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Phytoremediation Capacity Assessment of Common Tropical Vegetable (*Abelmoluscus esculentus*) on Crude Oil Impacted Soil

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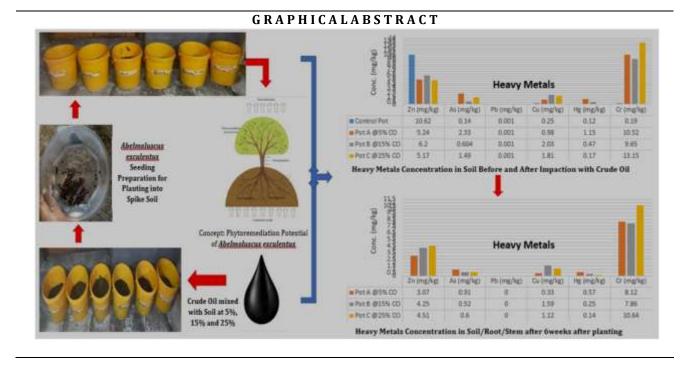
ABSTRACT

Environmental degradation due to crude oil pollution has several impacts, and various mechanisms have been adopted to clean up crude oil components in the environment. One such mechanism is the use of vegetables as a phytoremediation agent. The study investigated the phytoremediation capacity assessment of a common tropical vegetable (Abelmoluscus esculentus) on crude oil-impacted soil. Uptake response of Okra plant at different concentrations of crude oil (5%, 15% and 25%) in 4 kg using laboratory standard techniques, Bioaccumulation factor (BF) and Translocation factor (TF) ratio were analysed. Based on the outcome, the Okra plant showed a significant reduction in heavy metals (HM) concentration in the impacted soil. Hence, the plant showed heavy metals uptake capacity. At 5% crude oil concentration, the BFroot was >1 for all the heavy metals except Cr, while the TF showed that all HMs had <1 except for Hg (>1). For all other concentrations (15% and 25% crude oil), the BF showed <1, except for Cu of BFroot at 15%. Also, the TF showed <1 for other concentrations except Zn at 15%. The significant relationship between the soil and the root of the okra plant can be attributed to the closeness; as expected, the root draws its nutrients from the available soil. Okra (Abelmoschus esculentus) can clean up crude oil-contaminated soil as a costeffective and environmentally friendly method. The study recommended the prevention of crude oil spillage and further research to establish the most efficient plant period for better uptake capacity of the plant.



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Introduction

Soil contamination has become a global phenomenon as soil in many parts of the world has been contaminated by chemicals and heavy metals due to industrial and agricultural activities and unregulated waste disposal [1]. Global intensification of oil and gas activities comprising exploration, drilling, production, onshore storage, and petroleum transportation has increased the risk of spillage and leakage of crude oil into the environment [2]. Crude oil is a complex mixture of hydrocarbon and organic compounds such as benzene and polyaromatic hydrocarbons known to pose environmental and health hazards [3]. With rapid industrialisation and urbanisation, the abundance of heavy metals in the environment has increased enormously during the past decades, which raised significant concerns throughout the world [4-7]. Crude oil spillage is a common event in Nigeria, specifically in the Niger Delta region, leading to environmental pollution [8,9]. Soil oil spill contamination of the topsoil can render the soil unsuitable for plant growth by increasing the toxic contents of the soil [10] and exposing humans to various health risks [11]. The presence of heavy metal (HM) in soil due to crude oil impact affects soil fertility, and the non-degradability of the heavy metals implies a persistent presence in the soil over a long period [4, 10]. Therefore, it is necessary to take remediation measures to prevent heavy metals from entering into terrestrial, atmospheric and aquatic environments [6]. Various remediation approaches have been developed to reclaim heavy metal-contaminated soil. However, limitations have yet to be reported on these physicochemical approaches [12]. Therefore, there is a need to develop cost-effective, efficient environment-friendly and remediation technologies reclaim heavy metalto contaminated soil. Environmental restorations of metal-polluted soils using plant-based technology have attracted increasing interest in the last two decades [13, 14]. Phytoremediation has been developed as a cost-effective and environmentally friendly remediation method for contaminated soils [15]. It is an economically attractive approach to decontaminate soils polluted by heavy metals. Because of its

