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Evaluation of Lead and Arsenic content of *Azardirachta indica* seed oil and *Citrus sinensis* peel oil creams as mosquito repellent

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Abstract
There is an increase preference for plant-based repellents due to their effectiveness, friendliness to the environment and biodegradable nature. It is therefore necessary to ascertain the safety of these repellents by analysing their heavy metal content. This study indicates that the heavy metals, lead and arsenic content of *Azadirachta indica* seed oil cream and *Citrus sinensis* peel oil cream as mosquito repellents are significantly low therefore, they are very safe for use according to the Ghana Standard Authority specification. These results provide new insight into the safety of these mosquito repellents.

Keywords: Arsenic, Lead, *Azadirachta indica*, *Citrus sinensis*, mosquito repellent cream.

Introduction
The use of repellent is an effective and reliable method used in breaking Human-vector transmission cycle of pathogens [1]. Additionally, the application of repellent on clothing and bed nets have shown excellent results in Africa thus, conferring protection to the individual using them [2, 3]. Moreover, topical repellent is one of the most dependable ways of controlling outdoor biting of mosquitoes, especially in rural and farming communities [4].
Many users of repellent are concerned about the safety of DEET and hence plant-based repellents have become an ideal to customers [5]. Moreover, the easily biodegradative nature and friendliness to the environment has added to its preference [6, 7].
However, the heavy metal constituent of natural repellent creams could be toxic and in turn defeat its overall safety purpose. Exposure of the skin to arsenic can cause a variety of benign skin lesions including hyperpigmentation and hyperkeratosis [8]. It was indicated in a recent research that developing countries have high environmental pollution of Lead which may be absorbed by plants from which these natural repellents are made from. Organic Lead may be absorbed directly through the skin and its effects are devastating. High levels of lead in repellents when inhaled can result in decreased performance in some tests of cognitive performance that measure functions of the nervous system [9].
In the last two decades, an intensive effort has been made by several researchers to investigate the safety of diethyltoluamide (DEET), one of the most widely used and reliable synthetic insect repellents available [10]. However, many users of repellents are still concerned about the safety of DEET [5]. Furthermore, the easily biodegradative nature of plant-based repellents and friendliness to the environment has added to their preference by customers in recent years [6, 7] but the toxicity of some heavy metals in these topical repellents have not been given much attention.
This study seeks to evaluate the Arsenic and lead content of *Azadirachta indica* seed oil Cream and *Citrus sinensis* peel oil cream as mosquito repellent and as well compare it to the standard maximum allowable concentration requirement of these metals in topical repellents.

Materials and Methods
Study Design
This experimental study was conducted from January to February, 2020 at the Soil Science Laboratory of KNUST. The *Azadirachta indica* seed oil and *Citrus sinensis* peel were purchased from Agape Moringa Processing in Tamale, Ghana.

Sample materials and extraction of oils

The neem (*Azadirachta indica*) seed oil and sweet orange (*Citrus sinensis*) peel oil were purchased from Agape Moringa Processing in Tamale Northern Region of Ghana. The seed and the peels were collected from Tamale as well. Coloured rind of *C. sinensis* (two oranges) were put into Clevenger apparatus and distilled water of 100 cm^3 was added. The flask containing the peels was heated just below 100ºC and distillation took place steadily. Distillate of 50 cm^3 was collected in a measuring cylinder and the oil layer was removed with pipette completing the steam distillation method [11].This was done till 200ml of oil was obtained.

Azadirachta indica seeds from Tamale were dried and milled into powder. Oil was extracted from the powder (458.65 g) using Petroleum ether (2.2 L) with the Soxhlet apparatus for 4 days. The extract was concentrated using the rotary evaporator at temperature 45ºC and a percentage yield of 43.6% v/w obtained. The oil was then stored in an amber bottle at a cool dry place until ready for use.

$$\text{Yield of extract obtained} = \frac{Weight\ of\ neem\ seed\ oil\ x\ 100\%}{Weight\ of\ plant\ material\ taken}$$

Formulation of repellent creams

Three creams were formulated from the two oils (Neem seed oil cream, *C. sinensis* peel oil cream and Combined Neem and *C. sinensis* peel oil cream) at 30% concentrations respectively. A volume, 6mls of oil was added to 20g of Aqueous cream which was stirred and later homogenized to form the repellent creams. For the combined cream, 3mls of Neem seed oil and 3mls of *C. sinensis* peel oil were used.

Heavy metal analysis on the creams

Heavy metal analysis was conducted on all the three natural creams at the Soil Science laboratory of Faculty of Agriculture, KNUST using the 210 VGP Atomic Absorption Spectrophotometer, Buck Scientific, USA. Prior to the analysis 1g of each sample was digested using hydrochloric and nitric acid in the ratio 3:1 respectively. For Heavy metals, according to the Ghana Standard's Authority guidelines (catalogue code: GS 10902017) only Lead and Arsenic levels in repellents are tested for.

