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# Heavy Metal Levels in Insect Species Collected from Enyigba Community with Mining Activities in Southeast Nigeria

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**ABSTRACT:** Insects are the largest and most diverse group of organisms which are typically the overwhelming dominant invertebrate faunal group extensively used in biomonitoring and bioassessment. The aim of this paper was to evaluate the heavy metals levels in insect species collected from Enyigba Community with mining activities in Southeast Nigeria using appropriate standard methods. In this study, three sites namely: Upper Inyia-(SA), Lower Inyia (SB) and the Control Site (SC) were selected and insect specimens were collected and evaluated from July 2022 to February, 2023. The insect groups used were *Reticulitermes flavipes* (termite), *Zonocerus elegans* (grasshopper), *Acraea acrita* (butterfly) and *Cremtogaster* sp. (Ant). The selected insects' samples were analyzed using atomic absorption spectrophotometer (AAS). The results showed heavy metal accumulations on the selected insects particularly the toxic metals such as Cadmium (Cd), Copper (Cu), Manganese (Mn) and Mercury (Hg). Insects examined were *Reticulitermes flavipes, Zonocerus elegans, Acraea acrita,* and *Crematogaster* sp. collected from Enyigba mines. High concentrations of Cd was recorded in *Zonocerus elegans* at SA (0.13±0.042) and SB (0.17±0.007) which is far above WHO/FAO permissible limit. The result further revealed that there was also high Cd concentration (0.21±0.011) in *Reticulitermes flavipes,* which is also far above WHO/FAO permissible limit. Grasshopper, termites and butterfly had the highest accumulation of heavy metals among the studied insect groups, highlighting the challenges of mining on agriculture and food security in the region.

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Heavy metals are defined as metals with a high atomic weight and a density more than 5 g/cm$^3$ (Zhang *et al.*, 2019). However, when compared to their physical features, the chemical characteristics of heavy metals are the most useful (Su, 2014). Environmental toxicity that exceeds established maximum residue limits (MRL) has attracted more consideration from think tanks globally (Su, 2014). Lead (Pb), Copper (Cu), Cadmium (Cd), and Zinc (Zn) produce an alarming array of environmental and health issues (Zhang *et al.*, 2019; Su *et al.*, 2014). These heavy metals are released into the environment through a variety of natural processes, including volcanic eruptions, spring water, erosion, and bacterial activity, as well as

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anthropogenic activities such as fossil fuel combustion, industrial processes, agricultural activities, and feeding. These heavy metals bioaccumulate in living creatures and the human body through diverse processes, creating negative effects (Monisha *et al.*, 2014). In the human system, these heavy metals are transported and compartmentalised into body cells and tissues, attaching to proteins and nucleic acids, damaging these macromolecules and disturbing the cellular functioning (Monisha *et al.*, 2014). As a result, heavy metal poisoning can have a variety of effects in the human body. In extreme circumstances, it can disrupt the central neurological function, leading to mental disorders, harm the blood constituents, and may damage the lungs, liver, kidneys, and other essential organs, promoting various diseases (Monisha *et al*., 2014). Heavy metals come from a variety of sources, including mining, industry, and agriculture (Monisha *et al*., 2014). Fertilizers, insecticides, livestock dung, and wastewater are examples of agricultural pollution sources. In recent years, there has been an increase in ecological and worldwide public health concerns over metal poisoning of the environment. Furthermore, human exposure has increased considerably as a result of an exponential expansion in their use in a variety of industries, including agriculture and technology (Bradl, 2002). Heavy metals are typically difficult to remove once present in soil or water, and they are said to inflict irreparable damage to survival, feeding, growth, and the behaviour of organisms, including insects (Di *et al*., 2016. According to several studies, heavy metals can harm insect cell ultrastructure and genetic material, causing cell apoptosis, which can disturb cell vigour, proliferation, and result in mutation (Stolpe *et al*., 2017). Heavy metal ions have been found to enter insect bodies by respiration, air deposition, or dislodgeable residues on surfaces, as well as ingestion during feeding or grooming. Metal and metalloid pollution in the air, soil, and water is primarily caused by mining activities, industrial production, automobile exhaust, the combustion of leaded petrol, chemical and manure-based fertilisers, geological processes, some pesticides, and metal-containing plastic films (Lin *et al*., 2017; Vu and Wu, 2019). Heavy metal contamination in insects is well established in the literature (Dobermann *et al*. 2017). Insect consumption is endangered, particularly by anthropogenic pressures such as mining, which may have an impact on food safety due to insect toxicity caused by heavy metal pollution and allergies (Poma *et al*., 2017). Although other elements such as copper (Cu), zinc (Zn), and iron (Fe) are required for plant growth and human and animal nutrition, mining activities have considerably increased their concentrations in the environment to levels that exceed permitted limits (Sikamo *et al*. 2016). Metal pollution have numerous harmful effects on vertebrates, including cellular damage, carcinogenesis, and neurotoxicity (Chen *et al*., 2017). The high concentrations of heavy metals in the environment caused by intensive mining activities in Zambia's Copperbelt province have prompted concerns regarding the safety of edible insects and food plants (Nakayama *et al*., 2011). Biomagnification of heavy metals endangers insect diversity and decreases the safety of insects for human consumption. The study found significant variance in heavy metal concentrations among edible insects, likely due to changes in insect species, development stage, and heavy metal type (Schrögel and Wätjen, 2019).

