Alteration in Physicochemical Parameters of Soil Beneath Rabbit Carcass: Consequence of Carcass Decomposition

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Abstract

Forensic taphonomy involves the use of decomposition to estimate post-mortem interval (PMI) or locate cadaveric grave. The process associated with carcass decomposition in outdoor setting. Some other methods are available to determine the accurate post mortem interval (PMI). Instead of these methods, a soil-based approach one of the best methods to determine post mortem interval. As a consequence, we investigated the physical characteristics of the carcass, soil pH, soil moisture content, electrical conductivity and the concentration of total carbon, total nitrogen and soil-extractable phosphorus in soil beneath rabbit (*Oryctolagus cuniculus* L.) carcass which was placed on soil surface up to skeletal stage of decomposition. The significant increases were observed in concentration of soil pH, moisture content, soil-extractable phosphorus and total carbon.

Keywords: *Decomposition stages, Forensic taphonomy, rabbit carcass, physicochemical.*

Introduction

Forensic taphonomy is an applied science with clear aims: Use the processes associated with cadaver decomposition to estimate post mortem or post burial interval, determine the manner of death, locate graves and identify the deceased[1,2]. Forensic taphonomy derives these aims from taphonomy, a branch of palaeontology[3]. The majority of these studies have focused on the activity of above ground insects[4;5] and scavengers[6] whereas less attention has been given to the processes that occur in soils associated with cadaver breakdown[7].

The estimation of soil in a demise examination is frequently taken as cooperative proof. This mirrors the customary perspective of criminological science: Soil is a medium that can be characterized by natural substance, and physical properties[8]. Soil has evidential values, it contains mineral, vegetation (both vegetative and as pollen), and animal material (living and dead). The components like fossils, bone and glass are also found in soil, these all are rare and helpful for characterization. Now a day stereomicroscopic observation, scanning electron microscope, x-ray spectrometer, detection of soil colour (before and after heating soil samples) and macroscopic observation are being used for the soil studies. These attributes are generally performed to investigate a serious crime.

Decomposition is a process that commences immediately after death and, depending on environmental conditions, will proceed until skeletonization has occurred[9,10]. The chemistry associated with decomposition and the destruction of soft tissue is complex[11]. During decomposition chemical components are released from body through autolysis and putrefaction[12]. Carcasses are mainly decomposed by microbes and invertebrates[13]. Decomposition results the degradation of carbohydrates, lipids and protein, which will yield carbon-based, phosphorous-based and nitrogen-based products which may be retained in the soil from the body of carcass, therefore, Grave soils were found to have higher levels of total C and total N after burial.

Research model: **-** A rabbit (*Oryctolagus cuniculus* L.) carcass weighing about 2.5 kg was used as a research model to study alteration in physico-chemical parameters of soil beneath them. The rabbit carcass was procured from rabbit farm and placed on soil surface.

Experimental site: - The course of decomposition of carcass and its impact of soil was studied during March, 2016 at village Miani of District Hoshiarpur, Punjab. The site of experiment was located north side of Dasuya (31°42′15″N, 75°35′5″E). The climatic condition of experimental site during research period is; the normal annual rainfall of experimental site is 991 mm and average temperature is varying from 24°C to 42°C during experimental period. The soil of experimental site is loamy with pH vary from 7.4 to 8.2. The unwanted vegetation of experimental site was removed. The heavy iron cage was used to cover the carcass to protect them from animal scavengers.

The soil samples were collected with the help of rustproof stainless steel spatula which has no chemical interference with soil. During sampling, approximately 40 g of soil was collected below carcasses and placed into zip lock bags. The collected samples were sieved, placed in sterile vials and transferred to deep freezer (-20°C) for pending analysis[14].

Analysis of Physico-Chemical Characteristics of Soil

Soil pH was calculated with the help of pH meter. One part of soil and five parts of water (1:5) were taken for the analysis[14]. Before the analysis the instrument was calibrated with solutions of different pH i.e. 4.0, 7.0 and 10.0. Electrical conductivity of soil was calculated with the help of digital conductivity meter. Soil and water were taken in 1:5 ratios. Calibration was done with KOH solution[15]. Moisture content of the soil was estimated by heating methods. 5 grams of soil were taken and heated at temperature of 105°C for 24 hours in oven. Weight of pre heated and post heated soil was recorded with the help of weighing balance. Difference between initial and final weight of soil is moisture content of soil.