Results and Discussion

Table 1: Results of heavy metal content of creams

Sample Name	As(mg/kg)	Pb(mg/kg)
Orange peel oil cream I	0.00629	0.0039
Orange peel oil cream II	0.00809	0.0041
Orange peel oil cream III	0.00869	0.0040
Neem seed oil cream I	0.00225	0.0036
Neem seed oil cream II	0.00210	0.0036
Neem seed oil cream III	0.00270	0.0035
Neem seed oil and orange peel oil cream I	0.00150	0.0029
Neem seed oil and orange peel oil cream II	0.00132	0.0030
Neem seed oil and orange peel oil cream III	0.00165	0.0030

The values shown on Table 1 were the raw data obtained for the heavy metal Lead and arsenic content of all the three natural creams namely; neem seed oil Cream, Orange peel oil cream and combined neem seed oil and orange peel oil cream in triplicates.

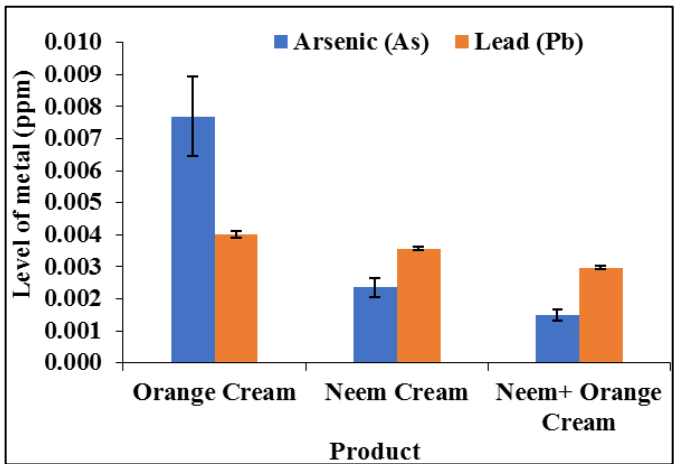

Fig 1: Comparison of Arsenic and lead content of Neem seed oil Cream, Orange peel oil Cream and the combination of neem seed oil and orange peel oil cream.

The level of Arsenic (As) recorded was highest in orange cream (0.008 ± 0.0012mg/kg), followed by neem cream (0.002 ± 0.0003 mg/kg) with neem and orange cream recording the least (0.001 ± 0.0002 mg/kg). There was a statistically significant difference between the level of Arsenic (As) in Orange cream vs. Neem cream ($p < 0.001$), and Orange cream vs. Neem and orange cream ($p < 0.001$).

With the level of Lead (Pb), both orange cream and neem cream had a concentration of 0.004 ± 0.0001 mg/kg while neem and orange cream recorded 0.003 ± 0.0001mg/kg as shown in Fig 1. Generally, there was a statistically significant ($p < 0.001$) difference between the levels of Lead (Pb) recorded in the three creams (Table 2).

It was observed that Arsenic (As) and Lead (Pb) concentrations in the combined Neem and orange cream was the least for both metals. This is as a result of half of the initial volume of 6mls used that is 3mls each of Orange and Neem oils in the cream formulation.

Both the levels of Arsenic (As) and Lead (Pb) recorded in all the creams were below the Ghana Standard Authority (GSA) guideline value of 5.0 mg/kg and 1.0 mg/kg respectively. The result from the Heavy metal analysis indicated that all the creams were 0.2% and 0.5% respectively for As and Pb of the standard maximum concentration requirement in topical creams. This proves that neem and orange peel oil creams are very safe to be applied on the human skin (Fig 1). There were no adverse effects observed during and after the experiment.

Supplementary Material

Statistical analysis for heavy metals

Table 2: One-way ANOVA between Arsenic (As) and Lead (Pb) concentrations in the three creams

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Arsenic	Between Groups	0.000	2	0.000	60.270	0.000
	Within Groups	0.000	6	0.000		
	Total	0.000	8			
Lead	Between Groups	0.000	2	0.000	145.400	0.000
	Within Groups	0.000	6	0.000		
	Total	0.000	8			

Table 3: Shows Multiple Comparisons

Multiple Comparisons							
Tukey HSD							
Dependent Variable	**(I) Product**	**(J) Product**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
						Lower Bound	**Upper Bound**
Arsenic	Orange	Neem	0.00534000*	0.00061188	0.000	0.0034626	0.0072174
		Neem + Orange	0.00620000*	0.00061188	0.000	0.0043226	0.0080774
	Neem	Orange	-0.00534000*	0.00061188	0.000	-0.0072174	-0.0034626
		Neem + Orange	0.00086000	0.00061188	0.396	-0.0010174	0.0027374
	Neem + Orange	Orange	-0.00620000*	0.00061188	0.000	-0.0080774	-0.0043226
		Neem	-0.00086000	0.00061188	0.396	-0.0027374	0.0010174
Lead	Orange	Neem	0.00043333*	0.00006086	0.001	0.0002466	0.0006201
		Neem + Orange	0.00103333*	0.00006086	0.000	0.0008466	0.0012201
	Neem	Orange	-0.00043333*	0.00006086	0.001	-0.0006201	-0.0002466
		Neem + Orange	0.00060000*	0.00006086	0.000	0.0004133	0.0007867
	Neem + Orange	Orange	-0.00103333*	0.00006086	0.000	-0.0012201	-0.0008466
		Neem	-0.00060000*	0.00006086	0.000	-0.0007867	-0.0004133

*. The mean difference is significant at the 0.05 level.

Conclusion

It can be concluded that the heavy metals, lead and arsenic content of *Azadirachta indica* seed oil cream and *Citrus sinensis* peel oil cream as mosquito repellent is significantly lower than the maximum allowable requirement and hence safe topical repellent cream.

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