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Ecotoxicological Evaluation of Industrial Degreaser on *Nitrobacter* sp.

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ABSTRACT The effects of industrial degreasers (Rigwash and Aquabreak) on nitrite utilization and percent log survival and log mortality of *Nitrobacter* were investigated. Nitrite utilization decreased with increase in concentration and exposure period of the toxicants. A 10% decrease in the logarithmic rate of growth of *Nitrobacter* after 72 hours of exposure to most of the toxicant was observed 0-10% mortality values were recorded when Aquabreak degreaser was used as toxicants. These results show that industrial degreaser inhibit the nitrification process in the ecosystem and elicit mortality rate of *Nitrobacter*. These may lead to reduced ability of affected ecosystems to support both aquatic and terrestrial plant growth.

Keywords: industrial degreaser, log survival, *Nitrobacter*

Introduction

Degreasers are chemicals that are used to clean metal by washing dirt, grease and oil from auto engine parts. A degreaser can either be oil-based or water-based. Oil-based degreasers are usually toxic and flammable. Even small amounts entering surface or groundwater can result in serious pollution. Many oil-based degreasers readily evaporate and contribute to smog or ground level ozone. Water based cleaners are generally safer for the user and the environment. They are less toxic than oil based degreasers and small amounts can be broken down in sewage treatment facilities. (Department of Energy and Environmental Protection, 2013). TRICHLOROETHYLENE (TCE) Is one of the compounds that is mainly used a degreaser. Chlorinated aliphatic hydrocarbons (CAH_S) may enter into water bodies and contaminate

water sources and affect human health in a direct or indirect manner. Many efforts have reported in removal of organic material from aqueous solutions (Mesdaghinia *et al*., 2007; Naghizadeh *et al.,* 2008). Trichloroethylene is one of (CAH_S) chlorinated aliphatic hydrocarbons that has been used as a degreaser (Watts, 1998). It is a volatile, colourless and nonflammable liquid and has different uses in many industries such as electronic and electrical, fabricated metal products and transport operations (ATSDR, 1995). Because of improper handling and disposal practices, TCE has been frequently detected in groundwater. TCE is considered as a probable carcinogenic chemical (Group B_2) to human (Watenberg *et al.,* 2000) it has also many other adverse effects on human and animals, (CEPA, 1993; Gist and Burg, 1995; Wartenberg *et al*., 2000). Due to its serious health effects, U.S Environmental protection Agency (U.S EPA) has set the maximal contaminant level (MCL) and maximum contaminant level goal (MCLG) for TCE as 0.005mg/L and zero respectively. Most conventional treatment processes such as coagulation, sedimentation, precipitate softening, filtration and chlorination are not efficient in removal of TCE (Russell *et al.,* 1992). Air stripping can induce secondary air pollution because of the phase shift of TCE. Although membrane processes and granular activated carbon are used most often, but they are expensive and transfer the contaminant to another phase (Nutt *et al.,* 2006). TCE is used mainly for vapour degreasing of fabricated metal parts in the automotive and metal industries. This study was designed to assess the effect of industrial degreasers on aquatic microorganism in marine water.

Materials and Methods

Source and isolation of Nitrobacter

Surface water sample was collected from Bonny River at Bonny with a 100ml sterile plastic container. This was used within 1 hour of collection for the isolation of *Nitrobacter* sp. employed in this study.

The method used for the isolation of *Nitrobacter* from the River water sample was adopted from (Colwell and Zambruski; 1972) using winogradsky agar medium as modified by (Okpokwasili and Odokuma, 1994).

The composition of this medium was

K_2HPO_4	-	0.5g
$MgSO_4.7H_20$	-	0.3g
$MnSO_4.H_20$	-	0.02g
$FeSO_4.6H_20$	-	0.02g
$NaNO_2$	-	0.05g
$ZnCl_2$	-	0.03g
Agar	-	0.3g
Distilled water	-	200ml

The Winogradsky agar medium was autoclaved at 121^0c for 15 minutes and aseptically transferred to sterile petri dishes after cooling to about 45^0C. The petri dishes were then inoculated with the river water and incubated aerobically for 4 days at room temperature (30 ± 2^0C), Greyish, mucoid, flat colonies were suggestive of Nitrobacter species.

