Microbial Response to Varying Concentrations of Crude Oil Pollution of Agricultural Soils in Ondo State, Nigeria

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Original Research Article

ABSTRACT

This research investigated the effects of varying concentrations of crude oil on the population of crude oil degrading microorganisms in crude oil polluted agricultural soils from Igodan- Lisa, Oba-Ile and Ido-Ani areas of Ondo State, Nigeria. The soil samples were exposed to 1- 4% (w/w) crude oil and analyzed monthly for six periods using standard microbiological techniques for the cultivation and enumeration of crude oil degrading bacteria and fungi. Results indicated that the crude oil degrading microbial populations were significantly altered. The population of crude oil degrading microbes were higher (1.03×10^5 - 1.10×10^6 cfu/g for bacteria and 1.07×10^4 – 8.67×10^5 sfu/g for fungi) in polluted than unpolluted (1.53×10^4 – 9.40×10^5 cfu/g for bacteria and 1.17×10^3 – 5.17×10^5 sfu/g for fungi) soils and also varied with increase in the amount of crude oil spilled and time. The mean count indicated that the microbiological status of the soil samples were not negatively impacted at 1-4% crude oil contamination and the effect on soil micro flora is a function of both concentration and contact time.

Keywords: Varying concentrations; crude oil; agricultural soils; microbial population; micro flora; contact time.

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1. INTRODUCTION

Crude oil contamination of the environment in the form of pollution and the consequent ecological and environmental impact is a global threat to environmental sustainability and hence a concern to environmental researchers. Pollution of the environment by petroleum occurs when petroleum or its derivatives are introduced into the environment at levels harmful either directly to the environment or indirectly to the dependents of the environment.

The sources of crude oil spill into the environment differ and the amount spilled vary from minor to disaster. The Niger- Delta Region of Nigeria frequently experience crude oil spill resulting from diverse human activities of exploration, exploitation, distribution and indiscriminate use and discharge of crude oil and its derivatives, sabotage, negligence during production operations and fuel tanker loading processes, pipeline vandalisation, corrosion and leakage of pipe lines and oil tanker terrestrial accidents. Crude oil is toxic in nature and no matter its quantity and size (minor, medium, major or disaster) may cause minor or severe damages to the environment and all forms biomass(including indigenous micro flora and micro fauna) dependent on the environment [1, 2,3]. Pollution of the soil environment with crude oil results in the devastation of arable agricultural lands in terms of its productive capacity as crude oil can sterilize the soil for a varying period of time [4,5,6,7,2], creating nutritional imbalances (especially of carbon-nitrogen ratio) as well as imbalance in the biological ecosystem and may impair or impede important biochemical processes. The enormous damages to the environment by spilled oil is due to the presence of many toxic compounds such as polycyclic aromatic hydrocarbons, benzene and its substituted and cycloalkane rings in relatively high concentration [8]. The overall implication of crude oil on agricultural land and the socio-economic lives of people residing in the affected areas is increased unemployment, poverty and hunger among the people who depend predominantly on sales from their farms for food and economy.

Several physicochemical and biological strategies have been applied to remediate polluted soil and water environments. The effects of crude oil spill and time for reclamation of crude oil polluted soil depends on the quantity and the concentrations of the pollutant [9,10]. Among the remediation techniques, bioremediation which relies on the use of microorganisms with diverse metabolic capabilities is an evolving and effective method that holds promise for the degradation and removal of many environmental pollutants. Microbial biodegradation can be carried out by autochthonous and allocthonous population. Microbial degradation of hydrocarbons by natural populations of microorganisms is the major and ultimate natural mechanism by which petroleum hydrocarbon pollutants can be cleaned up from the environment [11,12,2]. Ikuesan [13] reported that in a six months laboratory study, native microbial population was responsible for 52.33-58.74% crude oil removal in soil samples contaminated with 5% (w/w). This method of biological reduction in the complexity and mineralization of petroleum hydrocarbon involving primarily bacteria and fungi is eco-friendly, cost effective and simple relative to the physico-chemical methods. The microorganisms involved in bioremediation of crude oil polluted soil use petroleum hydrocarbon as carbon and energy source. However, this process is complex because it depends not only on the biodegrading capabilities of native microbial populations but also on other parameters such as environmental factors, soil type, nutrients, chemical composition, concentration and physical state of the pollutant the nature and amount of hydrocarbon present [14,2].

