidly. As the result, roots will have difficulty in taking nutrients so that the plants will suffer from nutrient deficiency.

The impacts of N deficiency affect several biological conditions such as chlorophyll contents on leaves (chlorosis), decrease in amino acids and amide formations. While P deficiency causes the plants suffering from chlorosis, stunted growth and energy transfer impairment. The role of K element is very important in photosynthesis, water balance and turgor regulations. Generally, K deficiency will be followed by micro nutrients deficiency particularly if the concentration of calcium is higher and the impacts will be very severe.

The impairment of the growth was also caused by the soil which becomes saline, subject to the produced water contamination. As the consequence the leaves were dehydrated. If the permeability decreases, the moisture of the soil will be affected. Case (1977) stated that if the moisture of the soil decreased, the salinity of soil solution increased. He informed that the root system was unable to tolerate salt concentrations more than 1.5% dry weight of soil.

Such treatments also made the plants in stress conditions. Stress is defined as an environmental change which induces reduction of plant growth. It was evident that under stress conditions which impair plant growth, the plant hormone (cytokinin) activity declined, while the activity of growth inhibitors, such as ABA increased. The hormonal changes enable the plants to adapt and survive under new environmental conditions. This study was carried out by Mizrahi (1980) using the tobacco plants in root medium (Hoagland solution) with 6.0 g/l of NaCl.

The effects of NaCl (50 and 100 ppm in Hoagland solution) on rice plants (*Oryza sativa*) have also been studied by Sasmitamihardja and Dasuki (1979) to find out the nutrients consumption pattern. They concluded that NaCl could increase or decrease mineral absorption capability depending on plant age and the sort of minerals absorbed by the plants.

Table 4
Results of observation on height

n	Ctrl	20 %	40 %	60 %	80 %	100 %	Σ
1.	30.83	31.78	27.86	25.72	23.72	20.14	160.05
2.	35.78	28.11	27.22	28.67	22.22	23.19	165.19
3.	27.44	30.67	29.33	28.72	23.78	21.72	161.66
4.	36.22	31.56	27.89	27.17	27.11	21.44	171.39
Σ	130.27	122.12	112.30	110.28	96.83	86.49	658.29
$\bar{n}$	32.57	30.53	28.08	27.57	24.21	21.62	

B. *The Width of the Canopy*

When the plants were at the age of 15 days the width of the canopy was 13.87 ± 0.73 cm. At day 12 of treatment the difference of the width started to show, though at the concentrations of 60% and 80% they had a relatively equal value. Until day 10 after treatments they gave significance difference in most treatments except in 20% concentration where no difference with the Control was observed. Presumably the small amount in concentration was unable to affect the width.

Tabel 4a
Analysis of variance
Observation on height

SOURCE	DF	SS	MS	F	F
FACTOR	5	322.20	64.44	13.26	0.000
ERROR	18	87.46	4.86		
TOTAL	23	409.67			

The results of observation on the width of the canopy are shown in Table 5.

The Anova (Table 5a) shows that F value (13.14) is higher than the values of 2.77 and 4.25 from the statistical table, meaning that almost all treatments were significantly different form the Controls, the exception being in 20% treatment.

The deficiency in nutrients as mentioned earlier affects the branch formation, its growth and the space area of leaves, so the width of canopy decreased. This process presumably affects the seeds production (yield) because the space area provided for photosynthesis will be narrower and limited.

Individual 95 PCT CI'S for mean based on pooled St Dev

Level	N	Mean	St Dev
C1	4	32.568	4.202
C2	4	30.530	1.683
C3	4	28.075	0.892
C4	4	27.570	1.428
C5	4	24.208	2.065
C6	4	21.622	1.251

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                        (---*----)
                    (----*----)
                 (---*----)
                (---*----)
          (---*----)
    (---*----)
--+---------+---------+---------+----
 20.0      25.0      30.0      35.0
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Pooled St Dev = 2.204

Table 5
Results of observation on width

n	Control	20 %	40 %	60 %	80 %	100 %	Σ
1.	24.28	24.67	25.44	17.17	22.22	12.29	126.07
2.	30.78	25.78	22.00	23.89	17.17	17.69	137.31
3.	29.17	25.78	23.22	23.61	18.39	17.50	137.67
4.	27.78	23.56	23.67	20.39	22.28	14.13	131.81
Σ	112.01	99.79	94.33	85.06	80.06	61.61	532.86
$\bar{n}$	28	24.95	23.58	21.27	20.02	15.4	

C. *Number of Leaves*

At the beginning of treatments the seedlings had 8,2 ± 0.5 pieces of leaves. The difference in the number of leaves occured at day 9 of treatments. But until day 12 no difference occured between 20% and 40% treatments up to the end of the experiment. Most treatments were significantly different from the Controls. The results of observation on the number of leaves shown in Table 6.

