Genotoxicity and Environmental Implications of Crude Oil-related Pollutants in Nigeria

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Review Paper

ABSTRACT

This paper reviews the mechanistic and genetic effects of spilt crude oil and associated drill site chemicals on some Nigerian plants. Methods that range from morphological and physical observations, physicochemical, anatomical, cuticular to cytological and cytogenetic analyses were reviewed. Quality data on various negative consequences of exposure of plants to pollution and contamination by crude oil-related pollutants were x-rayed. Proper legislation, monitoring and enforcement of sound environmental Laws by stakeholders have been advised; Government collaboration with research institutions in order to keep abreast with new research findings was also suggested as ways to help strengthen, manage and reinforce our environmental Laws in order to protect our sources of food.

Keywords: Crude oil, pollution, plants, cytogenetics, environment.

1. INTRODUCTION

Nigeria is a country endowed with many natural mineral resources; one of these resources is crude oil. In the bid to harness this resource, a large extent of the environment has been negatively impacted due to poorly managed operational activities of the oil and gas industries. To examine and ameliorate this impact, the World Bank and other organisations have recommended Strategic Environmental Assessment (SEA) [1] in the oil exploration and exploitation locations. This programme helps to show the number of genotoxic substances released into the environment and also measures the devastation done to the environment.

Over the years, drilling operations have introduced oil and a wide range of other complex chemical compounds into the environment through drilling fluids and muds [1]. Oil exploration exercises generally have the tendency to pollute the environment through accidental discharges of chemicals, oil spillages and indiscriminate disposal of formation water. As these fluids find their way into the environment the physical, chemical and microbiological properties of the soil are affected [2].

Genotoxic effects of chemicals using the plant as bioassay have gained attention over the years because of high industrialization and pollution by different professional bodies; this raised environmental and health concerns because of the genotoxic transfer from plants to humans by virtue of our food chain. To this effect, scientists around the globe have also geared their efforts towards ensuring environmental protection in order to support the sustainability of the earth. The environment is a life-supporting system and comprises the air, water, soil, plants, animals, micro-biota and humans living and interacting with each other. Introduction of any form of a pollutant into the environment will automatically and detrimentally affect its biophysical (soil property, aquatic ecosystem, biodiversity [livestock, wildlife and plant life]) and socio-economic components (such as health, income), and ultimately diminish the livelihood of the populace [3,4,5,6].

Consequently, different genotoxicity assays involving whole organisms, mammalian cells and other biological entities have been developed over time but, plant bioassay has been proven to have the highest sensitivity [7,8,9,10,11,12]. Genotoxic substances induce xenobiotic responses in the genetic system of an organism, thereby altering the molecular modes of action and the genetic framework of the organism. Several chemicals proven to have genotoxic abilities include; maleic acid hydrazine and N-methyl-N-nitroso-urea [13], boron [14], methyl tertiary-butyl ether [15]. Some metals also have genotoxic qualities, examples are; hexavalent chromium (Cr[VI]) [16], mercury (Hg), arsenic (As) and lead (Pb) [17], lead (Pb) and zinc (Zn) [18,19]. Moreover, industrial effluents [20,21], oilfield wastewater [7], crude oil [22,23], gasoline and diesel [24] and some medicinal plants [25,26, 27] have been reported to have genotoxic effects on plants.

Juchimiuk *et al.*[13], reiterated that the mutagens: maleic acid hydrazine and N-methyl-N-nitroso-urea have genotoxic effects on both plant and human cell nuclei. The expressivity of genotoxic attributes in plants, therefore, means that humans are in danger of primary and/or secondary exposure to products of mutation(s) if polluted plants are consumed for a long time. To this end, the expression of this mutation in humans has been reported to present itself in the form of cancer, neurological disorders and, malfunction of the kidney, liver and the central nervous system [28,19]. The relationship between human activities and the polluted environment has been strongly recognized hence careful handling of chemicals and environmental monitoring has been advocated [7,29,30].

A clearer understanding of the impact of crude oil-related pollutants on the environment could be achieved through an extensive look at various levels of the functional domains of exposed plants. Such domains could be cellular, tissue, organic or an entire organism. The level and location of effect determine the methodology or approach used to gain insight into the location and extent of the effect.

This article is aimed at reviewing the genotoxic effects and environmental implications of oil-related pollutants on some Nigerian plants and ecosystems.