relatively low costs, phytoremediation poses a viable approach to cleaning up soils [13]. The phytoremediation capacity of a plant can be determined based on its bioaccumulation factor (BF) and translocation factor (TF) capacity [16]. The BF is calculated value that indicates the ability of plants to remove metal compounds from the soil/substrate. Meanwhile, the TF is a value that indicates the ability of the compound to be transferred from plant roots to other organs [17]. Plants with bioconcentration and translocation factors >1 can be used as bioaccumulators [18]. BF values > 2 are considered to be high values [17]. Plants can be used as phytostabilizers if they have BF >1 and TF <1 and as phytoextractors if they have bioconcentration factors < 1 and translocation factors >1 [18]. Several phytoremediators (plants/vegetables) have been studied in the last decades to determine their phytoremediation potential. For instance, the potential of Hopea adorate [19], Lycopersicon esculentum (tomato), Rumex acetosa (sorrel), and Solanum melongena (garden egg) [13], Acacia pycnantha and Eucalyptus camaldulensis [20], Ipomoea aquatica (water spinach) and Abelmoschus esculentus (okro) [21], Abelmoschus esculentus, Amaranthus dubius and Hibiscus sabdariffa L [22], Artemisia alba Turra [23], Tetraena qataranse [24], Telforia Occidentalis and Abelmoschus esculentus, Cv.

Kirikou [25], *Pteris vittata*, *Epipremnum aureum*, Mucuna bracteata and Imperata cylindrical [2], and Pisum sativum L. [26] have been examined under different spike conditions of several concentration of heavy metals. The present research intends to assess the phytoremediation potential of Abelmoschus esculentus (Okra) from crude oil-contaminated soil under different spike concentrations. **O**kra Abelmoschus esculentus an economically important is vegetable crop grown in tropical and subtropical parts of the world. This crop is suitable for garden cultivation, on large commercial farms and is grown commercially in Nigeria [27].

Method and Materials *Study area*

The soil sample for the study was collected from undisturbed land at Rumuagholu at the coordinate of longitude 4°52'39" N and latitude 6°58'28" E located within the Obio Akpor Local Government Area of Rivers State. The LGA is part of the state's metropolitan area and a major centre of economic activities housing several industries. The soil sample for the study was sourced from an area where the soil is still in its natural state with no noticeable anthropogenic impact. The soil sample will be collected randomly at a uniform depth of 15cm with a hand trowel packed in a bag. The soil sample was then appropriately mixed and passed through a 4 mm sieve to remove impurities.

Soil preparation and planting of seed

From the sieved soil, 12 kg of the soil was weighed and mixed with five (5) concentrations of crude oil (that is, 5%, 15%, and 25% crude concentration). The concentration in volume is highlighted in Table 1. The mixing was adequately done to ensure uniformity of the impacted soil. The experiment was carried out with four (4) pot samples; three (3) ports contained the impacted samples, while the fourth port was the control.

Okra (*Abelmoschus esculentus*) was used for the study from a reputable seedling outlet. The seedling was planted a week after the soil preparation and directly in the potted soil. All pots were watered twice daily (with a measured amount of 50 ml of water) by spraying to maintain sufficient soil moisture. The pots were placed in areas shaded from rain but with access to sunlight.

S/N	Sample ID	Height of soil	Volume of crude oil	Concentration of crude				
1.	Control soil pot	4000 g	-	-				
2.	Sample pot A	4000 g	12.7 ml	5 mg/Kg				
3.	Sample pot B	4000 g	38 ml	15 mg/Kg				
4.	Sample pot C	4000 g	63.4 ml	25 mg/Kg				

Table1. Sample details and concentration conversion for crude oil

Data analysis

Descriptive statistic was adopted for the study, and the findings were presented in tabulation. Aside from the tabular representation of findings, the study adopted the use of graphs and charts as a means to summarise and describe findings in a manner that made it easier to understand their attributes, differences and patterns.

Soil analysis

(1) *Control Soil (CS) Sample*: A sample of the soil without crude content was collected and analysed to represent the background value of the soil at its natural state. The CS analysis will include pH, Electrical Conductivity and heavy metals (Arsenic (As), Copper (Cu), Mercury (Hg), Zinc (Zn), Lead (Pb), and transition metal-Chromium (Cr)).