Heavy metal concentrations were found in houseflies (*Musca domestica* Diptera: Muscidae), dragonflies (Libellula luctosa Odonata: Libellulidae), and phantom midges (*Chaoborus punctipennis* Diptera: Chaoboridae) (Butt *et al*., 2018). Certain insect species may avoid contaminated areas, particularly during oviposition. Females of *Drosophila melanogaster* (Diptera: Drosophilidae), *Plutella xylostella* (Lepidoptera: Plutellidae), and *Pieris rapae* (Lepidoptera: Pieridae), for example, avoided ovipositing on heavy metal-rich plant material (Freeman *et al*., 2006). Since the beginning of mining in Ikwo, Ebonyi State, the emission of mining dust and related waste discharges from the mining site has caused critical health conditions such as persistent heart problems, cancer, and a variety of other side effects on domestic and wild animals, as well as plants and insects in the surrounding area. This increasing consequential impacts of mining in the area, as well as the resulting consequences, necessitate an in-depth investigation to determine the impact or health implications of mining on the local human, animal, plant, and insect communities. This is because industrial pollution poses a severe threat to insects and ecosystems in general. In recent years, rapid industrialization and rising urbanisation have had a negative impact on all ecosystems, particularly soil dwelling organisms and other non-targeted organisms (Burden *et al*., 2019). These anthropogenic operations (mine activities) emit hazardous metals and metalloids into the soil and surrounding environment, affecting soil microflora and insect ecology, resulting in nutrient imbalances and soil acidification (Burden *et al*., 2019). Toxicity of these metals occurs across the food chain when contaminated species are consumed by another or uncontaminated organism at a higher trophic level (Sabiha-Javied, 2023). This is normally extremely hazardous to ecological receptors, and if taken by humans, it can cause major health problems in their bodies (Gu *et al*., 2018). Due to the negative effects of mines and mine waste on organisms, seasonal changes in insect population abundance and diversity are required to determine the drastic decline in insect population and diversity, metal accumulation on local entomofauna, and the health implications associated with edible insect consumption by area residents. Excavation of soil and minerals in order to extract useful minerals (Pb and Zn) for industrial manufacturing of explosive devices, device resistors, and cell batteries of all types, as well as roofing materials, has been a common occurrence in the area, with little regard for surrounding ecosystems, disrupting the balance of wildlife, plants, and water bodies.