2. METHODOLOGY

Different methods are adopted for genotoxicity testing using plants and soil as presented in Table 1. Each study followed an approach that gave access to the cytological or organic site of accumulation or action of the test pollutant(s).

Table 1. Overview of different genotoxic studies using plants and soil bioassay

Study parameter	Method employed	References
Oilfield wastewater on onion	Cytology	[7]
Spent lubrication oil on groundnut	Physico-chemical analysis and plant morphology	[31]
Crude oil on beans	Cytology and physicochemical analysis	[32]
Spent engine oil on maize and soil	Physico-chemical analyses on plant and soil samples	[9]
Diesel fuel and spent lubricating oil on maize	Plant morphology	[33]

Hexane on beans and soybeans	Plant morphology	[34]
Spent engine oil on beans	Plant morphology	[35]
Diesel and gasoline on bitter leaf	Cytology and plant morphology	[36]
Spent engine oil on groundnut	Plant morphology	[37]
Spent motor oil on groundnut	Physico-chemical analysis and plant morphology	[38]
Crude oil on *Jatropha curcas*	Physico-chemical and heavy metal analyses	[39]
Crude oil on maize and beans	Plant morphology	[22]
Crude oil on okra	Plant morphology and physicochemical analysis	[23]
Crude oil on the chilli pepper	Plant morphology	[12]
Spent engine oil and unused engine oil on beans	Plant morphology	[6]
Potassium chromate and sodium azide on cocoyam	Plant anatomy	[40]
Oilfield chemicals on cocoyam	Plant epidermis	[2]
Potassium chromate on beans	Plant morphology, epidermis and anatomy	[41]

3. RESULTS AND DISCUSSION

3.1 Plant morphological examination

This is the study of the physical attributes of plants, which includes plant height, leaf area, the shape of leaves, girth size, root length, fruit size and so on. Morphological assessments are very crucial when studying the effects of pollutants on plants, as morphological variations are among the first visible signs observed in plants under a polluted environment. Consequently, morphological assessments have been used by many researchers to characterize the effects of chemicals on plants [42,9,33,6,1,43].

3.1.1 Growth inhibition and stimulation

Growth inhibition is the suppression of plant height, it can occur in the form of reduced root growth, reduced root number, stunted shoots, reduced leaf number and size. Petroleum-related substances have been shown to significantly reduce seed germination, the emergence of seedling, growth and seed production of plants. Olayinka and Arinde [37], reported suppressed growth of groundnut plants grown in spent engine oil compared with the control (Table 2). They also showed that there was an increase in growth suppression with an increased concentration of the spent engine oil.

Similar features have also been reported by Odeigah *et al*. [7] with oilfield wastewater, Ogbemudia *et al*. [34] with whole and water saturated fractions of hydrocarbons. Nwakanma *et al*. [36] with diesel and gasoline, Olayinka and Arinde [37] with spent engine oil, Iyaba and Offor [22] and Osawaru *et al*., [23] with crude oil.

Table 2. Effects of spent engine oil on plant height, number of leaves, leaf area and stem girth of *Arachis hypogea* (Groundnut) [Source: Olayinka and Arinde, 2012]

Treatment (mL)	Plant height (cm)	No of leaves plant^{-1}	Stem girth (cm)	Leaf area (cm^2)
0	14.40	37.60	1.420	294.70

25	12.30	26.20	1.150	116.30
50	9.60	24.80	1.090	73.20
75	7.90	19.70	0.930	56.40
100	5.20	12.40	0.850	28.60

Growth inhibition in plants grown in an oil-polluted environment not only affects the morphology of such plant but also their chlorophyll content. Nwakanma *et al*. [36] reported low chlorophyll contents in plants growing in a polluted environment when compared to their controls; the low chlorophyll and mineral contents of plants growing in polluted environments explain why they have retarded growth. The pollution in the environment makes it difficult for plants to uptake basic mineral nutrients that aid in their metabolic activities hence, inhibition of photosynthesis and respiration abound [31].

