

Impact of carrion decomposition on topsoil chemistry and their eco-forensic implications in a Southern Nigerian ecosystem

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ABSTRACT. Post-mortem interval (PMI) is the period since death of human or animal remains upon discovery. Studying the soil chemical properties could provide a taphonomic approach of carrion decay. The present study aimed to examine the relationships between soil chemical properties and decomposition timeline of remains at both dry and wet seasons in a Southern Nigerian (Port Harcourt) ecosystem. Using an observational, analytical design during the dry (December 2022 to February 2023) and wet season (3rd to the 18th of April 2023), healthy pigs (*Sus scrofa domestica*) weighing between 40 – 60 kg were used. Upon euthanization, pigs were buried at different cadaver decomposition islands (CDIs) within the site and at weekly intervals. Pig carrion decomposition was scored quantitatively using the Keough et al. total body score (TBS) method based on the observed morphological appearance of selected body regions. Soil samples were collected, air-dried, sieved, and analyzed for chemical properties using standard analytical procedures. Statistical differences in soil chemical characteristics between post-mortem intervals were performed using both one-way analysis of variance (ANOVA) and Post Hoc tests. From the results, pH levels of dry and wet season gravesoil samples differed significantly at various PMIs ($p < 0.05$) in comparison with control soil samples. Calcium and magnesium levels were increased on days 7 (mean TBS of 16.3) and 14 (mean TBS of 21.3) while potassium concentrations elevated significantly at both days 14 in both seasons. There were significant increases in sodium concentration on day 7 (at a mean TBS of 19.3) for the wet season. Exchangeable acidity (EA) and effective cation exchange capacity (ECEC) values increased significantly in both study periods. The study showed significant associations between the post-mortem intervals of study pig carcasses and variations in chemistry of the gravesoil samples.

Keywords: Post-mortem interval; taphonomy; soil chemistry; carrion decomposition; Port Harcourt.

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Introduction

Moments after the death of a person, few visible changes to the environment are observed during the early stages of decomposition as cadaveric materials are contained within the deceased person until the skin ruptures or fluids purge from body orifices. For surface-deposited remains, once the body deflates, these fluids leach into the surrounding soil, and a cadaver decomposition island (CDI) form afterwards (Carter et al., 2007). In the early and active stages of decay procedures, there are quite a lot of buildups of bodily gases such as hydrogen sulfide, carbon dioxide, and methane, from the orifices of both internal and external organs of these carcasses. At this juncture, the compression from these gases ensures that the cadaver fluid leaks from these orifices and permeates into the soil (Aitkenhead-Peterson et al., 2015; Heo et al., 2021). The CDI is a darkened area of moistened soil. The vegetation within the CDI may die and disintegrate depending on the soil nutrient status (Wescott et al., 2024). Vegetation located further from the remains is less likely to be affected by these disturbances. Post-mortem interval (PMI) is known to be the period since death of these remains upon discovery (Adheke & Oghenemavwe, 2023). As the post-mortem interval (PMI) increases, the vegetation will regenerate, often healthier than it was originally, which can cause a clear delineation of the site in some cases (Wescott et al., 2024). Species assemblage of vegetation may become altered, especially where the vegetation cover is destroyed upon burial (Fernández-Jalvo et al., 2011; Provoost et al., 2011). This is often the response

of opportunistic plants colonizing areas of increased nutrient availability and some species may therefore act as grave indicators (Wescott et al., 2024).

Decay influences the encompassing soil chemistry by clearing out unstable and tireless compounds that are related to diverse stages of decomposition (Perrault et al., 2015; Fancher et al., 2017). The study of the chemical, as well as the physical properties of the soil, has provided scientists with pertinent information for manufacturing a host of taphonomic approaches for them to better apprehend the processes of any human or carrion decay occurring within any burial site (Dupras et al., 2011; Wescott, 2018). The mineral composition of soil, the organic matter within it and the environment, all are determined by the chemical properties of soil – however, physicochemical characteristics of different soils vary in space and time due to variation in topography, climate, and action of weathering, vegetation cover, microbial activities, and several other biotic and abiotic factors (Paudel & Sah, 2003). The applications of soil biochemical techniques have been regularly employed to estimate PMI and to locate clandestine graves (Vass, 2001; Carter & Tibbett, 2003; Tibbett et al., 2004). However, each grave location is distinctive in terms of its soil texture or structure which is based on ecological components such as vegetation or climate, geography, geology, and time for soil arrangement. The clay soil texture is associated with the hindrance of carrion breakdown as they are associated with a low rate of diffusion of emitted abdominal gases. Thus, when remains are surface buried in such soil, it can lead to an outcome that is synonymous with reduced decay processes since the exchange of diffusible gases such as oxygen and carbon dioxide are usually insufficient for effective soil microbe activities (Surabian, 2012; Obalum et al., 2017). An associated report by Forbes et al. (2005) noted that the soil textural differences between sandy and silt soils proved significantly relevant in the formation of adipocere.