## MATERIALS AND METHODS

*Study Area:* This study was carried out in Ikwo Local Government Area of Ebonyi State. It is predominantly a pre-urban setting with mainly teak trees, (*Tectona grandis*), palm trees (*Elaeis guineensis* Jacq), Gmelina tree (*Gmelina arborea* Roxb), neem trees (*Azadirachta indica* A.Juss), mango trees *Mangifera indica* Rouen van), grassy areas and houses. Ikwo is the second most populated local government area of 214,969 people situated at the eastern part of the State with a land mass of approximately 500 sq.km, with annual rainfall ranging from 1600-2000mm and shares borders with Abakaliki, Izzi and Ezza South Local Government Areas, National Population Commission (NPC, 2006). Ikwo has a semi-tropical climate characterized by two distinct season, the wet season which starts from the month of April-October (peak in July) and the dry season which starts from the month of November-March (peak in February), (Sikoki and Anyanwu, 2013). The study area has an annual mean temperature of 25 °C and 35 °C and seasonal temperature ranges between 22 °C - 30 °C to 23°C - 35°C for wet season and dry season respectively, and mean seasonal rainfall is 242 mm (Sikoki and Anyanwu, 2013). Leading industry in Ebonyi State is mining due to lead, zinc, and limestone deposits around Ikwo and Abakaliki L.G.As. Mineral deposit in Ikwo communities had led to mining and local quarrying activities resulting to preponderance of heavy metals and even in agricultural soil at the area.

*Study Location and Duration:* The study was carried out at Enyigba Mines in Ebonyi State, South-eastern Nigeria (Figure. 1). Enyigba is an important and leading industrial area of Ikwo/Abakaliki, Ebonyi State Nigeria. The coordinates of the study location falls within 6°10'49"N, 8°8'24E. For site A; 6° 10'44"N,8°8'30"E for site B; and 6°8'49"N, 8°8'37"E for Control Site which obtained using Google Maps GPS app on a Redmi 10C Smart phone and covered a land mass of about $70km^2$. The sampling points include, Upper Inyia-(Site A), which is 1 km away from the processing unit, Lower Inyia (Site B) and the Control Site (Site C) AKO which was 4 kilometres away from Site A and Site B. The sampling covered the wet and dry seasons, July, 2022 - February, 2023.

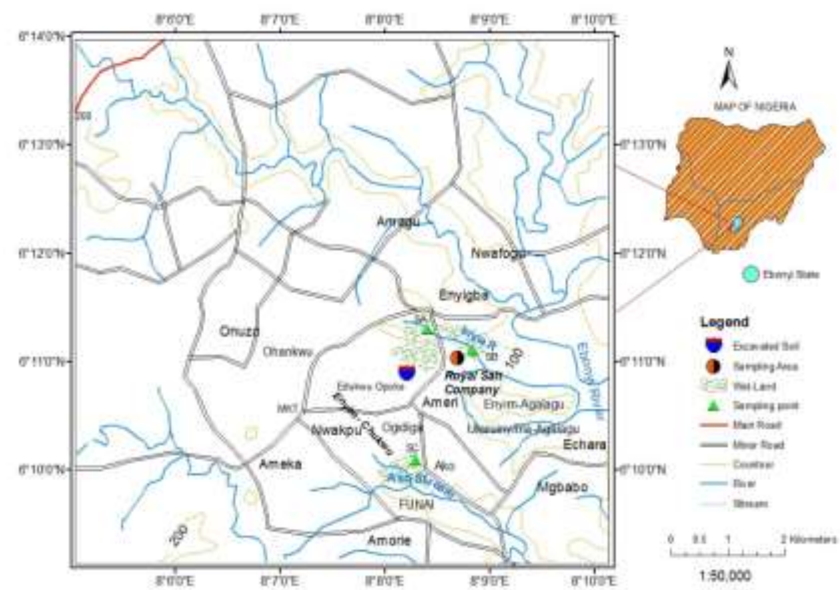


**Fig 1:** Map of Nigeria Showing Ebonyi State and the Sampled Area
(Source: Geology Department, EBSU, 2023)

*Insects Collection Methods:* Collection of insect from Enyigba mines took place from July, 2022 to February, 2023. Insects were collected three days weekly, namely: Monday, Wednesday and Friday for the period of the research and it was done in the morning hours (8am -11am) and the evening hours

(6pm) for light attracting insects (flying termite, moths). The insects collected were transferred to the killing bottle with 75% ethanol diluted with water while it awaits sorting and identification. However, multiple sampling techniques were adopted by applying same sampling methods in all sites. Specimens were collected by using pit fall traps, sweep net, beating method, hand picking method as in Akunne *et al.* (2014).