The extraction of phosphorus from soil has been done according to[16] soil extraction method. The acid-soluble forms of phosphorus were extracted by using combination of Hydrochloric acid and Ammonium fluoride. 1 g of oven dried soil was extracted with diluted of ammonium fluoride (NH_4F) and concentrated hydrochloric acid (HCl). The soil samples were centrifuged at 6000 rpm for 5 minutes. Dispense 0.50 mL of the supernatant plus 2.0 mL colourimetric reagent was added and mix into a test tube to stand for 30 minutes. Soil-extractable phosphorus absorbance was measured with the help of Spectrophotometer at wavelength of 880 nm.

A colourimetric reagent was prepared to dissolved 17.14 g ammonium molybdate $[(NH_4)6MO_7O_{24}.4H_2O]$ in 200 mL of warm deionised water, 0.392 g potassium antimonyl tartrate ($KSbO.C_4H_4O_6$) separately in 150 mL deionised water. Place 500 mL deionised water in a 2 L volumetric flask and slowly add 200 mL concentrated sulphuric acid with mixing.

Total carbon and total nitrogen content in soil was analysed by combustion with helium gas and estimated by using Vario EL Cube Elementar CHNS analyser. The temperature of combustion tube was maintained at 1150°C and 850°C for reduction tube. The analysis was conducted at Punjab Agriculture University, Ludhiana (Punjab).

Statistical analysis: - The statistical analysis was conducted by using software SPSS Version 15 to compare variation among parameters in experimental soil and control soil. The variance in parameters was tested by using Levene's test.

Results

Rabbit carcass took 9 days for complete decomposition. The decomposition was divided into different stages viz. fresh, bloated, active decay, dry decay and skeletal. The soil beneath carcass were taken from three different depths i.e. 0-5cm top, 6-10 cm middle and 11-15 cm in order to check pH, electrical conductivity and moisture content, but in case of total nitrogen, total carbon, soil-extractable phosphorus upper layer (0-5 cm) of soil were taken into account because most significant changes were occur in top most layer of soil only. This experiment was conducted in the month of March, 2016. The average temperature during experiment was 38±1° C and humidity was 27±1%.

Soil pH

A significant difference ($P < 0.05$) in pH of control soil and soil beneath carcass was observed (Fig. 1). Soil is become slightly acidic during active decay stage as compare to control soil due to seepage of fluid into the soil.

Electrical conductivity

There was no significant difference ($P > 0.05$) in electrical conductivity of control soil and experimental soil was observed (Fig. 2). Increase was seen during active decay stage of decomposition at depth of 5 cm and 10 cm of soil samples as compare to control soil.

Moisture content

A significant difference (P < 0.05) in moisture content of control soil and experimental soil was observed (Fig.3). The moisture content of experimental soil was high during bloated, active decay and dry decay stages of decomposition due to seepage of body fluid.

Soil-extractable phosphorus

There was a significant difference (P < 0.05) in soil-extractable phosphorus of control soil and experimental soil was observed (Fig. 4). This is due to the large number of components like enzymes, phospholipids, proteins; nucleic acids which were store in the form of phosphorus in the body and a decomposing carcass can release high amount of phosphorus in the soil beneath carcass.

Total carbon

A significant difference (P <0.05) observed in concentration of total carbon in experimental soil and control soil (Fig. 5). The increase in total carbon concentration of soil was observed during dry decay stage of decomposition. This is due to release of CO_2 gas during decomposition.

Total nitrogen

There was no significant difference (P > 0.05) was observed in total nitrogen content of soil beneath rabbit carcass and control soil (Fig. 6). The increase in total nitrogen concentration observed during dry decay stage of decomposition. However, the nitrogen concentration level returns to basal level by skeletal stage of decomposition.