The wet and dry seasons of a tropical Nigerian ecosystem are characterized by several ecological factors that engineer several processes that influence the edaphic components of the ecosystem (Osborne et al., 2018; Salisu et al., 2021). Fluctuations in rainfall and temperature are some of the factors that affect the chemical properties of soil (Fatubarin & Olojugba, 2014; Olojugba & Fatubarin, 2015). The impact of soil pH on the rate of breakdown of carrion in soils is profoundly accompanied by the level of abundance of soil microbes that play a role in enriching the soil ecosystem (Benninger et al., 2008). Related studies have shown that some of these properties such as ammonia content, dissolved organic carbon and nitrogen contents, as well as phosphates and nitrates tend to rise upon their accumulations in different CDIs across various parts of the world (Stokes et al., 2013; Szelecz et al., 2018; Yong et al., 2019). Meanwhile, as pertaining to the field of forensic science, a soil sample that is obtained from a particular crime scene can be investigated for the presence or quantity of soil nutrients for the purpose of solving the crime upon the discovery of remains (Benninger et al., 2008; Carter and Tibbett, 2008; Fitzpatrick, 2008). Hence, the current study was done to assess the influence of soil chemical characteristics in the decomposition timeline of pig carcasses on specified periods during the dry and rainy seasons in Choba, a Southern Nigerian ecosystem. There is arguably no current information that is available for local forensic anthropologists and scientists to have an enhanced comprehension of the impacts of various degrees of cadaver decomposition on Nigerian soil ecosystems to possibly predict the time of death – hence this study aim.

Materials and methods

This study was an observational and analytical study design that took place during the dry season (December 2022 to February 2023) and wet season periods (from 3rd to the 18th of April 2023). Healthy pigs (*Sus scrofa domesticus*) weighing between 40 – 60 kg were used as non-human proxies for the study. They were obtained from the Livestock Unit, Research and Teaching Farm of the Faculty of Agriculture, University of Port Harcourt, and were confirmed to be healthy by a veterinary doctor. The pigs were euthanized with sodium pentobarbiturate which was administered intramuscularly. After about five minutes of administration, there were signs of convulsion showed by uncontrolled muscle spasms, and after a while, the animal became still. Pig death was confirmed upon observations of cessation of heartbeat and pulse (as confirmed by a veterinary doctor). The exact date and time of death was then recorded. The pigs were taken to the study site for surface burials. Upon arrival at the burial site, each pig was allocated a number to ensure proper observation. A bamboo fence was erected around the burial site; the dimension of the fence was measuring 9m x 6m x 2m. The fence had a gate for controlled access. The purpose of fencing was to limit the entering of rodents or large scavengers and thieves that could remove or alter the position of the animals at the burial site thereby unduly influencing the decomposition process. The animals were placed three (3) meters apart at different points to

reduce the possibility of the migration of insects from one pig to another. This spacing helps to ensure insect colonies from one pig do not contribute towards the decay of another pig. The sampling technique employed in this study was a convenient, purposive type. Some of the pigs were used for the period of dry season and others were used for the period of rainy season. The sample size was considered due to stringent animal protection laws on animal rights in Nigeria. An ethical clearance was obtained (UPH/CEREMAD/REC/MM83/025) from the University Research Ethics Committee of the University of Port Harcourt.