In order to optimize agricultural yield and improve the livelihood of the people in the oil producing region where agricultural lands have been devastated due to crude oil pollution, it is expedient to return polluted soils to their pre-contamination status to support agricultural activities which is the main occupation of the people. Ikuesan [15] evaluated the crude oil biodegradation potentials of some indigenous soil microorganisms but the study does not focus on the effect of crude oil concentrations on the microbial population associated with crude oil degradation. The use of bioremediation strategy to return crude oil contaminated environment to its pristine state requires a thorough understanding of the effects of crude oil concentrations on the native microbial population with ability to metabolize petroleum hydrocarbon in order to enhance crude oil mineralization. Therefore, the overall objective of this research is to evaluate the effects of varying concentrations of crude oil on the population of crude oil degrading microorganisms associated with agricultural soils in order to develop appropriate

bioremediation approach of reclaiming polluted agricultural soil.

2. MATERIALS AND METHODS

2.1 Sample Collection

The samples used in this study were arable agricultural soils collected from Igodan- Lisa, Oba- Ile and Ido-Ani all in Ondo State, Nigeria. The samples were collected using the hand auger at depth of 15-20cm into sterile black cellophane bags and transported to the laboratory for analysis within 48 hours.

2.2 Microbiological Analysis of Soil Samples

2.2.1 Enumeration of microbial population

All media, distilled water and diluents were sterilized by autoclaving at 121°C for 15 minutes at 1.1 kg/cm^2 pressure. Glassware were sterilized in hot air-oven at 160°C for two hours. One gramme of each soil sample was serially diluted to 10^{10} using nutrient broth as diluent. Each dilution (1ml) was cultured using pour plate method on Bushnell-Hass (MSM) broth incorporated with 1.5% agar (for bacteria), 1.2% agar (for fungi). The media were also fortified with fungisol (10mg/lt) for bacteria and 50mg/lt of streptomycin for fungi after sterilization to determine the loads of Total Crude oil Degrading Bacteria and Fungi. Crude oil (2%) sterilized using 0.45 μm millipore filter served as carbon source. The pH of the medium was adjusted to 7.2 and 5.6 respectively for bacteria and fungi estimation. The MS-oil medium for crude oil degrading bacteria and fungi were then incubated at 28 ± 2°C respectively for 14 and 21 days. The colonies which developed on the plates were counts in which the number of colonies were less than 300 [16] and its triplicate for each sample was selected. The averaged count was then multiplied by the dilution factor at that dilution and expressed as colony forming unit (cfu/g) or spore forming unit (sfu/g) per gramme of sample bacteria and fungi respectively.

2.2.2 Soil treatments

For the preparation of crude oil contaminated soils, the method of Ekpo and Ebeagwu [17] and Njoku et al. [18] was adopted. Contamination with crude oil was done by thoroughly mixing crude oil with the soils in their respective plastic container to obtain 0-4% (w/w) crude oil contamination. The untreated samples (0% w/w) were the controls.

2.3 Effects of Crude oil Contamination on the Population of Crude Oil Degrading Microorganisms of Soil

The agricultural soil samples treated (0-4%) as described above were used to study the effects of varying concentrations of crude oil on the population of crude oil degrading bacteria and fungi. Samples were incubated at 28°C ± 2°C for 7 days to allow for acclimatization between oil and soil and then enumerated for crude oil degrading bacteria and fungi as day zero. Changes in microbial population of crude oil degrading microbes were monitored monthly for six periods using standard microbiological techniques for the enumeration of crude oil degrading microorganisms. The counts of bacteria and fungi were thus expressed as colony forming unit (cfu/g) and spore forming unit per gram (sfu/g) respectively.

2.4 Statistical Analysis

Data obtained on microbial counts were analyzed by one way Analysis of Variance (ANOVA) using SPSS version 18.0 (2010).

Note: Values are means of triplicate determinations.

3. RESULTS

3.1 Effects of Varying Concentrations of Crude Oil on the Population of Crude Oil Degrading Microorganisms

The results of this study revealed the effects of varying concentrations of crude oil on the population of crude oil degrading microbes of soil samples. Treatment of soil samples with crude oil at 1 - 4% (w/w) contamination level showed that the population of crude oil degrading microorganisms of the agricultural soils were significantly altered.