*Sample processing:* The four (4) selected insects' samples *Crematogaster* sp., *Reticulitermes flavipes, Acraea acrita* and *Zonocerus elegans* were relaxed with hot water and transferred into sampling bottle after sorting, capped properly and were immediately taken to St. Paul Laboratory Services Umudike Abia State for heavy metal analysis. On arrival, the samples were registered and dried in an electric oven set at 65 $^{o}$C and milled with Thomans Willey milling machine through a 0.6mm sieve and stored in an envelope for further treatment (Azam *et al.*, 2015).

*Sample Digestion by the Multiple Nutrients:* The processed sample was weighed 0.1g (100mg) into a 150 ml pyrex conical flask, and 5.0 ml of the multiple nutrient extraction mixture ($H_2S0_4$- selenium salicylic acid) was added to the sample, and allowed to stand for 24 hours (overnight). The sample was placed on a heat in digestion block set at 32$^{o}$C and allowed to heat for 4 hours under the fume cupboard. Then, 5 ml of cone Hydrochloric acid (HCl) was added to the sample and the heating temperature increased to 80 $^{o}$C. The sample was allowed to heat vigorously until the appearance of a white perchloric fume appeared and a clear solution obtained then the digestion terminated (Azam *et al.*, 2015). Thereafter, it was brought down from the hot plate and allowed to cool and finally transferred into a 50 ml volumetric flask using distilled water to wash down and make up to the mark.

*Atomic Absorption Spectrophotometer (AAS) determination of heavy metals in samples:* The digestion was used to determine the various metals using the USA Bulk scientific Absorption spectrophotometer (AAS).

*Statistical Analysis:* Data on insect collection were subjected to one way Analysis of Variance (ANOVA), was used to compare the mean concentration of heavy metals of insects collected in Enyigba mines. Sample means were separated using Post Hoc test (Duncan Multiple Range Test) at 5% significant level

## RESULTS AND DISCUSSION

*Concentration of Heavy Metals on Reticulitermes flavipes (Termites) Recovered at Enyigba Mines*: The concentration of heavy metals on *R. flavipes* recovered at Enyigba mines is shown in Table 1. The result revealed that Zn recorded highest concentration (0.27±0.018) in SA followed by SB (0.26±0.02) while least in SC (0.20±0.028). Mercury was highest in SA and SB (0.15±0.000) while least in SC (0.01±0.001). (0.21±0.011, 0.21±0.011) in SA and SB respectively. Hg recorded (0.15±0.000, 0.15±0.000, 0.01±0.001). Cu recorded 15±0.078, 0.13±0.071, 0±0.000). However, there was a significant difference between Enyigba mines and control. In comparison with the WHO acceptable limit for Cd, concentration of Cd on termite recovered from SA and SB is higher than WHO.

**Table 1:** Concentration of Heavy Metals in *R. flavipes* Found in in Enyigba Mine Sites

| **Heavy metals** | **Mean concentration of heavy metal in *R. flavipes* per sites** | | | **WHO/FAO, 2001 Standard (ppm)** |
|---|---|---|---|---|
| | **SA** | **SB** | **SC** | |
| Zn | 0.27±0.018 | 0.26±0.02 | 0.20±0.028 | 30 |
| Hg | 0.15±0.000 | 0.15±0.000 | 0.01±0.001 | 0.5 |
| Cu | 0.15±0.078 | 0.13±0.071 | 0.00±0.000 | 3 |
| Cd | 0.21±0.011 | 0.21±0.011 | 0.04±0.311 | 0.05 |

*Row sharing similar superscript are not significantly different at p <0.05*

*Concentration of Heavy Metals in Crematogaster sp. (Ant) Recovered at Enyigba Mines:* The concentration of heavy metal on *Crematogaster* sp. recovered in Enyigba mines is shown in table 2. The result revealed that Cu recorded highest concentration (1.93±0.106, 1.92±0.11, 0.87±0.028) in SA, SB and SC respectively followed by Cd (0.13±0.035, 0.52±0.537, 0.04±0.000). Zn recorded (0.07±0.021, 0.06±0.018, 0.03±0.035). Also Hg recorded 0.03±0.035, 0.03±0.035, 0±0.000) respectively. However in comparison with WHO acceptable limit, the concentration for Zn, Cu, Fe, Pb, Mn in the three studied sites were lower but concentration of Cd, Hg were higher than WHO permissible limit.