The study was carried out in the Anthropological/Burial Farm of the Department of Anatomy Faculty of Basic Medical Sciences, University of Port Harcourt, located in Rivers State in the tropical rainforest vegetation zone. The area has tall trees (at least 30m high), moderate to high precipitation (mean of 20%), high humidity (average of 84%), and a warm temperature (average of 27 degrees Celsius) during the rainy season and hot and cloudy, with a mean precipitation value of 3%, average low humidity of 63%, and colder temperatures (with an average of 22 degrees Centigrade) during the dry season. The edaphic nature of this vegetation usually varies from loamy, sandy, and clayey forms of soil, with varying levels of humus contents.

Total body score (TBS)

This study used the amended method of Megyesi et al (2005) for pig models, developed by Keough et al. (2017). This was used to score decomposition based on the morphological appearance of three (3) body regions, head and neck, trunk, and limbs. The stages of decomposition are first assessed qualitatively and then converted into quantitative scores from these three regions in the body. The allotted point value of each region was then added to determine the TBS which represents the overall stage of decomposition of each pig from a minimum of 3 to a maximum of 35 points (as shown in Figure 1).



Figure 1. Stages of decomposition. A – Fresh stage, B – Bloat (early) stage, C – Active decay stage, and D – Advanced decay stage.

Evaluation of soil physicochemical characteristics

Soil samples were collected and later air-dried at room temperature for a few days, sieved through a 2mm sieve, and analyzed for chemical properties using standard analytical procedures. The soil particle size distributions were determined using the standard hydrometer and pipette technique (Kettler et al., 2001). Soil texture was determined using the soil textural triangle based on the percentages of different soil particle sizes (Sutherland and de Jong, 1990). The pH of the soil samples was determined using the standard water extraction method with a standard glass electrode in water to soil ratio of 1:2.5 mixed with distilled water suspension (McLean, 1982). The exchangeable bioavailable potassium (K) of the soil samples was extracted with neutral normal ammonium acetate buffered at pH 7 after shaking for 2 hours (Rhoades, 1982). Exchangeable calcium (Ca) and magnesium (Mg) were determined by EDTA complexometric titration (Heald, 1965) while sodium (Na) was determined using EDTA complexometric titration (Knudsen et al., 1992). Exchangeable acidity (EA) was determined from 0.1NaCl extracts and titrated with 1.0N hydrochloric acid. Effective cation exchange capacity (ECEC) was determined by summing up total exchangeable bases and total exchangeable acidity.

Statistical analysis

Quantitative data was obtained and later analyzed using the Statistical Package for Social Sciences (SPSS) IBM version 23.0. The results of data analyses were expressed in the form of tables and figures. Both descriptive and inferential statistical tools were utilized for proper representation and understanding of results. Statistical differences in soil physicochemical characteristics between post-mortem intervals were performed using both one-way analysis of variance (ANOVA) and Post Hoc tests. A statistical significance of less than 0.05 was accepted.

Results and discussion

In Table 1, the pH levels of gravesoil samples differed significantly with increases on days 7, 14, and 21 ($p < 0.05$). In comparison with the day 0 samples, the concentrations of both Ca and Mg increased significantly on days 7 and 21. The concentrations of K increased significantly on both days 14 and 21 in comparison with day 0 control soil samples. There were slight increases in the concentrations of Na at days 7, 14 and 21 in close relationship with day 0. The EA levels increased on various experimental days, however, their values differed significantly when day 0 was compared with postmortem day 14 and 21. Finally, ECEC values increased significantly on both days 7 and 21 in close association with day 0 values.

Table 1. Effects of changes in soil chemical parameters on postmortem interval (Dry Season).

PMI (Days)	TBS	pH (H ₂ O)	Ca (mg kg ⁻¹)	Mg (mg kg ⁻¹)	K (mg kg ⁻¹)	Na (mg kg ⁻¹)	EA (Cmol kg ⁻¹)	ECEC (Cmol kg ⁻¹)
	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M
Day 0		5.23 ± 0.04	4.10 ± 0.29	2.20 ± 0.12	0.12 ± 0.00	0.17 ± 0.00	0.80 ± 0.00	7.39 ± 0.40
Day 7	16.3 ± 1.31	6.54 ± 0.12 ^a	17.60 ± 2.89 ^a	8.10 ± 0.47 ^a	0.14 ± 0.01	0.16 ± 0.01	1.05 ± 0.12	27.06 ± 2.58 ^a
Day 14	21.3 ± 0.86	6.51 ± 0.15 ^a	8.28 ± 1.05 ^b	3.93 ± 0.13 ^b	0.19 ± 0.02 ^{a,b}	0.20 ± 0.02	1.22 ± 0.09 ^a	13.81 ± 1.09 ^b
Day 21	26.7 ± 0.48	6.78 ± 0.12 ^a	21.98 ± 0.38 ^a	10.50 ± 2.37 ^{a,c}	0.20 ± 0.00 ^{a,b}	0.20 ± 0.01	1.57 ± 0.09 ^{a,b}	34.43 ± 2.81 ^{a,c}