(2) *Impacted Soil*: Soil analysis was carried out on the impacted soil after six (6) weeks of planting. The soil was analysed for interested heavy metals.

Okra plant

Analysis of the okra plant (root and shoot/stem) for the extent of heavy metal uptake was carried out six (6) weeks after planting (AP). According to the United States Environmental Protection Agency (US EPA) [28], the heavy metals of interest are considered to have carcinogenic risk in humans. These heavy metals include As, Cu, Hg, Zn, Pb, and transition metal-Cr. The metals were determined following atomic absorption spectrophotometry as described by [29].

Bioaccumulation Factor (BF): BF is computed as heavy metal accumulated in each plant tissue to

that dissolved in the soil medium as shown below;

Root bioaccumulation factor is computed based on the Equation (1).

$$BF_{root} = \frac{C_{root}}{C_{soil}}$$
(1)

Shoot bioaccumulation factor is computed based on the Equation (2).

$$BF_{shoot} = \frac{C_{shoot}}{C_{soil}}$$
(2)

Translocation factor (TF)

TF is computed based on the Equation (3).

$$TF = \frac{BF_{shoot}}{BF_{root}}$$
(3)

Statistical analysis

The relationship between the crude oil concentration and Okra plant (root and stem) was tested through inferential statistics Pearson Product Momentum Correlation (PPMC) at 95% level of significant. As inferential statistics, PPMC allows for exploring the statistically significant differences between more than two sets of variances.

Results and Discussion

Physicochemical properties of crude oil concentration in soil samples

The physiochemical, heavy metals, and polycyclic aromatic hydrocarbons (PAHs) concentrations before and after impaction with crude oil of the soil samples were presented in Table 2. The pH of the control pot was 7.64, indicating its alkaline nature. However, the pH decreased and moved towards acidic as the

crude concentration increased from 5% (7.45) 15% concentration (7.19) and 25% to concentration (6.08) which is acidic in nature. This indicates the impact of crude in the soil. This showed similarity with the study conducted by [30], where the pH of the soil studied became acidic (5.6) due to the oil spill's impact. [1] reported a similar finding in their study, where the pH was acidic (4.80) due to crude oil contamination of soil. The EC indicated that Pot C (6.2 μ s/cm) has the lowest EC followed by Pot B (49.3 μ s/cm) while Pot A has the highest EC (188 μ s/cm) among the impacted pots while the control pot has an EC of 16.1 μ s/cm. The high EC indicates high ions (salt), which can be attributed to the impact of crude oil [31]. The THC of the soil showed that Pot C has 2256110 mg/kg THC, while the control pot has the least THC of 0.02. The outcome indicated that the crude oil concentration influences the amount of THC in the soil. The outcome is similar to that of [25], where there was a sudden increase in the THC of their sample due to crude oil impact. Similarly, the total PAHs of the soil showed a sudden increase with an increase in the crude oil concentration of the soil, with pot C of 25%

concentration having the highest total PAHs with the control pot with the least. This can also be attributed to the crude oil impaction on the soil. Concerning the heavy metals of the soil, the Zn concentration of the control pot has the highest concentration of 10.62mg/kg, while the lowest was pot C with 25% crude concentration. This outcome could be attributed to the natural phenomenon around the soil area, which could have influenced the Zn concentration of the soil. The As of the soil showed an increase from the control pot of 0.14 mg/kg of As to 2.33 mg/kg found in pot A. All the soil samples maintained a Pb concentration of 0.001 mg/kg across all the pots.

The Cu of the soil showed an increase from the control pot of 0.25 mg/kg of Cu to 2.03 mg/kg found in pot B. The Hg of the soil showed an increase from the control pot of 0.12 mg/kg of Hg to 0.47 mg/kg found in pot B. The Cr of the soil showed an increase from the control pot of 0.19 mg/kg of As to 13.15mg/kg found in pot C. A similar study was conducted by [21,25], where there was an influence on the concentration level of the heavy metals in the soil after being impacted with crude oil.