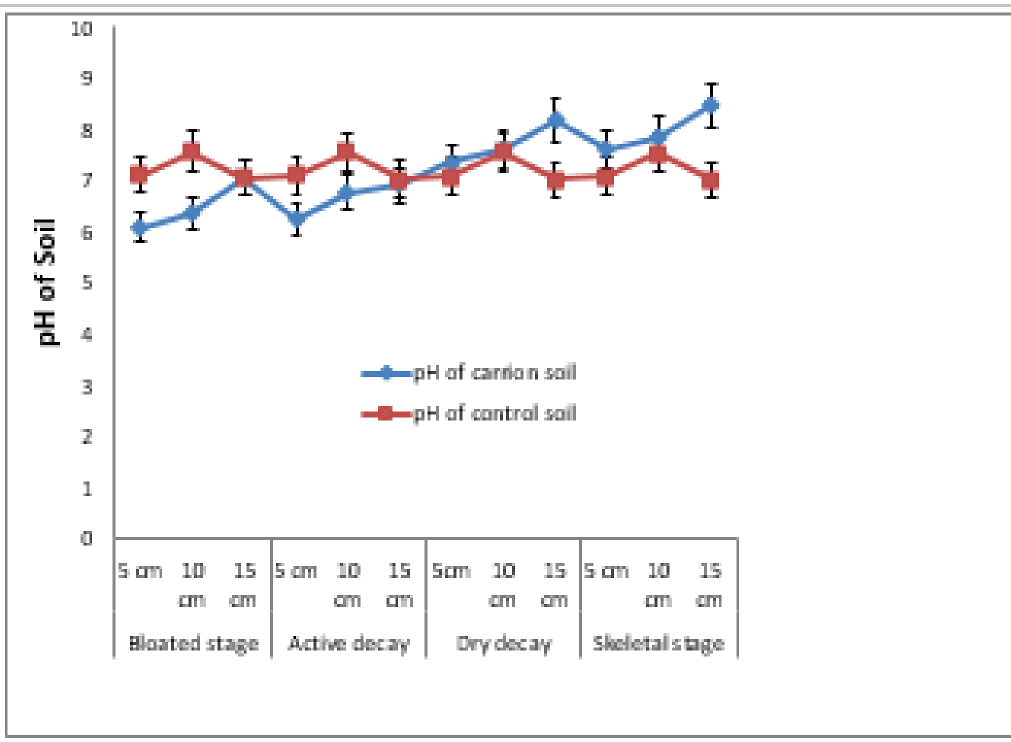

Fig. 1. pH of control soil and soil beneath rabbit carcass

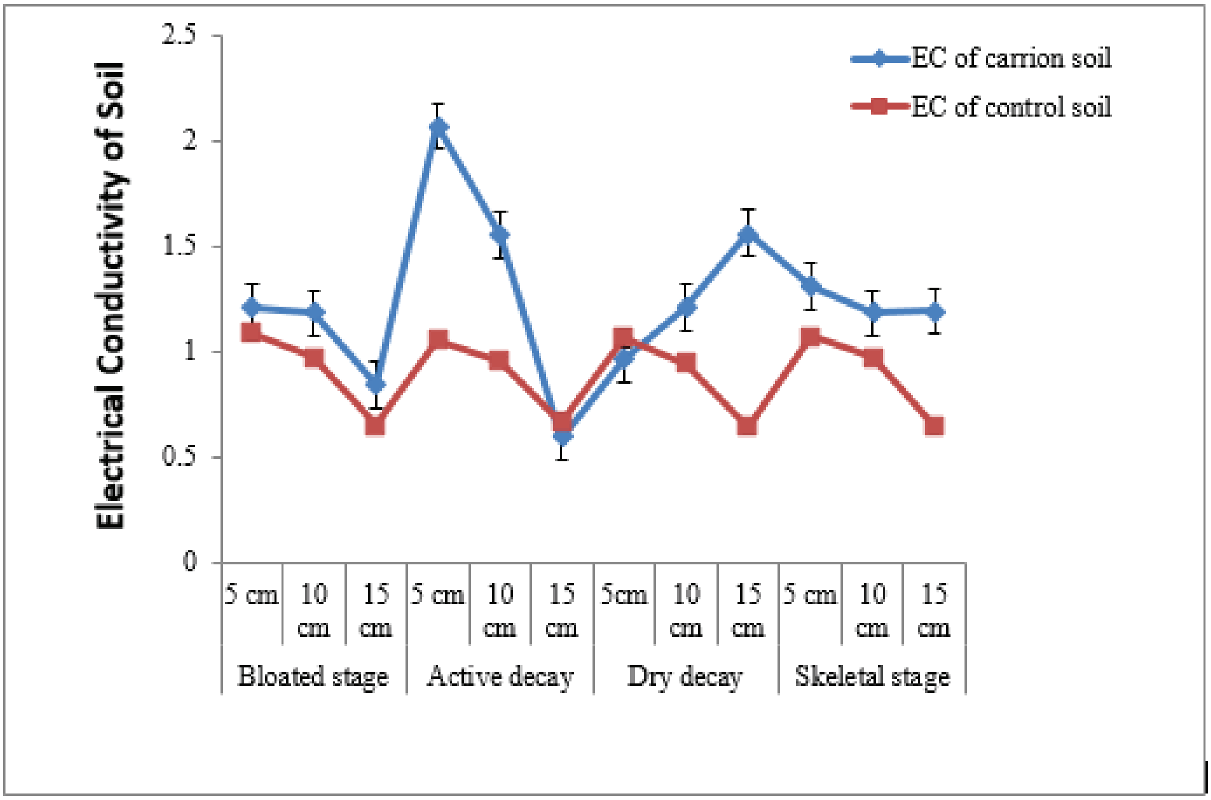