Malformation of leaves, change in the number of leaves per plant stand as well as death are some of the effects of oil-related pollutants on plants. These characters were reported by Adu *et al*., [6] with spent and unspent engine oil on *Vigna unguiculata*, Ajah and Obute [41] with potassium chromate (oilfield chemical) on *Vigna unguiculata* and Ajah and Osuji [44] with sodium thiosulphate and graphite powder (oilfield chemicals) on *Colocasia esculenta* and *Xanthosoma maffafa*. Ajah and Obute [41] reckoned that malformed leaves are caused by chromosome breakage, reduction in the auxin level, changes in enzyme activities and variation in ascorbic acid concentration. Change in the number of leaves per plant has been attributed to the induction of metabolic disorder by the pollutants [9], which cause the inability of the seedlings to absorb water due to the change in the physical and chemical structures of the soil [39]. Plant death is opined to be caused by the destruction of microbes present in the soil due to insufficient aeration following a decrease in the air-filled pore space [38] by the oil-related pollutants.

Growth stimulation is the expression of more vigorous vegetative features in plants under the polluted condition when compared to plants in an unpolluted environment. Treated plants with taller heights, increased leaf area and increased yield, when compared with their respective controls had been reported by Anoliefo and Vwioko [42] with *Capsicum annum,* Bamidele and Sijuade [45] with *Colocasia esculenta* and, Eze and Dambo [46] with *Zea mays.* This phenomenon poses great danger and health concerns because it is believed that some traces of these pollutants are taken up by these plants; when these polluting plants are being consumed by animals and man, there would be a genotoxic transfer and this would eventually lead to increased incidences of terminal illnesses.

3.2 Plant cytological examination

Cytology is the study of cells [47] especially with reference to its sub-cellular structures [48]. Cytogenetics deals with genetic events within the cell, which is the basic building block of living organisms [49]. Cytogenetic studies provide information about the genetic architecture of plants, including the physical location of genes on chromosomes [50,51]. Chromosomal characters are important because chromosomes contain genetic information expressed in the phenotype [52]. Cytology and cytogenetic studies have proved invaluable in characterizing the mutagenic effects of chemicals on plants [36,18,15]. Cytological studies use the root tips of plants because the root system of plants houses the most vigorous cytological activity at the root tips, which are usually the first contact of any environmental pollutant [7]. To this end, scholars had used cytological examinations to study the cytotoxicity potentials of many chemical compounds [53,54].

3.2.1 Chromosomal aberration

Chromosomal aberration is a shift from the normal karyomorphological observations of the chromosome, particularly during different mitotic phases. The introduction of genotoxic substances poisons the spindle fibres causing mitodepressive (i.e. mitosis depressive) effects, decreased mitotic index, anti-mitotic activities, abnormal cell division, aberrant cells, nuclear lesions, anaphase bridges among others.

Odeigah *et al*. [7] on oilfield wastewater with *Allium cepa* reported decreased mitotic index that further decreased with increased concentration of the oilfield wastewater. Chromosomal aberrations were also reported to be significantly high; ranging from sticky chromosomes, vagrant chromosomes, multipolar anaphases to anaphase-telophase bridges with or without acentric fragments.

Related findings were reported by Nwakanma *et al*. [36] on *Vernonia amygdalina* with diesel and gasoline. They recorded decreased mitotic index, vagrant chromosomes, aberrant mitotic cells, chromosomal bridges and stickiness as well as decreased mitotic cells (Table 3) that decreased with increased concentration. In the same vein, Saikia *et al*. [16] studied the effects of hexavalent chromium on *Bacopa monnieri* and reported RNA damage. Furthermore, chromosomal aberration, decreased mitotic index and reduced fertility were also reported by Oladele *et al*. [18] in *Vigna subterranean* treated with lead and zinc. Similarly, Ismail and Morsi [15] reported chromosomal aberration, decreased mitotic index and anti-mitotic activities in *Vicia faba* using Methyl Tert-butyl Ether as a test agent.

The cytotoxic effects of oilfield chemicals on plant roots imply that similar fate awaits humans and animals because of our food chain link. It is therefore important that we guard against the accidental or intentional release of chemical mutagens into the environment because their introduction will affect the physical, chemical and soil microbial properties. This will eventually lead to the buildup of essential, non-essential elements and heavy metals in the soil which are taken up and translocated to different plant tissues [9].