a = differ significantly from Day 0 at $p < 0.05$; b = differ significantly from Day 7 at $p < 0.05$; c = differ significantly from Day 14 at $p < 0.05$.

As shown in Table 2, the pH levels of gravesoil samples increased at both days 7 and 14, however, differences in pH levels were significant on day 14 ($p < 0.05$) in comparison with day 0 while the pH levels at day 14 differed significantly to that of day 7. The values of Ca and Mg were increased on both days 7 and 14. The concentrations of K increased significantly on both days 7 and 14 in comparison with day 0 control soil samples. The concentrations of Na at day 7 differed significantly to that of day 0, although, the concentrations of Na at day 14 differed significantly to that of day 7 ($p < 0.05$). Both EA and ECEC values increased on both days 7 and 14 in close association with day 0 values.

Table 2. Effects of changes in soil chemical parameters on postmortem interval (Wet Season).

PMI (Days)	TBS	pH (H ₂ O)	Ca (mg kg ⁻¹)	Mg (mg kg ⁻¹)	K (mg kg ⁻¹)	Na (mg kg ⁻¹)	EA (Cmol kg ⁻¹)	ECEC (Cmol kg ⁻¹)
	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M	Mean ± S.E.M
Day 0		5.55 ± 0.03	5.40 ± 0.12	2.60 ± 0.12	0.09 ± 0.00	0.18 ± 0.00	0.80 ± 0.00	9.07 ± 0.01
Day 7	19.3 ± 1.07	6.04 ± 0.33	6.00 ± 1.51	3.80 ± 0.93	0.10 ± 0.00 ^a	0.14 ± 0.01 ^a	0.88 ± 0.21	10.92 ± 2.22
Day 14	31.3 ± 0.80	7.60 ± 0.04 ^{a,b}	6.90 ± 0.99	4.10 ± 0.76	0.13 ± 0.00 ^{a,b}	0.17 ± 0.01 ^b	1.08 ± 0.10	12.38 ± 1.63

a = differ significantly from Day 0 at $p < 0.05$; b = differ significantly from Day 7 at $p < 0.05$.

In the present study, the pHs of gravesoil samples after day 0 for both seasonal periods were significantly changed at subsequent postmortem intervals (PMIs). Changes in the pH can affect soil chemistry and potentially impact the interactions between soil particles, organic matter, and nutrients (Dupras et al., 2011; Wescott, 2018). This current study demonstrated that the pH levels of burial soils in close association with the control soils were significantly lower at different post-mortem intervals for both dry and rainy seasons. The decomposition process can release organic acids and other compounds that may influence the pH of the soil (Perrault et al., 2015). The rate of organic acid generation increases with the rate of carcass decomposition, which may cause the pH of the soil to drop more quickly (Irish et al., 2019). Previous research conducted by Melis et al. (2007) after examining burial soils of animal carcasses within a temperate region was able to show some levels of significance in the increased levels of pH concentrations. Benninger et al. (2008) and Meyer et al. (2013) noted from their findings that significant increases in burial soil pH were

observed upon day 14 of pig death – which agrees with this present study. It has been suggested that an increase in soil pH could be due to accumulations of ammonium ions (Benninger et al., 2008). The nitrification of ammonium to nitrates by soil bacteria can lower soil pH and these changes can, in turn, influence the decomposition process. Certain microbial communities thrive in specific pH ranges, and changes in pH can favor or inhibit the growth of decomposers (Averill & Waring, 2018; Qiu et al., 2018; Dhaliwal et al., 2019; Anthony et al., 2020).