Fig. 2 Electrical conductivity of control soil and soil beneath rabbit carcass

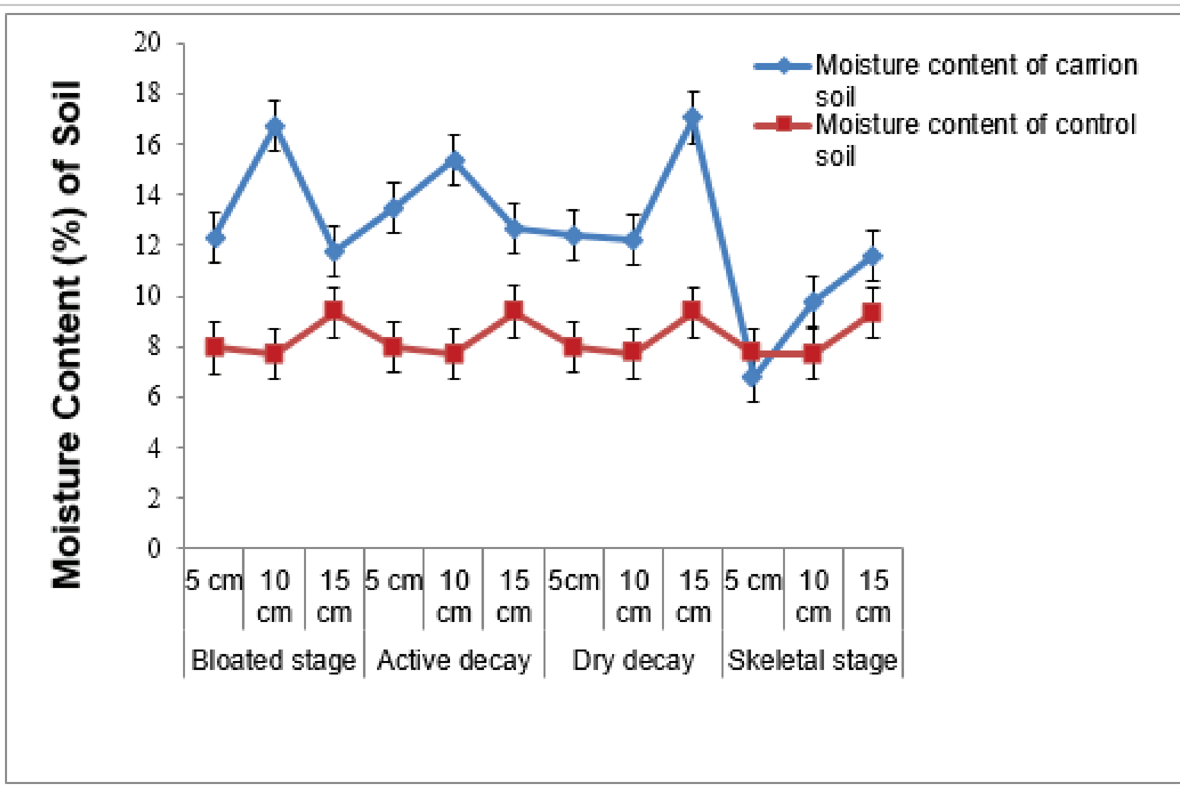

Fig. 3. Moisture content (%) of control soil and soil