3.3 Plant cuticular examination

This is the study of the epidermal architecture of plants. This study involves the use of the epidermal tissues of the leaves to study plant behaviour. Cuticular studies are important because they yield critical information about stomata, trichomes and epidermal cells of a plant [55]. Cuticular examinations of plants are very important when investigating the effects of pollutants on plant species because they are sensitive to any shift in the environment [41,56]. Morphological variations are among the first signs observed in plants under polluted conditions but, studies have shown that some plants that grow in the polluted environment do not show any morphological defects and may even have more vegetative growth than plants in unpolluted environments [35,57]. Based on this trend, many scholars resorted to using epidermal and anatomical characters of plants for environmental monitoring [58,59,60,61,62] due to their conservative potentials. The reason for this is that such characters are considered as bioindicators of environmental quality [63] and are believed to be of much value at the levels where other classical methods cannot be applied [64].

3.3.1 Epidermal modification

Epidermal characters are of high taxonomic value and are often altered when plants are assaulted with chemicals. Distortions in the sizes and numbers of stomata and trichomes affect the morphology, respiratory and fertility responses in plants. Consequently, Omosun *et al*. [4], on the effects of crude oil on *Amaranthus hybridus* reported a shift from the isodiametric shape of the epidermal cells of the control plant to isodiametric polygonal to irregular shapes of the treated plants. They, however, reported irregularity in the shape of the epidermal cells which worsened with further increase of crude oil concentration. Additionally, decrease in stomatal index and change in the nature of the anticlinal walls from straight to curved and to sinuous were some of the variations they reported, these variations become pronounced with a higher concentration of the pollutant.

Table 3. Mitotic effects of diesel and gasoline polluted soil on the root tips of *Vernonia amygdalina* (Bitter leaf) [Source: Nwakanma *et al.*, 2011]

Conc. (mL)	No of cells	No of dividing cells	No of dividing cells P	M	A	T	Stickiness	C-mitosis	Bridges and Fragments	Vagrant	Binucleus	Mitotic index (%)
Control	500	37	6	13	7	11	0	0	0	0	0	7.40
5 (p)	335	10	2	3	2	3	1	0	1	5	0	2.99
10 (p)	319	10	2	4	2	2	2	0	2	2	0	3.13
15 (p)	291	12	0	5	3	4	4	0	3	3	2	4.12
20 (p)	265	9	0	3	2	4	3	0	2	3	0	3.40
25 (p)	213	7	0	2	3	2	2	0	2	2	0	3.29
5 (d)	317	10	1	3	3	3	3	0	2	3	1	3.15
10 (d)	313	8	1	3	2	2	1	0	2	3	0	2.56
15 (d)	275	8	0	3	2	3	2	0	2	2	0	2.91
20 (d)	251	5	0	2	1	2	1	0	2	2	0	1.99
25 (d)	207	5	0	3	1	1	1	0	2	2	0	2.42

Keys: [(p) = petrol, (d) = diesel, P = prophase, M = metaphase, A = anaphase, T = telophase]

Reduced stomatal index and frequency due to pollution was also reported by Ajah and Obute [41] with *Vigna unguiculata*; they adduced that the reduction in the number of stomata in the polluted plant means reduced gaseous exchange hence, reduced photosynthetic activities; thus, the degree of sensitivity and fertility of plant species depend on its nutritional status and environmental factors [65].

Change in the nature of the anticlinal wall was also reported by Ajah *et al.* [56] with potassium chromate treatment on the abaxial epidermis of *Xanthosoma maffafa* (Plate 1). This trend means that the pollutant exerted pressure on the stomata of the plant in the course of its development and this also correlates to the fertility potential of the affected plants. Generally, the ability of an introduced substance into the environment to change the natural architecture of plants means that such substance is mutagenic hence, proper handling of such substance should be emphasized.