Table 5a
Analysis of variance
Observation on width

SOURCE	DF	SS	MS	F	p
FACTOR	5	379.93	75.99	13.14	0.000
ERROR	18	104.06	5.78		
TOTAL	23	483.99			

The Anova (Table 6a) shows that the F value (= 23.95) is higher than 2.77 and 4.25 from statistical table, meaning that it is in agreement with the statement earlier.

Chlorosis occured at day 9 of treatments and increased in numbers until the end of experiment. At day 12 of treatments necrosis occured and increased in numbers until the end of experiment. After treatment at day 6 two plants died. The mortality increased (3 plants) and other 3 plants withered at the end of experiment.

Chlorophyl examination as an additional of experiment was also carried out and the contents appeared to decrease as well. The results of examination on chlorophyl contents as shown in Table 7.

Individual 95 PCT CI'S for mean based on pooled St Dev

Level	N	Mean	St Dev
C1	4	28.003	2.768
C2	4	24.948	1.063
C3	4	23.583	1.425
C4	4	21.265	3.153
C5	4	20.015	2.623
C6	4	15.403	2.642

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                            (----*----)
                      (----*----)
                   (----*----)
              (-----*----)
           (----*----)
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-----+---------+---------+---------+-
    15.0      20.0      25.0      30.0
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Pooled St Dev = 2.404