The population of crude oil degrading microbes were higher (1.03 x 10^5 – 1.10 x 10^6cfu/g for bacteria and 1.07 x 10^4 – 8.67 x 10^5 sfu/g for fungi) in polluted than unpolluted (1.53 x 10^4 – 9.40 x 10^5 cfu/g for bacteria and 1.17 x 10^3 – 5.17 x 10^5 sfu/g for fungi) soils. Tables 1(a- c) show the trend of the effects of varying

concentrations of crude oil on the bacterial population within the sampling period of 150 days. The bacterial mean counts ranged 1.80×10^4 cfu/g – 3.45×10^5 cfu/g, 3.00×10^4 cfu/g – 9.40×10^5 cfu/g and 1.5×10^4 cfu/g – 3.40×10^5 cfu/g respectively for Igodan-Lisa, Oba-Ile and Idoani control samples. The counts of bacteria at 1% (w/w), 2% (w/w), 3% (w/w) and 4% (w/w) crude oil treated soils were 1.30×10^5 cfu/g – 1.1×10^6 cfu/g, 1.40×10^5 cfu/g – 9.77×10^5 cfu/g, 1.03×10^5 cfu/g – 8.70×10^5 cfu/g and 1.47×10^5 cfu/g – 8.37×10^5 cfu/g respectively. The fungal counts ranged between 1.67×10^4 sfu/g – 8.67×10^5 sfu/g, 1.83 sfu/g – 6.60×10^5 sfu/g, 1.20×10^4 sfu/g – 4.20×10^5 sfu/g and 1.07×10^4 sfu/g – 4.50×10^5 sfu/g for soils exposed respectively to 1% (w/w), 2% (w/w), 3% (w/w) and 4% (w/w) of crude oil (Tables 2a - c). The highest counts of bacteria and fungi were observed at 1 - 3% (w/w) of crude oil pollution. The effect of contact time on microbial population was also significant. The highest population count was at days 60 - 90 for bacteria and 90 - 120 for fungi.

Table 1(a). Effects of varying concentrations of crude oil on the crude oil degrading bacterial counts ($\times 10^5$ cfu/g) of Igodan-Lisa soil sample

Time (Days)	SS$_3$	SS$_{3A}$	SS$_{3B}$	SS$_{3C}$	SS$_{3D}$
0	0.18 ± 0.00^a	1.53 ± 0.15^a	2.26 ± 0.88^b	$4.57 \pm .0.29^e$	1.50 ± 0.15^a
30	3.07 ± 0.12^d	1.30 ± 0.20^a	1.60 ± 0.17^a	4.00 ± 0.12^d	1.93 ± 0.20^b
60	3.10 ± 0.21^d	2.47 ± 0.35^b	2.87 ± 0.14^c	2.30 ± 0.12^b	3.17 ± 0.12^d
90	2.53 ± 0.09^c	2.80 ± 0.20^c	3.27 ± 0.15^d	2.57 ± 0.07^c	2.50 ± 0.12^c
120	1.67 ± 0.15^b	2.30 ± 0.20^b	3.03 ± 0.03^c	1.93 ± 0.07^b	1.63 ± 0.15^a
150	3.45 ± 0.15^d	2.37 ± 0.15^b	1.60 ± 0.06^a	1.03 ± 0.08^a	2.27 ± 0.37^{ab}

Legend: SS$_3$: Igodan- Lisa soil without 0%(w/w)crude oil contamination (Control); SS$_{3A}$: Igodan- Lisa soil with 1% (w/w) crude oil contamination; SS$_{3B}$: Igodan- Lisa soil with 2% (w/w) crude oil contamination; SS$_{3C}$: Igodan- Lisa soil with 3% (w/w) crude oil contamination; SS$_{3D}$: Igodan- Lisa soil with 4% (w/w) crude oil contamination

Table 1(b). Effects of varying concentrations of crude oil on the crude oil degrading bacterial counts ($\times 10^5$ cfu/g of Oba-Ile soil sample