beneath rabbit carcass

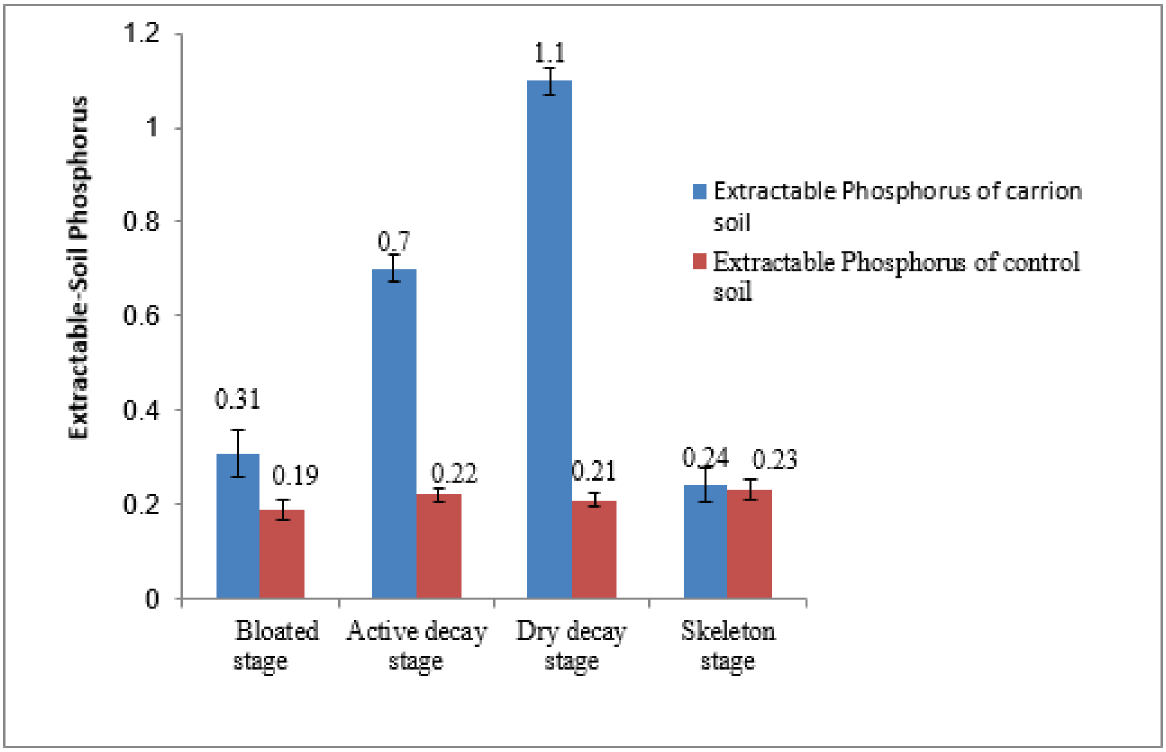

Fig. 4. Extractable Phosphorus of control soil and soil beneath decomposed rabbit carcass