*Concentration of Heavy Metals on Acraea acrita (Butterfly) Recovered at Enyigba Mines:* The results of the concentration of heavy metals on *A. acrita recovered* at Enyigba mines is shown in table 3. The result revealed that Cu had highest concentration (7.04±0.768, 1.7.±0.042) in SA, SB and SC respectively. Followed by Mn (1.9±0.636, 1.85±0.707, 0.3±0.021). Zn recorded (0.28±0.035, 0.26±0.028, 0.1±0.000) Cd recorded (0.25±0.27, 0.4±0.27, 0.02±1.269) in SA and SB respectively. There was a significant difference at (P<0.05) in Cu, Zn concentration on *A. acrita* in SA, SB and SC. However, in comparison with the WHO acceptable limit for Cu, Cd, Mn concentration on *A. acrita* recovered from SA and SB was higher than WHO permissible limit but Zn, Hg was lower than WHO acceptable limit.

**Table 2:** Concentration of Heavy Metals on *Crematogaster* sp. (Ant) Recovered at Enyigba Mines

| **Heavy metals** | **Mean concentration of heavy metal in *Crematogaster* sp. Per sites** | | | **WHO/FAO, 2001 Standard (ppm)** |
|---|---|---|---|---|
| | **SA** | **SB** | **SC** | |
| Zn | 0.07±0.021 | 0.06±0.018 | 0.03±0.035 | 30 |
| Hg | 0.03±0.035 | 0.03±0.035 | 0±0.000 | 0.5 |
| Cu | 1.93±0.106 | 1.92±0.11 | 0.87±0.028 | 3 |
| Cd | 0.13±0.035 | 0.52±0.537 | 0.04±0.000 | 0.05 |

*Rows sharing similar superscript are not significantly different at p <0.05*

**Table 3:** Concentration of Heavy Metals on *A. acrita* (Butterfly) Recovered at Enyigba mines

| | **Mean concentrations of heavy metals in *Acraea acrita* by sites** | | | |
|---|---|---|---|---|
| **Heavy metals** | **SA** | **SB** | **SC** | **WHO/FAO, 2001 Standard (ppm)** |
| Zn | 0.28±0.035 | 0.26±0.028 | 0.10±0.000 | 30 |
| Hg | 0.07±0.00 | 0.05±0.000 | 0.02±0.00 | 0.5 |
| Mn | 1.90±0.636 | 1.85±0.707 | 0.30±0.021 | 0.05 |
| Cu | 7.10±0.707 | 7.04±0.764 | 1.70±0.042 | 3 |
| Cd | 0.25±0.279 | 0.4±0.071 | 0.02±1.269 | 0.05 |

*Rows sharing similar superscript are not significantly different at p <0.05*

*Concentration of Heavy Hetals on Zonocerus elegans (Grasshopper) Recovered at Enyigba Mines:* The concentration of heavy metals on *Z. elegans* recovered at Enyigba mines is shown in table 4. The result revealed that Mn had highest concentration (10.95±0.768, 10.94.±0.085, 5.38±0.035) in SA, SB and SC respectively. Followed by CU (1.65±0.354, 1.64±0.354, 0.49±0.014). The result reported that Zn had (0.15±0.014, 0.16±0.014, 0.02±0.071). Fe had (0.11±0.014, 0.11±0.014, 0.1±0.014). Pb recorded (0.08±0.00, 0.08±0.004, 0.04±0.064). The result also reported that Cd recorded high concentration (0.13±0.042, 0.17±0.007, 0.04±0.001) at SA and SB respectively. There was a significant difference in the concentration of Mn, Cd in *Z. elegans* between Enyigba mines (SA, SB) and control (SC) at P<0.05 but no significant difference was observed between SA and SB. However in comparison with the WHO acceptable limit for Cd, the concentration of Mn, Cd in *Z. elegans* from SA, SB and SC was higher than WHO permissible limit but the concentration of Cu, Fe, Pb, and Zn were lower than WHO acceptable limit.

