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Toxicity of Workplace Aluminum Particles: Insights from Earthworm (*Eisenia fetida*) Tests in Soil Mixtures

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ABSTRACT: Lifestyle changes have led to increasing use of alternative materials in building construction, fabrication of furniture and household appliances. Apart from the associated light weight and aesthetics, Aluminum products endure various pressures that range from climatic factors to pest attacks; hence, they are more durable than wood and other conventional materials. Activities of fabricators are widespread in many Nigerian cities and these result in traces of Aluminum particles derived from cutting, shaping and surface filing. The resulting recalcitrant dust particles can exert adverse consequences on biota. Therefore, this study examined the effects of different levels of Aluminum particles on earthworm in soil mixtures by assessing their behaviour, mobility and mortality in a five-week ecotoxicity test. Worms became sluggish after only two-week exposure and this culminated in loss of mobility and ultimately mortality in exposed organisms. Mortality of worms was highest (80 – 100%) in soil mixtures with the most proportion of Aluminum particles and decreased correspondingly with contaminant levels. However, there was no mortality of worms in soil mixtures without any Aluminum particles. Lethal concentration (LC50) values of 2.564g/kg, 0.995g/kg and 0.851/kg were determined at two, four and five weeks, respectively. The results suggest that worms in the course of foraging in soil, can internalize contaminating Aluminum particles, which may lead to adverse consequences in exposed population. Considering the role of earthworms in breakdown of soil organic matter and nutrient cycling, indiscriminate disposal of Aluminum particles across various landscapes may have consequences on soil fertility, food security and sustainability.

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Occurrence of natural and anthropogenic pollutants is widespread, while their impacts appear to worsen with increasing human population and industrial activities (Hou *et al.,* 2020). As challenges associated with priority pollutants including plastics, personal care products, electronic wastes, agrochemicals, heavy metals, pharmaceuticals, nanoparticles, airborne carbon and soothe particles continue to emerge, mankind strives with lifestyle adjustments in order to alleviate potential negative impacts on vegetation, wildlife, micro ecosystems as well as human populations (Doyle *et al*. 2020, Singh *et al.,* 2021). Recent adjustments in building constructions in Nigeria have recorded the use of different alternatives and these include replacements of wooden windows and doors with aluminium fabricated frames and glass. Apart from improved aesthetics, aluminum-fabricated windows and doors are easier to make and may endure climatic factors and attacks from insects and termites. In actual fact, availability of aluminum-fabricated windows and doors is widespread worldwide, even though various artisans working in this field either cut their materials manually with hand-held tools, utilize simple electric cutting devices or may not wear protective gears during operations. Apart from exposing fabricators to workplace hazards, these practices can cause emerging fine aluminum dust particles to escape into the outer environment where they may threaten soil health and resident organisms. Unfortunately, fine particles of aluminum can travel considerable distances and are well known for their recalcitrance in the outer environment. Enormous diffusivity of nanoparticles appears to facilitate their mobility in porous media (Wiesner *et al*., 2006). Due to poor enforcement of regulations, local workshops specialized in aluminum fabrications are widespread in cities and town where they hope to meet the needs of a thriving real estate industry in Nigeria. Concerns are even more dismal considering that Aluminum particles can occur in micro and nano scales, thereby raising concerns on their fate and ecotoxicity in terrestrial ecosystems. Coleman and co-workers

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(2010) have assessed the fate and effects of nano-sized aluminum oxide on earthworm (*Eisenia fetida*); however, the impact of actual workplace aluminum particles on terrestrial organisms is poorly understood. Other studies have shown evidences of inflammatory responses in rats exposed to particles of ferric oxide (Zhu *et al*., 2008), growth inhibition in microalgae treated with tin, titanium and zinc oxides (Aruoja *et al*., 2009) and adverse effects on crustaceans and bacteria (Heinlaan *et al*., 2008). Considering the widespread use of aluminum and occurrence of the particulates in their original form generated by artisans and fabricators, it is necessary to investigate their fate and likely consequences on terrestrial organisms. This is especially critical for soil living organisms including earthworms that eat soil whole, thereby predisposing them to possible health effects including damage to coelomocytes and immunological cells. Therefore, the objective of this study is to investigate how particles of aluminum obtained from a commercial workshop and spiked on a loamy soil may affect earthworms in a five-week ecotoxicity test.