Table2. Soil properties concentration before and after impaction with crude oil								
Parameters	Control Pot	Pot A @5% CO	Pot B @15% CO	Pot C @25% CO				
рН	7.64	7.45	7.19	6.08				
EC (µs/cm)	16.1	188	49.3	6.2				
THC (mg/kg)	0.02	80.424	423400	2256110				
Total PAHs(mg/kg)	<0.01	3.45	3.43	19.96				
Zn (mg/kg)	10.62	5.24	6.2	5.17				
As (mg/kg)	0.14	2.33	0.604	1.49				
Pb (mg/kg)	0.001	0.001	0.001	0.001				
Cu (mg/kg)	0.25	0.98	2.03	1.81				
Hg (mg/kg)	0.12	1.15	0.47	0.17				
Cr (mg/kg)	0.19	10.52	9.65	13.15				

Table? Soil properties concentration before and after impaction with crude oil

Determine the heavy metal concentration in the okra plant (soil, root, and stem)

After planting (6 weeks), each pot was tested for the heavy metal concentration in the soil, root and stem (consisting of parts of the plant above the soil) and the outcome was presented in Table 3. The pot A of 5% concentration indicated that after six weeks of planting, the concentration of Zn was 3.07mg/kg (from 5.24mg/kg before planting), the concentration of As was 0.91 mg/kg (from 2.33 mg/kg before planting), the concentration of Cu was 0.33 mg/kg (from 0.98 mg/kg before planting), the Hg concentration was 0.57 mg/kg (from 1.15 mg/kg before planting) while the concentration of Cr was 8.12 mg/kg (from 10.52 mg/kg before planting).

This implies the Zn, As, Cu, Hg, and Cr had a reduction in concentration by 2.17 mg/kg, 1.42 mg/kg, 0.65 mg/kg, 0.58 mg/kg, and 2.4 mg/kg, respectively. The pot B of 15% concentration indicated that after six weeks of planting, the Zn concentration was 4.25 mg/kg (from 6.2 mg/kg before planting), the As concentration was 0.52 mg/kg (from 0.604 mg/kg before planting), the Cu concentration was 1.59 mg/kg (from 2.03 mg/kg before planting), the Hg concentration was 0.25 mg/kg (from 0.47 mg/kg before planting) while the Cr concentration was 8.12 mg/kg (from 10.52 mg/kg before planting). This implies the Zn, As, Cu, Hg, and Cr had a reduction in concentration by 1.95 mg/kg, 0.084 mg/kg, 0.44 mg/kg, 0.22 mg/kg, and 1.79 mg/kg, respectively.

Table3. Heavy metal concentration in the Okra plant (soil, root, and stem) after planting

		6Weeks	6 AP @5	%	6	Weeks	AP @ 15	%	6	Weeks	AP @ 25	5%
HM	Soil	Root	Stem	Total	Soil	Root	Stem	Total	Soil	Root	Stem	Total
Zn	0.75	1.51	0.81	3.07	2.06	0.97	1.22	4.25	2.65	1.24	0.62	4.51
As	0.33	0.53	0.05	0.91	0.48	0.04	0.001	0.52	0.34	0.21	0.05	0.60
Pb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cu	0.09	0.24	0.001	0.33	0.14	0.84	0.61	1.59	0.86	0.19	0.07	1.12
Hg	0.01	0.14	0.42	0.57	0.21	0.04	0.001	0.25	0.09	0.05	ND	0.14
Cr	4.62	2.54	0.96	8.12	5.21	1.81	0.84	7.86	8.04	1.64	1.01	10.64

Table4. Bioaccumulation and translocation factor of Okra for phytoremediation capacity

6Weeks AP @5%				6Weeks AP @ 15%			6Weeks AP @ 25%		
HM	BFroot	BFshoot	TF	BFroot	BFshoot	TF	BFroot	BFshoot	TF
Zn	2.01	1.08	0.53	0.47	0.59	1.26	0.47	0.23	0.49
As	1.61	0.15	0.09	0.08	0.002	0.03	0.62	0.15	0.24
Pb	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cu	2.67	0.01	0.004	6	4.36	0.73	0.22	0.08	0.36
Hg	14	42	3	0.19	0.005	0.03	0.56	-	-
Cr	0.55	0.21	0.38	0.35	0.16	0.46	0.20	0.13	0.65
	6We	eeks AP @5	5%	6Weeks AP @ 15%			6Weeks AP @ 25%		
HM	BFroot	BFshoot	TF	BFroot	BFshoot	TF	BFroot	BFshoot	TF
Zn	2.01	1.08	0.53	0.47	0.59	1.26	0.47	0.23	0.49
As	1.61	0.15	0.09	0.08	0.002	0.03	0.62	0.15	0.24
Pb	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cu	2.67	0.01	0.004	6	4.36	0.73	0.22	0.08	0.36
Hg	14	42	3	0.19	0.005	0.03	0.56	-	-