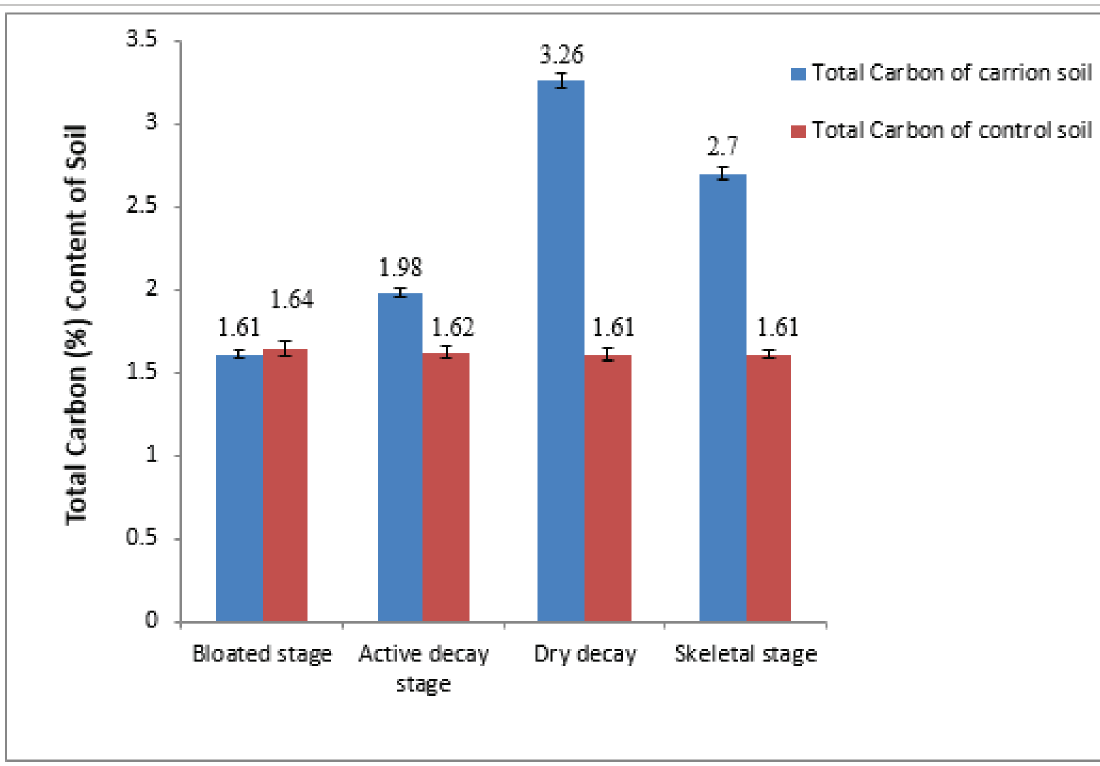

Fig. 5. Total Carbon (%) content of control soil and soil beneath rabbit carcass

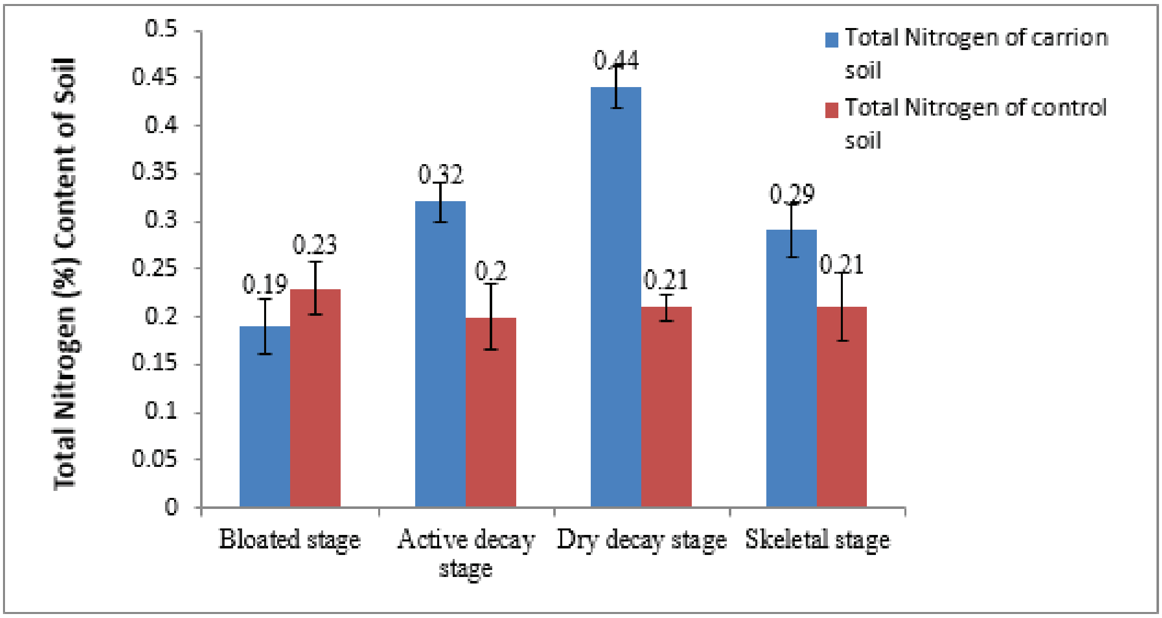

Fig. 6. Total Nitrogen (%) content of control soil and soil beneath rabbit carcass