Gram staining of the colonies revealed pear-shaped, Gram negative organisms indicative of nitrobacter (Colwell and Zambruski 1992). The colonies were aseptically streaked on fresh winogradsky agar and incubated for 2 days. Greyish, mucoid, flat colonies were once more obtained and were aseptically transferred from the six petri dishes into 200ml Erlenmeyer flasks containing the growth medium and incubated for 24 hours at room temperature.

A solution of 0.25mg sodium nitrite ($NaNO_2$) per liter was autoclave at 121^0c for 15 minutes and 100ml of it was transferred aseptically into 250ml Erlemeyer flasks. This served as diluents for the effect determination.

Toxicity Test Procedure

Preparation of effluent

The effluent was prepared following the procedure outlined in APHA, (1992). 75%, 50%, 25%, 15%, 5% and the control (10m of diluent) concentrations of the Rigwash and Aguabrak (degreaser 's effluent) were prepared.

Test procedure for the bacterium

0.5ml of the degreaser was added to 9.ml of the diluent, 1.5 of the degreaser was also added to 8.5ml of the diluent, 2.5ml degreaser was added to 7.5ml diluent, 5ml degreaser was adder to 5ml of diluents and 7.5ml degreaser was added to 2.5ml diluents of the toxicant concentration (75%, 50%, 25%, 15%, 5% and % respectively) and plated out immediately after inoculation in triplicates on appropriate media. This is know as zero hour count plating. These were incubated at room temperature (28 ± 2^oC).

Aliquot (1ml) of each concentration of the effluent was then plated out after 4 hours, 8 hours, 12 hours and 24hours in triplicates on appropriate media. This was followed by incubation for 72 hours (Nitrobacter). The plates were then counted.

Percentage mortality of organisms

Brackish water was use in the study as a specimen to assess the probable toxic effect degreaser could have on aquatic environment. The formular for the calculation of percentage mortality was adopted from APHA (1992). The

percentage mortality was done by using the zero toxicant concentration to subtract the % log survival.
Thus: %Mortality = Zero toxicant Concentration - % log survival.

Percentage log survival of the bacterial isolate in degreaser diluent

The percentage log survival of the bacterial isolates in the degreaser diluent used in the study was calculated using the formular adopted from Williamson and Johnson (1981). The percentage log survival of the bacterial isolates in the diluent was calculated by obtaining the log of the count in each toxicant concentration, dividing by the log of the count in the zero toxicant concentration and multiplying by 100. Thus:

$$\% \text{ log survival} = \frac{\log C}{Logc} \; x \; 100$$

Results

Estimation of *Nitrobacter* sp. using standard plate count of water samples and degreaser –Rigwash and Aquabreak are shown in table 1-2.

Table 1: Lethal toxicity of degreaser - Rigwash on survival counts of Nitrobacter sp.

	0hr	4hrs	8hrs	12hrs	24hrs	48hrs	72hrs
Control	50	58	50	78	30	30	90
5%	400	300	180	250	200	60	40
15%	92	80	49	40	15	50	30
25%	204	120	30	40	66	40	100
50%	32	40	50	51	60	80	40
75%	70	75	40	83	50	40	20

Table 2: Lethal toxicity of degreaser - Aquabreak on survival counts of Nitrobacter sp.