C	0 55	0.01	0.00	0.25	0.1.0	0.46	0.00	0.10	0.65
Cr	0.55	0.21	0.38	0.35	0.16	0.46	0.20	0.13	0.65

The pot C of 25% concentration indicated that after 6 weeks of planting, the concentration of Zn was 4.51 mg/kg (from 5.17 mg/kg before planting), the As concentration was 0.60 mg/kg (from 1.49 mg/kg before planting), the Cu concentration was 1.12 mg/kg (from 1.81 mg/kg before planting), the Hg concentration was 0.1 4mg/kg (from 0.17 mg/kg before planting) while the Cr concentration was 10.64 mg/kg (from 13.15 mg/kg before planting). This implies the Zn, As, Cu, Hg, and Cr had a reduction in concentration by 0.66 mg/kg, 0.89 mg/kg, 0.69 mg/kg, 0.03 mg/kg, and 1.23 mg/kg, respectively.

This form of a significant reduction in HM concentration was also reported in the study by [25]. Also, [22] reported a similar finding to Okra's capacity to significantly reduce the concentration of heavy metals in a spike/impacted soil.

Bioaccumulation and translocation factor of okra for phytoremediation capacity

Table 4 presented the phytoremediation capacity of the okra plant based on the bioaccumulation (BFroot and BFshoot) and translocation factor. The Okra's ability to transfer accumulated HMs through the soil to root and stem/shoot is based on the BF and TF. The TF is more specific about the movement of HMs from the root to the shoot/stems of the plant. All plants with BF and TF greater than one (>1) have potential for phytoremediation/phytoextraction capacity, although this capacity varies towards different HMs. Based on the outcome, the BFroot at 5% CO indicated that the Okra phytoremediation capacity descended as Hg< Cu< Zn <As< Cr, Hg<Zn<Cr<As<Cu for BFshoot while the TF showed that all HMs had <1 except for Hg. The BF showed <1 for all other concentrations except for Cu of BFroot at 15%. Furthermore, the TF showed <1 for other concentrations except Zn at 15%. The finding showed that Okra could be a good phytoremediation /phytoextraction agent at low concentrations, which was corroborated by [25].

According to Akpokodje and Uguru [25], some heavy metals at low doses are essential micronutrients for plants, but in higher doses, they may cause metabolic disorders and growth inhibition for most plant species. Palmroth et al. [32] reported that plant root exudates help degrade toxic organic chemicals and act as substrates for soil bacteria, improving the plants' phytoremediation potential. Atlas and Bartha [33] suggested that the interaction between plants and microorganisms is the primary mechanism responsible for petrochemical degradation in phytoremediation efforts. In addition, [24] noted that the accumulation of heavy metals in plants is highly complex as various biotic and abiotic factors influence the mechanisms likelv of phytoremediation.

Correlation analysis of crude oil concentration and okra plant (root and stem)

From Table 5, the possible relationship between the concentration and the Okra plant and the extent of the relationship was determined based on Pearson correlation (r) and using the decision rule ($p \le 0.05$, Ho *Rejected* and p > 0.05, Ho Accepted). For Crude oil 5% concentration, there was a significant relationship between the concentration of heavy metals in soil and root of okra plant (where $p \le 0.05$, where p=0.029); however, there was no significant relationship between the concentration of heavy metals in soil and stem (p > 0.05, where p=0.165) and between the concentration of heavy metals in stem and root of okra plant (p > 0.05, where p=0.061). The extent of relationships among the mediums (soil, root, and stem) showed a strong relation as r tends to 1 (r= 0.916, 0.726 and 0.861). For Crude oil 15% concentration, there was a significant relationship between the concentration of heavy metals in soil and root of okra plant (where $p \le 0.05$, where p=0.052); however, there was no significant relationship between the concentration of heavy metals in soil and stem (p > 0.05, where p=0.310) and between the concentration of heavy metals in stem and root of okra plant (p > 0.05, where p=0.116). The relationships among the soil, root and stem are strong as r tends to 1 (r= 0.876, 0.576 and 0.785). For Crude oil with 25% concentration, there significant was а