Discussion

The present study has significance from forensic point of view and has implication in PMI estimation. In the entire study the biochemical attributes were considered, the alteration in these attributes support the forensic investigation in estimation of time since death as well as place of death by comparing the contents of control soil and soil beneath corpse. The significant increase in concentration of nutrients occurs during bloated stage, active decay stage and dry decay stage of decomposition. Currently forensic entomology is considered as most accurate means to estimate PMI. However, forensic entomology will not able to answer all the questions after emergence of adult blow flies. If proper experimentation occurs then nutrient cycle in soil could be an important tool in cases where bodies remain undiscovered for extended period of time. In current study rabbit carcass was used as research model. Five decomposition stages were observed during whole course of study. Previous decomposition studies provided conflicting results. pH is almost remain same in control soil as well as in experimental soil during fresh stage of decomposition due to negligible seepage of nutrient into the soil. In previous study the increase in pH values was observed during decomposition of carcass[14]. In some other decomposition studies by also provide variation in pH level of soil beneath carcasses[17,18]. Soil pH may decrease during early stages of decomposition as a result of the fermentative processes carried out by anaerobic bacteria in the gastrointestinal (GI) tract as well as in soil[19] and subsequently increase due to carcass proteolysis[18]. The reduced level of pH did not increase over time as compare to control soil. The significant changes in soil was seen only in upper layer (0-5 cm) of soil as compare to middle and bottom layers of soil and this is due to the rapid conversion of the Nitrate to Ammonium ion by the microbes. Variations in pH level not able to demonstrate consistent correlation with process of decomposition.

The concentration of soil-extractable phosphorus was significantly increases during the decomposition of carcass. The significant increase detected in soil-extractable phosphorus concentrations beneath a decomposing carcass of pig (*Sus scrofa*) during 100 days of decomposition study of grave soil[14]. A significant increase in Phosphorus concentration beneath a decomposing ungulate (*Bos bison*) carcass and given the results that there was a increase in Phosphorus level of soil at carcass site as compare to control soil, upto 3 years post-mortem[20]. In addition the large number of components likes enzymes, phospholipids, proteins; nucleic acids are store in the form of phosphorus in the body. After death these all components flow out from the body in form of fluid and mix up with soil. Therefore a decomposing carcass can release high amount of phosphorus in the soil beneath carcasses.

In present study the total carbon content would increase during bloated and active decay stages of decomposition. Otherwise there was no significant difference between total carbon content of control soil and experimental soil occurred during other stages of decomposition. There was no significant difference of total carbon concentration between control and grave soil was observed beneath decomposed carcass[14]. The total carbon concentration in soil was due to carbon dioxide gas could release from the body of carcass and mixed in soil or lost in environment.

A significant increase in total nitrogen content has been reported in present study which was similar with the previous study which was conducted on gravesoil[14]. The significant increase was observed in total nitrogen concentration of soil beneath decomposing carcass[21]. There is an either organic or inorganic form, i.e. NO_3^-N and NH_4^-N of nitrogen which helps to increase the level of nitrogen. A decomposing carcass is a rich source of nitrogen.

Conclusion

The present study satisfactorily demonstrated that decomposition of carcass influxes a significant amount of nutrients into the soil beneath carcass. There is a significant variation in some physico-chemical parameters of soil beneath carcass as compare to control soil viz. Soil pH, moisture content, soil-extractable phosphorus and total carbon due to influx of nutrients into the soil. There is also variation among parameters according to stages of decomposition, which assists in PMI estimation in case where decomposed remains scavenged or displaced from its original position.

The results of this study are only applicable to the local soil environment, region and area from where soil samples were taken and cannot be extrapolated to other soil types or conditions. Hence, future studies will also investigate the nutrient influx from decomposing remains in a range of soil types to determine whether those patterns observed in this study are reproducible in different soil conditions.

We conclude that the effect of a rabbit carcass on the nutrient concentrations of soil is evident in this part of world. We believe that the decomposition of human remains in this environment would also increase nutrients level into the soil. This information has the potential to assist investigations in confirming the presence of decomposed remains where remains have been scavenged or dragged from their original site, or in shallow grave sites where relocation of the remains has occurred post-mortem. In spite not include in the present study there is a suggestion of utilizing soil microorganisms to estimate the PMI. Soil microbiology along with carcass microbiology plays important role in flawless evaluation of PMI.

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Ethical Clearance: Not required.

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