Time (Days)	SS$_4$	SS$_{4A}$	SS$_{4B}$	SS$_{4C}$	SS$_{4D}$
0	0.30 ± 0.01^a	3.17 ± 0.22^b	2.50 ± 0.06^b	2.63 ± 0.08^b	3.50 ± 0.03^c
30	2.83 ± 0.12^c	5.80 ± 0.28^c	3.00 ± 0.12^c	3.13 ± 0.09^b	1.47 ± 0.12^a
60	1.26 ± 0.67^b	11.00 ± 1.00^e	9.77 ± 0.07^e	8.70 ± 0.12^d	4.07 ± 0.09^d
90	9.40 ± 0.12^e	9.27 ± 0.15^d	4.53 ± 0.09^d	3.10 ± 0.12^b	1.70 ± 0.12^a
120	6.60 ± 1.53^d	2.33 ± 0.32^a	3.20 ± 0.12^b	4.47 ± 0.15^c	2.20 ± 0.12^a
150	1.38 ± 0.24^b	1.70 ± 0.12^a	1.40 ± 0.12^a	2.00 ± 0.12^a	2.60 ± 0.21^b

Legend: SS$_4$: Oba-Ile soil without crude oil contamination (Control); SS$_{4A}$: Oba-Ile soil with 1% (w/w) crude oil contamination; SS$_{4B}$: Oba-Ile soil with 2% (w/w) crude oil contamination; SS$_{4C}$: Oba-Ile soil with 3% (w/w) crude oil contamination; SS$_{4D}$: Oba-Ile soil with 4% (w/w) crude oil contamination

Table 1(c). Effects of varying concentrations of crude oil on the crude oil degrading bacterial counts ($\times 10^5$ cfu/g) of Ido-Ani soil sample

Time (Days)	SS$_5$	SS$_{5A}$	SS$_{5B}$	SS$_{5C}$	SS$_{5D}$
0	0.15 ± 0.01^a	9.40 ± 1.15^f	4.40 ± 1.53^c	5.70 ± 0.12^d	8.37 ± 0.09^c
30	2.00 ± 1.73^b	2.83 ± 0.12^b	3.47 ± 1.45^b	3.10 ± 0.00^a	5.83 ± 0.60^b
60	2.40 ± 1.15^b	6.43 ± 1.20^c	4.27 ± 1.45^c	4.47 ± 0.12^c	4.07 ± 0.07^a
90	2.63 ± 1.45^c	7.07 ± 0.67^d	4.23 ± 1.45^c	3.90 ± 0.12^b	3.80 ± 0.12^a
120	3.13 ± 1.45^c	8.20 ± 0.12^e	1.52 ± 0.64^a	2.97 ± 0.15^a	4.27 ± 0.15^a
150	3.40 ± 0.12^c	1.77 ± 0.07^a	6.53 ± 0.12^d	2.87 ± 0.15^a	4.13 ± 0.09^a

Legend: SS$_5$: Ido- Ani soil without crude oil contamination (Control); SS$_{5A}$: Ido- Ani soil with 1% (w/w) crude oil contamination; SS$_{5B}$: Ido- Ani soil with 2% (w/w) crude oil contamination; SS$_{5C}$: Ido- Ani soil with 3% (w/w) crude oil contamination; SS$_{5D}$: Ido- Ani soil with 4% (w/w) crude oil contamination

Table 2(a). Effects of varying concentrations of crude oil on the crude oil degrading fungal counts (x10^4 sfu/g) of Igodan- Lisa soil sample

Time (Days)	SS$_3$	SS$_{3A}$	SS$_{3B}$	SS$_{3C}$	SS$_{3D}$
0	1.33 ± 0.33[a]	4.33 ± 0.33[c]	2.77 ± 0.39[b]	4.33 ± 0.33[b]	1.07 ± 0.07[a]
30	3.50± 0.29[d]	1.67 ± 0.33[a]	1.83 ± 0.17[a]	2.33 ± 0.33[a]	3.43 ± 0.30[b]
60	4.33 ± 0.33[e]	3.33± 0.33[b]	4.50 ± 0.29[d]	3.83 ± 0.44[b]	3.33 ± 0.35[b]
90	3.67± 0.67[d]	5.50± 0.29[d]	7.53 ± 0.29[e]	5.20 ± 0.12[c]	3.27 ± 0.15[b]
120	1.50± 0.28[b]	2.50± 0.29[a]	8.53 ± 0.29[f]	7.87 ± 0.13[d]	5.60± 0.30[d]
150	3.33± 0.33[c]	4.33 ± 0.33[c]	4.13± 0.09[c]	4.43± 0.30[b]	5.33 ± 0.67[c]