**Table 4:** Concentration of Heavy Metals on *Z. elegans* (Grasshopper) recovered at Enyigba mines

| **Heavy metals** | **Mean concentrations of heavy metals in *Z. elegans* ± SD** | | | **WHO/ FAO, 2001 Standard (ppm)** |
|---|---|---|---|---|
| | **SA** | **SB** | **SC** | |
| Zn | 0.15±0.014 | 0.16±0.014 | 0.02±0.071 | 30 |
| Mn | 10.95±0.071 | 10.94±0.085 | 5.38±0.035 | 0.05 |
| Pb | 0.08±0.00 | 0.08±0.004 | 0.04±0.064 | 0.1 |
| Fe | 0.11±0.014 | 0.11±0.014 | 0.1±0.014 | 30 |
| Cu | 1.65±0.354 | 1.64±0.354 | 0.49±0.014 | 3 |
| Cd | 0.13±0.042 | 0.17±0.007 | 0.04±0.001 | 0.05 |

*Rows sharing similar superscript are not significantly different at p <0.05*

Heavy metals detected in our examined species are as a result of cumulative mine waste/discharge emanated ongoing mining activities at the study area which has made the environment an ecological danger zone. The findings of this study revealed that there was high level of heavy metals in *Z. elegans*, *R. flavipes,* and *A. acrita* and termed good potential metal accumulator, this is in agreement with (Hofer *et al.*, 2010) who reported that several studies spotlighted considerable differences in metals accumulation and excretion in organism. This may be associated with variations in their detoxification capacity or antioxidant defense. Population increase in some sites may be attributed to decrease in pollution, predators and competitors. This is in accordance with previous work which reported that populations may increase due to the removal of predators/competitors (Nahmani and Lavelle, 2002; Nahmani, *et al*., 2003). However, decrease in population in some site could be as a result of increase in predators this is in agreement with Van-Huis, (2013) predators (parasitoid) such as Ichneumonid species *T. talibarti* provide direct economic benefits in population control by predating on few other organisms.

Data showed that all the insect taxa clearly show site and species dependent metal accumulation pattern for Cd, Ni, Zn, Cu, Fe, Cr, and Mn. High metals concentration detected in selected insects species analyzed could be as a result of industrial effluents from the mining site. However, it should be noted that these metals are persistent and cannot be degraded by insect metabolism, they accumulate at upper trophic level. The result of the findings revealed that high concentration of metals were detected on sampled insects this is in agreement with Butt *et al.* (2018) who reported that relatively high concentrations of heavy metals were detected in housefly (*Musca domestica* Diptera: Muscidae), dragonfly (*Libellula luctosa* Odonata: Libellulidae), and phantom midge (*Chaoborus punctipennis* Diptera: Chaoboridae). In this study, high accumulation of metals were measured for insects collected at the mine contaminated sites. In *Z. elegans* as a polyphagous and omnivorous insect, relatively higher concentrations of heavy metals were found followed by *R. flavipes* and *A. acrita*. *Crematogaster* spp. recorded the least metal concentration even when it has the closest contact with the contaminated/polluted soil compared to other insects. This could be as a result of its genetic make-up. Eeva and Sorvari (2004) who reported that ants and termites tend to be resistant to severe pollutions. It might also be assumed that ants particularly possess physiological mechanisms, which aid in the regulation of metal ion concentrations and prevent metal contamination (Hofer *et al.*, 2010). Furthermore, all the insect taxa clearly show site and species dependent metal accumulation patterns for Cu, Pb, Mn, Zn, Cd, Fe, and Hg. High concentration Cd was recorded in termite, grasshopper, butterfly, at SA and SB, It is now obvious that the complaining of ill-health by inhabitants of the area (while interacting with the people of the area during the sampling) could be as a result of high metal concentration in their body systems. This is in agreement with Sobha *et al*. (2007) who reported high Cadmium (Cd) toxicity is mostly obvious in organs such as liver, kidneys, brain, lungs, placenta, and as well bones.

*Conclusion:* This study revealed that heavy metal accumulation is dependent on insect species and sites. Grasshopper, termites and butterfly had the highest accumulation of heavy metals among the studied insect groups, highlighting the challenges of mining on agriculture and food security in the area. The study also revealed the insects are good bioindicators of pollution hence consistent monitoring of environmental health is of great essence. The relevant government agencies should regulate the mining activities in the study area and employ bioremediation measurers timely to avoid adverse effects of heavy metals in the study area.

*Declaration of Conflict of Interest:* The authors declare no conflict of interest.

*Data Availability Statement:* Data are available upon request from the corresponding author

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