MATERIALS AND METHODS

Sources of earthworm, Aluminum particles and experimental soil: Earthworms were collected from a field site in Saint Mary's Primary School, Oye-Ekiti, Ekiti State, Nigeria. During the rainy reason of 2018, worm casts were located and dug up in order to locate and gently pick the worms from beneath the soil using a pair of forceps. The earthworms were transported quickly in transparent plastic vessels and maintained in the laboratory of the department of Animal and Environmental Biology, Federal University Oye-Ekiti until use. At first, the worms were rinsed in clean water and later transferred into the control experimental soil in a plastic container with depth, width and length of 24 x 30 x 40cm until use. The worms were allowed a one week acclimatization period in the test soil.

Characterization and preparation of soils: Soils dried in open air were processed and characterized for different parameters (Table 1). Prior to analysis, soil samples were passed through a sieve having pore size diameter of < 5mm to filter away debris and coarse stones. Aluminum particles used in this study were obtained from T.J. Aluminum Company in Warri, Delta State, Nigeria (Fig. 1). The particles were transported to the laboratory in aluminum foil properly wrapped and later stored at room temperature until use. On the other hand, the experimental soil was obtained from a relatively clean reference site with very minimal human activity in Phase II campus of the Federal University Oye-Ekiti at geographical coordinates of N 07^{o} 46.481', E 05^{o} 18.952' (*etrex 10* GARMIN GPS device). Soil was taken from a depth of about 15cm and transported to the laboratory. Later, the soil was allowed to dry in open air for a week prior to sorting and sieving to remove pebbles and large soil aggregates.

Table 1: Characteristics of experimental soils

Parameter	Soil
Colour	Brown
pH	7.10
Conductivity (μs/cm)	287.90
Porosity (%)	44.44
Salinity (ppt)	0.15
Bulk Density (g/cm3)	1.43
Texture	Sandy Loam
TOC (%)	1.70
Particle size distribution	
Sand	89.15
Silt	10.60
Clay	0.25

Soil preparation and exposure of earthworm: Aluminum particles of ≤ 150mm diameter were obtained by passing Aluminum dust through an appropriate sieve and later spiked on soil at concentrations of 0.32g/kg, 0.63g/kg, 1.25g/kg, 2.5g/kg, 5.0g/kg and 10g/kg. For each treatment level, a corresponding quantity of Aluminum particles was measured into a 2kg of dry weight soil and thoroughly mixed for two days prior to dispensing the treated soil mixture into five replicate exposure vessels (Fig. 1). Prior to replication and after spiked soil had been mixed thoroughly, the treated soil was allowed a two-week stabilization time at room temperature in the laboratory.

Fig. 1: Aluminum particles yield at a fabricator's workshop (A), collected Aluminum debris (B), soil contaminated with Aluminum particles in ambient environment (C), and five replications of control soil as well different concentrations of Aluminum spiked on soil in exposure vessels (D).

Briefly, the test soil and Aluminum particles were manually mixed continuously for several hours which lasted for two days prior to addition of water to dampen spiked soil. Subsequently, 2kg of each category of spiked soil was weighed into rectangular exposure vessels of 7cm (depth) x 10cm (width) x 15cm (length) in five replicates prior to introduction of the test organisms (Fig. 1). Additionally, 2kg of soil not spiked with Aluminum was weighed into similar exposure vessels as for the treatment category and marked as the experimental control. In total, thirty five samples were obtained and observed for a period of five weeks.

Five worms were randomly recovered from acclimatization soil and introduced into each of the spiked soil in vessels having different concentrations of Aluminum particles (Fig.1). Afterwards, the exposure vessels were covered with net mesh designed to restrict worms in soil and prevent them from crawling out. Worms in exposure vessels were assessed at two, four and five weeks to ascertain the state of exposed organisms and determine mortality. Additional food was not provided considering that worms burrowing through soil feed on available soil organic matter (Vavoulidou *et al*., 2009). Soil moisture level was maintained by regular addition of water every other day, while the study lasted for a period of five weeks. Mortality, physical activity and mobility of worms were noted throughout the exposure. Mortality data from the treatment population were compared with control worms at ≤ 0.05 level of significance, using version 22 of the Statistical Package for the Social Science (SPSS), while the bar graph was prepared in GraphPad Prism 5.

RESULTS AND DISCUSSION

At the end of the exposure period, earthworms were carefully recovered from the test soil and mortalities determined. Mortality of earthworm was most pronounced in soil treatment with the highest proportion of Aluminum particles (Fig. 2). The difference in mortality of worms from vessels with the highest concentration of Aluminum particles was significant compared to the control population ($P \leq 0.05$). However, mortality of worms reduced as the proportion of Aluminum particles decreased in the test soil, while no mortality was recorded in the control population (Fig. 2). The results show that contaminating Aluminum particles spiked on soil resulted in corresponding increase in mortality of exposed worms at two, four and five weeks of exposure. The results suggest that worms in the course of foraging for food and organic matter have developed mechanisms to internalize contaminating Aluminum particles, which ultimately lead to adverse consequences.