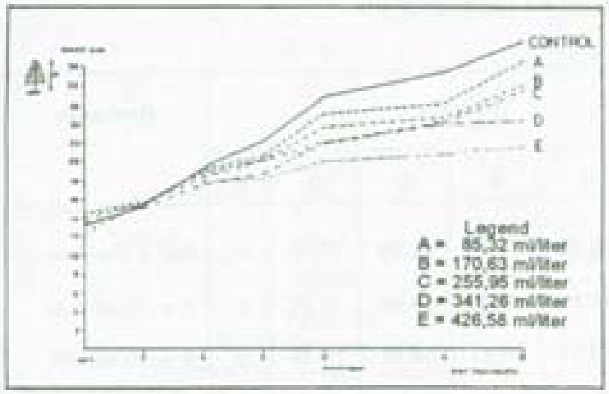

Figure 1
Plant growth within 12 days of treatments until day 10 after treatments

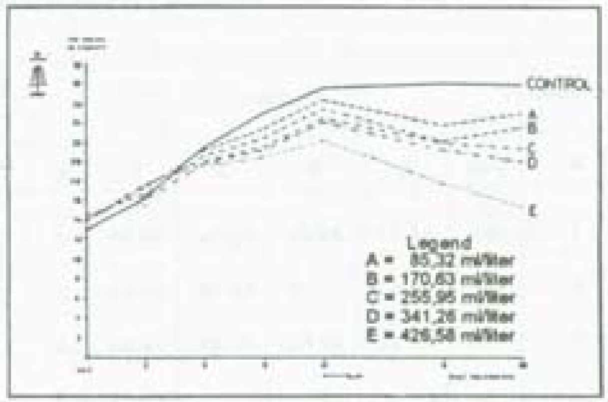

Figure 2
Average width of canopy

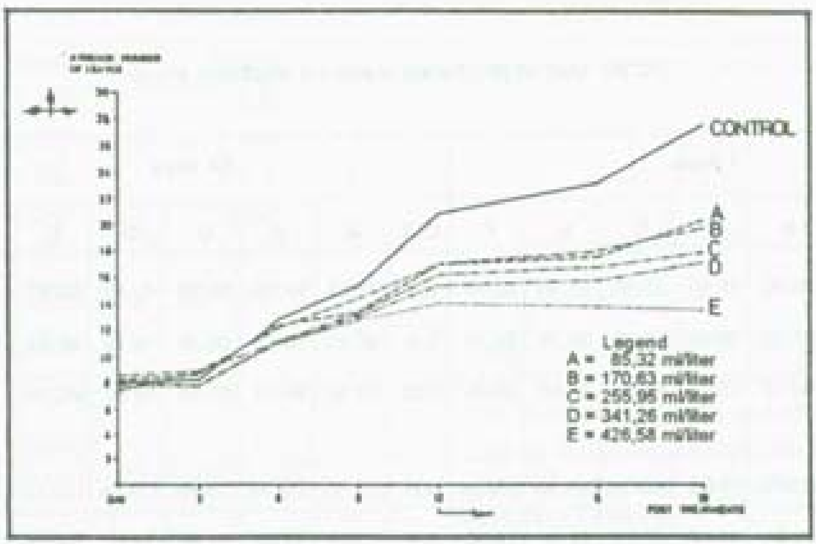

Figure 3
Average amount of leaves

Appendix 1

Preliminary test of produced water on soybean plant

Duration	7 days						14 days						Remarks
N	Ctrl	A	B	C	D	E	Ctrl	A	B	C	D	E	
1	100	91.35	93.68	63.94	32.04	0	100	85.56	83.84	60.06	17.30	0	Ctrl = Control
2	100	94.50	82.28	62.98	26.88	0	100	94.32	85.88	59.66	19.30	0	A = 10.00 ml/l
3	100	87.10	80.80	63.01	29.89	0	100	79.61	79.61	58.01	17.80	0	B = 50.00 ml/l
													C = 100.00 ml/l
Total	300	272.95	256.76	189.93	88.81	0	300	249.33	249.33	177.93	54.40	0	D = 500.00 ml/l
Average	100	91.00	85.60	63.30	29.60	0	100	83.10	83.10	59.30	18.10	0	E = 1000.00 ml/l

Note : *The figures represent score percentage of the plant vs control plant*

Appendix 2

DC 50 test of produced water on soybean plant

Duration	7 days							14 days							Remarks
N	Ctrl	A	B	C	D	E	F	Ctrl	A	B	C	D	E	F	
1	100	99.75	96.95	93.40	85.35	91.30	80.45	100	77.55	64.45	56.30	63.45	60.30	46.81	Ctrl = Control
2	100	98.10	97.20	98.50	92.35	85.25	80.45	100	95.45	80.15	60.78	79.75	66.53	60.78	A = 130.77 ml/l
3	100	99.45	84.37	75.60	86.45	84.89	59.05	100	78.30	84.14	61.60	65.60	552.50	39.4	B = 171.01 ml/l
															C = 223.63 ml/l
Total	300	297.3	278.52	267.5	264.15	261.48	219.95	300	251.30	228.75	178.68	178.68	179.33	146.99	D = 292.44 ml/l
Average	100	99.10	92.84	89.17	88.05	87.16	73.30	100	83.80	76.30	59.80	59.80	59.80	49.00	E = 1382.42 ml/l
															F = 499.76 ml/l

Note : *The figures represent score percentage of the undamage plant vs control plant*

Table 6
Results of observation on number of leaves

n	Control	20 %	40 %	60 %	80 %	100 %	Σ
1.	25.11	19.11	21.57	16.56	19.22	11.43	113.1
2.	27.67	17.33	19.44	16.33	14.89	13.50	109.16
3.	28.22	23.56	20.33	19.33	16.11	14.89	122.44
4.	28.67	21.33	18.33	19.89	18.11	14.44	120.77
Σ	109.67	81.33	79.77	72.11	68.33	54.26	465.47
$\bar{n}$	27.42	20.33	19.94	18.03	17.08	13.57	

Tabel 6a
Analysis of variance
Observation on number of leaves

SOURCE	DF	SS	MS	F	p
FACTOR	5	426.98	85.40	23.95	0.000
ERROR	18	64.17	3.57		
TOTAL	23	491.15			

Individual 95 PCT CI'S for mean based on pooled St Dev

Level	N	Mean	St Dev
C1	4	27.417	1.592
C2	4	20.333	2.703
C3	4	19.193	1.413
C4	4	18.028	1.844
C5	4	17.083	1.947
C6	4	13.565	1.537

Pooled St Dev = 2.404

```
-------+---------+---------+---------
                              (--*----)
                (---*---)
             (---*---)
        (---*---)
     (---*---)
(---*---)
-------+---------+---------+---------
     15.0      20.0      25.0
```

Table 7
Results of examination on chlorophyll (mg/g)

Treatment	Chl.a	Chl.b	Total	% of Ctrl.
Control	0.154	0.076	0.230	–
20 %	0.148	0.073	0.221	96.1
40 %	0.11	0.061	0.171	74.4
60 %	0.109	0.056	0.165	71.7
80 %	0.056	0.051	0.107	46.5
100 %	0.035	0.030	0.065	28.3

V. CONCLUSION

1. The damage concentration (DC 50) of produced water was 426.58 ml/l.
2. According to Palmer Classification, the produced water used in this study was Class III (S1 S2 A2).
3. Most treatments were significantly different from the Controls up to the end of the experiment. The higher the concentrations, the more severe the impacts that occured on height, width of canopy and number of leaves.
4. It is necessary to continue the study to find out on generative growth so that the yield amongst treatments can be compared.

This protocol hopefully might be used to support the application of RPL (Environmental Monitoring Plan) and RKL (Environmental Management Plan) within the area of oil production activities.

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