	0hr	4hrs	8hrs	12hrs	24hrs	48hrs	72hrs
Control	100	31	1	3	3	5	0
5%	28	0	0	0	0	0	0
15%	3	0	0	0	0	0	0
25%	1	2	0	0	0	0	0
50%	1	0	0	0	0	0	0
75%	0	0	0	0	0	0	0

Table 3: Percentage (%) Lethal Toxicity Result of degreaser-Rigwash

Conc.	**5%**	**15%**	**25%**	**50%**	**75%**
Control (%)	100	100	100	100	100

Start hour

% log surviving	800	184	408	64	140
% log mortality	-700	-84	-308	36	-40

4hrs

% log surviving	517.2	137.9	206.9	68.9	129.3
% log mortality	-417.2	-37.9	-106.9	31.1	-29.3

8hrs

% log surviving	360	98	60	100	80
% log mortality	-260	2	40	0	20

12hr

% log surviving	32.05	51.3	51.3	65.4	106.4
% log mortality	-220.5	48.7	48.7	34.6	-6.4

24hrs

% log surviving	666.7	50	220	200	166.7
% log mortality	-556.7	50	-120	-100	-66.7

48hrs

% log surviving	200	166.6	133.3	266.7	133.3
% log mortality	-100	-66.6	-33.3	-166.7	-33.3

72hrs

% log surviving	44.4	33.3	111.1	44.4	22.2
% log mortality	55.6	66.7	-11.1	55.6	77.8

Table 4: Percentage (%) Lethal toxicity Result of degreaser –Aquabreak

Conc.	**5%**	**15%**	**25%**	**50%**	**75%**
Control (%)	100	100	100	100	100

Start 0hour

% log surviving	100	3	1	1	0
% log mortality	0	97	99	99	0

4hrs

% log surviving	0	6.5	6.5	0	0
% log mortality	100	93.5	93.5	0	0

8hrs

% log surviving	0	500	900	0	0
% log mortality	0	-400	-800	0	0

12hr

% log surviving	0	0	0	0	0
% log mortality	0	0	0	0	0

24hrs

% log surviving	0	0	0	0	0
% log mortality	0	0	0	0	0

48hrs

% log surviving	0	0	0	0	0
% log mortality	0	0	0	0	0

72hrs

% log surviving	0	0	0	0	0
% log mortality	0	0	0	0	0

Discussion

These results showed that certain toxicant concentrations were stimulating (increase the activity) while others were inhibiting to nitrite utilization by *Nitrobacter*. Similar observations have been reported (Wang and Reed, 1983; Wang, 1985, Obire and Nrior, 2014). A good increase in the loss of nitrites with increasing exposure time was observed with the degreasers. The increase in nitrite utilization with increase in toxicant exposure time was not in uniform with the degreaser. Aquabreak degreaser was more toxic than Rigwash degreaser. At 72 hours 20% reduction in the viable count was observed when *Nitrobacter* was exposed to Rigwash degreaser and 0% reduction in the viable count was also observed when *Nitrobacter* was exposed to Aquabreak degreaser (Table 1-4). This might have resulted from degreaser effect on any of the target sites.

It is known that the site of action of a toxicant is a function of the nature of the toxicant (Sander *et al.,* 1985). The percent log survival of *Nitrobacter* during 0hr, 4hr, 8hr, 12hr, 24hr and 72hr exposure periods to these industrial degreasers carried out revealed that degreaser Righwash exhibited little effect while Aquabreak showed significant effect on the percent log survival of the organism. Careful examination showed that Aquabreak reduced the percent log survival more than Rigwash. The percent log mortality of *Nitrobacter* during 0hr, 4hr, 8hr, 12hr, 24hr and 72hr exposure periods to the industrial degreasers shows that the mortality rate on Aquabreak is higher than that of Rigwash (Table 3-4). According to (Okpokwashili and Odokuma 1996) the results of this study suggest that degreaser (Aquabreak) caused cell mortality. This has led to the reduction in the viable counts. This may be due to inhibition of the nitrification process within the 72hour exposure period. The degreaser Righwash did not elicit any appreciable reduction in viable counts though Aquabreak did. This clearly suggests that the mode of action of chemical degreaser is not limited to inhibition of the nitrification process by *Nitrobacter* but also involved cell death.

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