relationship between the concentration of heavy metals in the soil and root of the okra plant (where $p \le 0.05$, where p=0.031) and between the concentration of heavy metals in soil and stem of okra plant (where $p \le 0.05$, where p=0.012). However, there was no significant relationship between the concentration of heavy metals in root and stem (p > 0.05, where p= no output). The extent of relationships among the mediums (soil, root and stem) showed a strong relation as r tends to 1 (r= 0.916, 0.954, and 0.992).

	Table5. Pearson's co			
		Soil @5% CO	Root @5% CO	Stem @5% CO
	Pearson Correlation	1		
Soil @5% CO	Sig. (2-tailed)			
	Ν	5		
	Pearson Correlation	0.916*	1	
Root @5% CO	Sig. (2-tailed)	0.029		
	Ν	5	5	
	Pearson Correlation	0.726	0.861	1
Stem @5% CO	Sig. (2-tailed)	0.165	0.061	
	Ν	5	5	5
		Soil @15% CO	Root @15% CO	Stem @15% CO
	Pearson Correlation	1		
Soil @15% CO	Sig. (2-tailed)			
	Ν	5		
	Pearson Correlation	0.876*	1	
Root @15% CO	Sig. (2-tailed)	0.052		
	Ν	5	5	5
	Pearson Correlation	0.576	0.785	1
Stem @15% CO	Sig. (2-tailed)	0.310	0.116	
	Ν	5	5	5
		Soil @25% CO	Root @25% CO	Stem @25% CO
	Pearson Correlation	1		
Soil @25% CO	Sig. (2-tailed)			
	Ν	5		
	Pearson Correlation	0.912*	1	
Root @25% CO	Sig. (2-tailed)	0.031		
	Ν	5	5	5
	Pearson Correlation	0.954*	0.992**	1
Stem @25% CO	Sig. (2-tailed)	0.012	0.001	
	Ν	5	5	5

Table5. Pearson's correlation	coefficient (PCC) analysis
	i coemercine (i ooj analysis

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed)

The significant relationship between the soil and the root of the okra plant can be attributed to the closeness. As expected, the root draws its nutrients from the available soil. Consequently, such a relationship might not be possible between soil and stem, considering the distance, and the supplier of nutrients to the stem is basically through the root, which explains the possible relationship between root and stem HMs concentration. The strong relationship among the soil, root and stem could be attributed to their sources since they share the same sources under similar conditions.

Conclusion

Concerning the extent of crude impacted sites in the Niger Delta region as many mechanisms available for remediation are expensive with possible secondary impact on the environment. There is a need for cost-effective and environmentally friendly mechanisms for remediation activity. Phytoremediation is an effective and affordable technology that removes inactive metals and metal pollutants from contaminated soil and water. Okra (Abelmoschus esculentus) was planted in soil with three different concentrations of crude oil (5%, 15% and 25%) to examine the plant uptake capacity for heavy metals after 6 weeks of planting. The plant showed various degrees of uptake potential across the concentration, and this potential can be more significant over a more extended period of planting. The outcome demonstrates the practical application of okra plants to uptake heavy metal in crude oilcontaminated soil. Therefore, Okra (Abelmoschus esculentus) can clean up crude oil-contaminated soil as a cost-effective and environmentally friendly method.

Authorship Contribution

Ugwuechendu, TT: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing, Visualization, and Project administration. **Osuji, LC:** Supervision, Conceptualization, Methodology, Validation, Formal analysis, Data curation, Review, and Visualization. **Adejoh, IE:** Formal analysis, Investigation, Resources, Data curation, Second Draft, Visualization, and Project administration.

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Declarations

Conflict of interest: The authors have no relevant financial or non-financial interests to disclose. *Ethical approval:* Not applicable. *Consent to participate:* Not applicable. *Consent for publication:* Not applicable

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