Legend: SS$_3$: Igodan- Lisa soil without crude oil contamination (Control); SS$_{3A}$: Igodan- Lisa soil with 1% (w/w) crude oil contamination; SS$_{3B}$: Igodan- Lisa soil with 2% (w/w) crude oil contamination; SS$_{3C}$: Igodan- Lisa soil with 3% (w/w) crude oil contamination; SS$_{3D}$: Igodan- Lisa soil with 4% (w/w) crude oil contamination

Table 2(b). Effects of varying concentrations of crude oil on the crude oil degrading fungal counts (x10^4 fu/g) of Oba-Ile soil sample

Time (Days)	SS$_4$	SS$_{4A}$	SS$_{4B}$	SS$_{4C}$	SS$_{4D}$
0	0.12 ± 0.02[a]	30.00 ± 0.12[b]	17.67 ± 0.15[c]	1.20 ± 0.12[a]	6.00± 0.59[a]
30	5.33 ± 0.33[e]	12.67 ± 0.15[a]	10.67 ± 0.88[a]	12.33 ± 0.17[c]	10.67 ± 0.67[b]
60	2.53 ± 0.12[c]	46.67 ± 0.15[d]	29.67 ± 1.45[d]	15.00 ± 0.29[d]	15.67 ± 0.18[c]
90	3.67 ± 0.33[d]	80.67 ± 0.07[e]	65.00 ± 0.28[e]	35.33± 0.29[e]	6.60 ± 0.64[a]
120	4.10± 0.10[d]	86.67 ± 0.33[f]	13.00 ± 2.31[b]	2.10 ± 0.59[b]	31.33 ± 0.09[e]
150	2.33 ± 0.33[b]	40.00 ± 0.58[c]	66.00 ± 0.31[f]	1.90 ± 0.06[b]	18.00± 1.25[d]

Legend: SS$_4$: Oba-Ile soil without crude oil contamination (Control); SS$_{4A}$: Oba-Ile soil with 1% (w/w) crude oil contamination; SS$_{4B}$: Oba-Ile soil with 2% (w/w) crude oil contamination; SS$_{4C}$: Oba-Ile soil with 3% (w/w) crude oil contamination; SS$_{4D}$: Oba-Ile soil with 4% (w/w) crude oil contamination

Table 2(c). Effects of varying concentration of crude oil on the crude oil degrading fungal counts (x10^4 sfu/g) of Ido-Ani soil sample

Time (Days)	SS$_5$	SS$_{5A}$	SS$_{5B}$	SS$_{5C}$	SS$_{5D}$
0	32.33 ± 1.45[d]	42.67 ± 1.45[e]	35.67 ± 4.26[b]	3.50 ± 0.29[a]	35.00 ± 2.89[c]
30	13.00 ± 1.15[b]	24.33 ± 1.20[d]	9.67 ± 0.33[a]	35.00 ± 2.89[d]	36.00 ± 6.11[c]
60	11.67 ± 1.20[a]	5.50 ± 0.29[a]	12.33 ± 0.67[a]	25.00 ± 2.89[c]	45.33 ± 1.76[d]
90	51.67 ± 1.67[e]	6.27 ± 0.15[b]	32.67 ± 1.45[b]	42.33 ± 1.45[e]	27.00 ± 1.76[b]
120	13.67 ± 1.45[c]	6.50 ± 0.29[b]	39.00 ± 1.00[c]	2.83 ± 0.44[a]	20.33 ± 4.6[a]
150	11.67 ± 0.67[a]	10.67 ± 0.33[c]	60.03 ± 1.76[d]	5.00 ± 0.58[b]	19.00 ± 1.15[a]

Legend: SS$_5$: Ido- Ani soil without crude oil contamination (Control); SS$_{5A}$: Ido- Ani soil with 1% (w/w) crude oil contamination; SS$_{5B}$: Ido- Ani soil with 2% (w/w) crude oil contamination; SS$_{5C}$: Ido- Ani soil with 3% (w/w) crude oil contamination; SS$_{5D}$: Ido- Ani soil with 4% (w/w) crude oil contamination