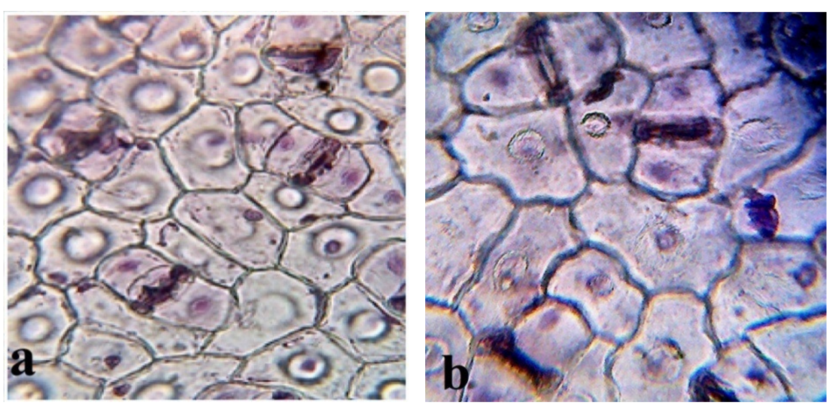

Plate 1. Nature of the anticlinal walls of the abaxial epidermis of *Xanthosoma maffafa*: a) Straight epidermal walls of the control b) Wavy epidermal walls of the treated plant [Source: Ajah *et al.*, 2017c]

3.4 Plant anatomical examination

This is the study of the internal structures of plants. Anatomical characters have proved relevant in the recognition and identification of species [40,44]. Anatomical features are known to be very reliable in determining the effects of pollutants in plants, as the anatomical features are rarely affected by environmental factors [52]. The use of anatomical characters of plants for environmental monitoring has been carried out with *Cynodon dactylon* [58], *Gazania harlequin* [66], *Abutilon indicum, Croton sparsiflorus*, *Cassia occidentalis* [62], *Colocasia esculenta* and *Xanthosoma maffafa* [40,44].

Anatomical examinations of plant species are considered highly informative and of taxonomic value because of the conservative value of internal features. Anatomical changes in plants are variable and differ from species to species. Leaf anatomical features have been observed to be very informative about the physiological conditions of plants under adverse conditions. Dias *et al.* [67], believed that the anatomy of plants contribute to our understanding of the physiological and structural changes they undergo thereby, providing information on how they adapt to their dynamic environment.

3.4.1 Anatomical distortion

This is the modification of the internal structures of plants. Saikia *et al.* [16] working with hexavalent chromium reported different alterations in the cortical and parenchyma tissues of *Bacopa monnieri*. They observed intracellular accumulation of Cr (VI) in the cortical tissues, hypertrophied cortical cells and formation of dark-ringed structures within the cortical cells and intercellular locations. A strong reduction of the meristematic activity in the hypertrophy of cortical parenchyma was also reported. In the same vein, Omosun *et al*. [4] on the effects of crude oil on *Amaranthus hybridus* reported reduced parenchyma cells of the stem with reduced intercellular air spaces that further reduced with increased concentration of crude oil. The cortical cells of the roots and stems of *A. hybridus* were also reported to be flattened tangentially and smaller in size when compared with the control. The parenchyma cells of both the stem and roots were also reportedly smaller in size when compared with the control. This trend further worsened with increased concentration of crude oil.

Furthermore, Ajah and Osuji [40] on the effects of sodium azide and potassium chromate on *C. esculenta* and *X. maffafa* reported various changes in the treated plants; these changes include distortions in the shape of the epidermal cells, thickness of the cuticle, presence of oxalate crystals, shape of vascular bundles, absence of medullary rays, occurrence of Siamese (contiguous) vascular bundles and vascular bundles with connecting tissues as well as change in the number of vascular bundles in the treated accessions when compared with their various controls. According to the study, plants exposed to these chemicals tend to have accelerated photosynthetic and growth abilities because of the change from barrel-shaped epidermal cells to convexly shaped cells. Convex shaped epidermal cells have been known to enhance the ability of leaf to capture light energy which promotes photosynthetic capacity in plants [65]. The treated plants were also reported to have increased defensive mechanism against herbivores and pathogenic attacks more than the controls because of their thickened cuticles and a higher number of oxalate crystals. The thickness of the cuticle helps in building plant's defensive mechanisms against herbivores and pathogenic attacks [68] while oxalate crystals help plants ward off herbivores [49,69].

The report of Ajah and Osuji [40] is worrisome as polluted plants tend to have more desirable qualities than the controls; this means that humans are exposed to the polluted food source and, prolonged consumption of such foods will lead to serious terminal diseases. To circumvent this, proper care must be taken to ensure that portions of land where cocoyam must be planted should undergo adequate evaluation so as to ensure the safety of our food sources.