Both soil calcium (Ca) and magnesium (Mg) levels were shown in the current study to have significant increases especially during the dry season period at days 7 and 21 in close association with the control soil samples. However, results based on the wet season revealed that both Ca and Mg levels did increase but not significantly. A similar study showed from their survey that calcium (Ca) was significantly elevated in the experimental cadaver decomposition islands (CDI) in the burial soils present in the CDI relative to the control soils (Aitkenhead-Peterson et al., 2012). Calcium is relatively immobile in soil compared to other nutrients like nitrogen (Kumar et al., 2020). As a result, the release of calcium from the cadaver might not be as influenced by leaching during the dry season. However, the slower decomposition might lead to a prolonged period of nutrient mineralization, with calcium gradually becoming available for plant uptake. Chemically, Mg acts as cation by interacting with other soil particles and molecules, whereby properties such as nutrient availability, cation exchange capacity (CEC), and electrical conductivity, are influenced by the presence of these cations like magnesium (Oliver et al., 2013; Gondek et al., 2019; Kumari & Mohan, 2021). These processes might be more dominant during the dry season, leading to a decoupling of their correlation with magnesium levels. In other words, the influence of magnesium on these parameters might be overridden by the stronger effects of these environmental conditions during that specific season. Furthermore, dry season conditions can lead to reduced soil moisture, which can affect the availability of nutrients and the activity of soil microorganisms (Sugihara et al., 2010; Abera et al., 2012).

The levels of potassium (K) in the gravesoil samples were shown to have elevated significantly at subsequent PMIs during both seasons in this current study. Related literature done by Aitkenhead-Peterson et al. (2012) and Szelecz et al. (2018) discovered from their outcomes that potassium (K) concentrations were significantly higher in their experimental burial soils compared to control soils. Soil sodium (Na) levels were known to increase in the gravesoil samples of dry season while that of the wet season slightly decreased significantly. Elevated sodium levels in soil can interfere with the availability of essential nutrients for decomposers, such as bacteria and fungi. These microorganisms play a vital role in the decomposition process by breaking down complex organic compounds into simpler nutrients that can be absorbed by plants and other organisms (Singh, 2016; Camenzind et al., 2018). High sodium content may negatively affect microbial populations, leading to a slower rate of decomposition (Singh, 2016). As explained by some studies, sodium can impact soil pH levels since excess sodium levels can affect the activity of decomposer organisms in decomposing soils that are slightly alkaline. These alkaline conditions in the soil may inhibit the growth and activity of decomposers, further slowing down the decomposition of the carcass (Ardahanlioglu et al., 2003; Ng et al., 2022).

Soil exchange acidity (EA) refers to the concentration of exchangeable hydrogen ions in the soil, which can influence soil pH and overall soil health (Sobola, 2023). During hot conditions, the release of organic acids from the decomposing cadaver could lead to gradual changes in soil pH, resulting in a mild increase in soil exchange acidity. Thus, this study revealed that the EA levels of both dry and wet gravesoil samples were elevated – however, significant changes were observed in that of dry season at different PMIs. The effective cation exchange capacity (ECEC) of soil refers to the ability of soil to retain and exchange positively charged ions (cations) like calcium, magnesium, potassium, and others (Oyebiyi et al., 2018). Similar to EA concentration levels in gravesoils of dry season, the present study also showed significant increases in ECEC levels. As carcass decomposition progresses during the dry season, this could lead to an increase in cations available for exchange, potentially contributing to an increase in ECEC. In the wet season, increased moisture and favorable conditions for microbial activity can lead to faster cadaver decomposition thereby resulting in a more rapid release of nutrients from the cadaver tissues into the soil. Changes in pH can alter the charge characteristics of soil particles thereby affecting their ability to hold cations and thus influencing ECEC. Certini (2005) suggested that a fall in organic matter, clay particle size, and clay fraction is brought about by intense temperature levels which could further cause a decrease in the ECEC of the soil.

Conclusion

The study concluded that there were significant associations between the postmortem intervals of study pig carcasses and the fluctuations in soil chemistry. The chemical properties of the gravesoil samples of the dry season study period showed more significant associations with PMIs in comparison to those of the rainy season. In comparison to day 0, the wet season findings showed that there were significant differences in pH, Ca, Mg, K, EA and ECEC levels, while the dry season findings from the study suggested that there were significant differences in pH, K, and Na concentrations. In line with the study's conclusions, comparative analyses on different gravesoil samples upon surface burials of pigs at different geographical locations within Nigeria should be considered to understand variations in soil chemistry.

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