4. DISCUSSION

One of the considerations critical for returning environmentally polluted soils to it pristine state is a thorough knowledge of the impact of oil pollution on the technological parameters for its elimination. In this study, the effect of varying concentrations of crude oil on the population of crude oil degrading microbes of soil was evaluated. Crude oil contamination of the three arable experimental agricultural soil samples of Igodan-Lisa, Oba- Ile and Ido- Ani at 1-4% (w/w) caused alterations in the bacterial and fungal counts of the soils. This finding is in line with those of Ijah and Antai [19] that oil spills cause alterations in microbiological properties of the soil.

The findings in this research revealed that the ecosystem from which samples were collected

for this study harbour considerable numbers of crude oil degrading bacteria and fungi. The counts of petroleum hydrocarbon degrading microbes in samples is suggestive of previous exposure to crude oil or other forms of hydrocarbon as a result of various anthropogenic activities which undoubtedly boosts the supply of carbon in the soils hence favours the growth and multiplication of these microbes. This assertion corroborates the report of Atlas [20] that the microbial populations of hydrocarbon degrading microorganisms in an ecosystem quantitatively reflect the degree or extent of exposure of that ecosystem to hydrocarbon contamination.

Result showed that there was a gradual increase in the population of both bacteria and fungi showing response to moderate levels of crude oil addition. The populations of crude oil degrading microbes were higher in polluted than unpolluted soils. Microbial population varied with increase in the amount of crude oil spilled and contact time. The changes in microbial counts may be the presence of crude oil which boosts the carbon supply in the soils, hence favour the growth of these organisms or due to changes in the physico-chemical properties of the soils especially the provision of carbon and nitrogen. The increased microbial counts suggestively represented an immediate response to the added organic carbon present in petroleum hydrocarbon which acted as additional carbon substrate for microbial growth, activity and multiplication. This finding supports the reports of Linkins et al. [21] and Ekpo and Ebeagwu [18] that microbial population show rapid increase in response to moderate level of oil with a more delayed increase to high level of oil. Prolonged contact with crude oil resulted in an unstable rise and fall in microbial population. This implies that prolonged contact of microorganisms with petroleum hydrocarbon may have deleterious effects on microbial population at any given concentration. This unstable trend of microbial population may be attributed to toxic components of petroleum or other metabolic products which selectively inhibit microorganisms resulting in a shift in population size and species diversity within the microbial community. Odu [22] also noted that this unstable trend in microbial population may also arise from selective destruction of aerobic microorganisms leaving the resistant and adaptive microbial strains to multiply. It has been reported that population levels of hydrocarbon utilizers and their population within the microbial community appear to be a sensitive index of environmental exposure to hydrocarbon [23]. The findings from this study therefore revealed that crude oil contamination of soils does not negatively impact on soil micro flora at moderate levels of contamination (1-4%) as microbial counts at the end of the study period were even at 4% higher than the control. This finding however deviates from the assertion of Osuji et al. [24] that beyond 3% concentration, oil has increasing deleterious effects on soil biota. Also, the highest counts of bacteria and fungi were obtained at days 60- 90 and 90-120 of contact for bacteria and fungi respectively.

5. CONCLUSION AND RECOMMENDATION

This study concludes that the microbiological status of the soil samples were not negatively impacted at 1-4% crude oil contamination. The population of both bacteria and fungi were significantly increased at the end of experiment at 1-4% w/w crude oil addition. Also, this study asserts that the effect of crude oil on soil micro biota is a function of both concentration and contact time. The occurrence of these bacteria and fungi with capacity to actively metabolize crude oil is indicative of potential use of indigenous microorganisms in the cleanup of hydrocarbon polluted sites.

Apart from the type and population of indigenous microorganisms in soil, other technological parameters critical for the application of bioremediation strategy in the elimination of hydrocarbon pollutant in soil are environmental factors, soil type, nutrients, chemical composition, concentration and physical state of the pollutant. Therefore, future study should investigate the effect of crude oil concentrations on soil physicochemical properties such as those that directly affect the growth, survival and multiplication of microorganisms in order to optimize their efficiency in bioremediation.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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