3.5 Plant physicochemical examination

This can simply be said to be the quantitative/qualitative analysis of plant extracts; it involves the extraction, isolation, identification and characterization of an extract of a/some parts of a photosynthetic plant (leaves, stem, flowers, fruits, roots). Physico-chemical analyses are usually carried out before and after pollution, results are then compared to evaluate the presence of substances that were not present before pollution; they are known to be reliable in the genotoxic assessment of plants [9,23,31,32].

3.5.1 ***Presence of heavy metals***

Nkwocha *et al*. [70] investigated the impact of oil pollution on some plants and soil of some communities in Imo State; higher levels of heavy metals were reported in the impacted soil and plants when compared to their controls. The observation and report of the presence of lead, chromium, nickel and cadmium in the soil and leaves of pumpkin and cassava (Table 4) call for further and urgent toxicological studies in surrounding areas where oil exploration activities take place.

Agbogidi *et al.* [39] reported significant accumulation of heavy metals in the tissues of *Jatropha curcas* when compared to their controls; they believed that this accumulation is gradual but with time could rise to a dangerous or lethal level with their inherent health hazard. In the same vein, Ogbuehi *et al*. [71] reported that diesel oil contaminated soil significantly reduced the macro-element composition (nitrogen, phosphorus and potassium) of soybeans; the decrease in the macro-element contents of this plant was reported as a result of the inhibitory properties of diesel oil which consequently affected the quality of food material produced by this plant. Agbogidi *et al.* [39] stated that contamination of soil with heavy metals causes changes in the composition of soil microbial community and, other biological and biochemical properties of the soil thereby making the soil very acidic and unfit for plant propagation.

The presence of heavy metals in our food sources even at small doses is dangerous because continuous consumption of such polluted food materials may lead to heavy metal accumulation in the human system. Lead has been found to be a causative agent of cancer [28]. Long-time exposure to chromium VI can cause damage of kidney, liver and nerve tissues. Nickel has been shown to be injurious to human health [72], while the accumulation of cadmium is said to cause tabular dysfunction and disturbances in calcium homeostasis and metabolism [30]. Iron accumulation has also been linked to an increased risk of colorectal cancer [72].

Table 4. Average concentrations of heavy metals in the soils and leaves of plants in some communities in Imo State [Source: Nkwocha and Duru, 2010]

Sites	Parameter	Cadmium (mg/Kg)	Chromium (mg/Kg)	Nickel (mg/Kg)	Lead (mg/Kg)
Awara (polluted)	Cassava	0.16	0.57	0.03	0.11
	Pumpkin	0.07	0.70	0.04	0.22
	Soil	1.50	10.75	14.77	48.20
Awara (control)	Cassava	0.01	0.01	0.01	0.00
	Pumpkin	0.00	0.00	0.02	0.00
	Soil	0.10	1.50	2.03	5.21
Izomba (polluted)	Cassava	0.17	0.70	0.04	0.16
	Pumpkin	0.06	0.85	0.03	0.20

	Soil	1.98	9.81	14.80	46.73
Izomba (control)	Cassava	0.00	0.01	0.01	0.02
	Pumpkin	0.01	0.00	0.01	0.00
	Soil	0.08	1.69	1.62	5.28

4. CONCLUSION

The study looked at how crude oil-related pollutants negatively affect our environment especially plants, our major food source. In Nigeria, there seems to be negligence on the part of the government to protect this source of food. This has a direct link to the increased rate of terminal illnesses like cancer, diabetes, liver and kidney failures. This translates to heavy dependence on chemotherapy [cancer], dialysis and organ transplants [kidney] thus, paving way for so much medical tourism overseas; this also affects the life expectancy ratio in Nigeria. The government should, therefore, rise up to their responsibility and protect our environment and stop paying lip services to environmental issues. To this end, the authors wish to recommend that every farm produce must thoroughly be analyzed by appropriate agencies to ensure that they pass the safety test before they are released to the public. Therefore, the Ministry of Environment, as well as Environmental Protection Agencies of various States across Nigeria, must ensure that there is total compliance to environmental legislation and regulations. The government should also ensure that environmental and agricultural extension officers are trained on how to monitor and enforce the International Standard Organization (ISO) on Environmental management. The government should also continue to collaborate with research institutions in order to keep abreast with new research findings that would help in strengthening our environmental Laws, so as to protect our sources of food.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Author 'FOA' managed the literature searches and, wrote the first draft of the manuscript. All the authors participated fully in the production of this work; all read and approved the final manuscript.

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