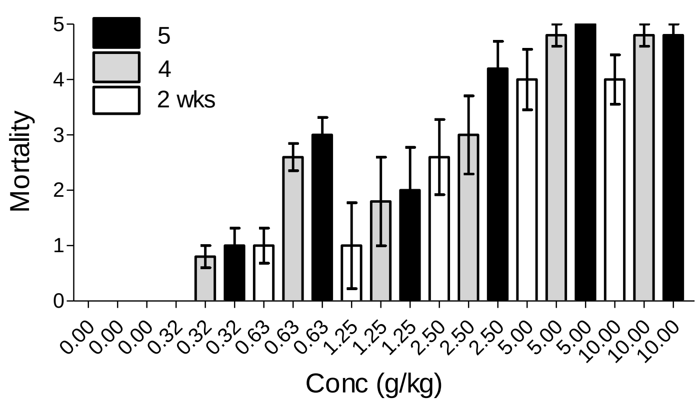

Fig. 2: Mortality of earthworm shown as mean of five replicate values following exposure in different concentrations of Aluminum in soil mixtures in a continuous test for 2, 4 and 5 weeks. Bars depict mean of triplicate determinations with Standard Error of Mean (SEM) values.

Further analysis revealed that concentration (LC_{50}) values of 2.564g/kg, 0.995g/kg and 0.851/kg lead to mortality of 50% of worms exposed to the contaminants at two, four and five weeks, respectively. These results suggest that the longer the exposure period at each treatment level, the less the tolerance of worms to Aluminum particles. At two weeks, worms were sluggish and this culminated in immobility due to exposure to Aluminum particles. The observed sub-lethal effects became worse and culminated in mortality of worms as contaminant concentrations increased in soil mixtures. Results from this study revealed that fine particles of Aluminum generated from industries and road-side artisans constitute enormous hazards to soil ecosystems and may have access into the aquatic environment. Despite the threat posed to soil dwellers, Aluminum particles can be taken up in crops and in turn transferred across the food chain. When one considers the control population of worms at the three assessment intervals of 2, 4 and 5 weeks, there was no mortality recorded throughout the duration of the experiment. However, mortalities appeared to increase as exposure concentrations and duration increased, thus leading to death of organisms in lower treatment levels (Fig. 2). It is possible that worms may have suffered other sub-lethal effects which did not culminate in death of exposed organisms. Therefore, mobility of worms is an important assessment endpoint and slowed down at low treatment levels, which caused them to become sluggish when taken out of the exposure vessels. Despite the threat posed by Aluminum particles, these contaminants are indiscriminately disposed into the outer environment where their recalcitrance drives availability and distribution in different environmental matrices. Concerns due to prevalence of this class of contaminants are even greater in developing countries where poor business etiquette, indiscriminate disposal of Aluminum particles, and poorly implemented environmental regulations contribute to their presence in soil and water bodies. Unlike plastics and other contaminants of concern, Aluminum particles are not even easily noticed when present in the environment, which may lead to their accumulation in different matrices of soil, sediment and water systems. Usually, the particles are swept away onto surrounding soil with potential consequences on various terrestrial organisms including earthworm, ants and millipedes that may eat soil whole with the corresponding contaminants. Considering that recalcitrant contaminants can be taken up and internalized in tissues of exposed organisms to accumulate higher up in the food chain, there is potential risk of bioaccumulation, biomagnification and threat to food security. Apart from prevailing threats due to Aluminum particles, exposures in workplace constitute enormous concerns particularly because of negligence in strict compliance with the use of protective gears among Nigerian workers. Unfortunately, the predominantly artisans that constitute a significant workforce in the industry may not be aware of numerous concerns and likely respiratory complications associated with exposure to Aluminum particles. Aside the tendency to become inhaled and consequently lead to inflammations in brain tissues and blood, human exposure to Aluminum particles have been associated with neurotoxicity, loss of memory and psychological concentration (Bondy *et al*., 2010; Mandour *et al*., 2011; Riihimäki *et al*., 2012).

Conclusion: Results from this study revealed that behavioural changes and immobility occurred in earthworms, which culminated in mortality of exposed organisms. Severity of the effects increased with Aluminum levels in soil mixtures, considering that worms eat soil whole to internalize potential particulate contaminants. However, no toxic response was observed in the control population. Given the role of earthworm in bioturbation and nutrient cycling, indiscriminate disposal of Aluminum particles across various landscapes may have consequences on biodiversity, soil fertility and food security.

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