

Time-course analysis of chemical and physical properties of organic fertilizer from *Tithonia diversifolia* leaves

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Abstract

Purpose Application of *Tithonia diversifolia* leaves as organic fertilizer is common in most rural regions in Africa. There is usually time period for curing between preparation and application stage. However, this curing period leads to reduction in fertilizer efficiency. This study aimed at analyzing the change in functional groups, particle morphology, size and elemental composition from preparation and application stages of *T. diversifolia* leaves as organic fertilizer.

Method The fertilizer was synthesized from *T. diversifolia* leaves. The synthesized fertilizer was stored at room temperature and pressure conditions for a grace period of 5 days; as is norm. Characterization of the samples at the two stages was done by FT-IR (functional groups), SEM (morphology), TEM (particle size) and EDS (elemental composition).

Results The results indicated reduction or complete loss of amine groups and carboxylic groups over this period. The fertilizer particles agglomerated over the storage period. Particle size increased from 112 nm to 133 nm diameter. There was a significant variation in the elemental composition between preparation and application time of the fertilizer samples.

Conclusion These findings prompt for better storage techniques to be used or reduction in curing and storage period of the fertilizers to minimize loss of plant nutrients.

Keywords *Tithonia diversifolia*, Organic fertilizer, Preparation, Application stage

Introduction

Food demand in the world is constantly growing with increasing human population. Use of fertilizers to supplement soil nutrients is seen as one of the remedies to this challenge (Fedoroff 2015; Shiferaw et al. 2011). However, most of the fertilizers used are inorganic and make the soil heavily reliant on them (Lin et al. 2019). These fertilizers are also expensive and associated with

soil and water pollution (Reynolds et al. 2015). To fill this gap, there is more need for exploitation of organic fertilizers especially in rural and marginalized areas where the Gross Domestic Product, GDP of its residents is low (Castelli 2018) and there is abundant organic matter to make organic fertilizers. Un.org (2017) predicts that the global population will hit 10 billion people by 2050. Despite the population increment, food production resources such as arable land, clean water and environment and fertile soils are fixed (Pretty and Barucha 2014). Actually, there is more competition for use of arable land to be used for other activities (Smith et al. 2010). It is thus pertinent to optimize on the available resources to maximize on food production. Use of fertilizers is one such technique that has successfully been used to increase food production over time (Beddington 2010).

Fertilizers are compounds that replenish available plant nutrients either in the soil or directly to a plant.

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Some fertilizers also restore the right soil conditions to boost plant growth, such as urea and lime which restore soil alkalinity (Goulding 2016). Fertilizers supply most of the basic plant requirements (especially nitrogen, phosphorus and potassium). However, some fertilizers are tailored to supply only specific nutrients to plants (Yousaf et al. 2017). Whereas mineral fertilizers are inorganic and chemically manufactured, organic fertilizers have a biological background with little or no chemical preparation required. Organic fertilizers have relatively less plant nutrient concentrations compared to the mineral ones (Han et al. 2016). They are however eco-friendlier and cheaper to prepare (Baghdadi et al. 2018).

Organic fertilizers have several environmental merits over the organic ones. Some of these merits include: more degradability in the soil, less soil poisoning as well as less chances of causing water and air pollution. Continuous application of mineral fertilizers onto a piece of land cause interference of the chemicals in the fertilizer with the soil composition leading to reduced soil fertility. High concentration of the chemicals also toxify the ecology of important soil micro-organisms chasing them away. The mode of application of several mineral fertilizers is also a source of water and air pollution. Top-dressing and foliar application of mineral fertilizers in windy conditions carry away some of the volatile compounds in the fertilizers, such as ammonia. These chemicals undergo photoreactions and are cleaved to yield harmful radicals that lead to ozone depletion (Chaka and Osano 2019). Other chemicals prevent radiation of heat from the earth and cause global warming while others react with atmospheric moisture and precipitate down as acid rain. Mineral fertilizers applied on the surface of the soil might get carried away by running rain water to leach into water bodies and cause water pollution (Maghanga et al. 2013). Organic fertilizers have their chemical nutrients embedded in cellulosic organic biomass. The nutrients can thus not be easily ionized out lowering their chances of volatilization into the atmosphere or being leached out by running water.

Organic fertilizers are prepared from both animal and plant sources. One of the most exploited sources of organic fertilizers is leaves droppings. *T. diversifolia* leaves are commonly used in several regions to prepare organic fertilizers. The plant grows in both humid and sub-humid conditions in tropical regions (Jama et al. 2000). The leaves are not only rich in nitrogenous com-

pounds (Reis et al. 2016) but also quite neutral thus do not interfere with soil pH (George et al. 2002). Preparation of organic fertilizers from *T. diversifolia* is also quite easy. Foliar from the leaves are prepared by soaking the leaves in water in an anoxic media for about one week. The crushed leaves of *T. diversifolia* can also be mixed with other animal manure to form a composite manure useful in healing the soil.

Like other organic fertilizers, the functional properties of *T. diversifolia* leaves are also affected by storage conditions. Even at room temperature and pressure conditions, away from direct sunlight, some bio-chemical reactions still proceed. Heat and sunlight are both known to induce or accelerate chemical reactions in fertilizers. Fertilizers with appreciable concentrations of volatile compounds such as ammonia change when exposed to these factors. Change in the initial presence and concentration of chemical ions interfere with the availability of other ions present (Hribar et al. 2002). It is therefore pertinent to elucidate the exact variations in available plant nutrients during preparation of organic fertilizers. This study aimed at analyzing the variations in chemical composition, functional groups, particle size and morphology of *T. diversifolia* organic fertilizer between the time of preparation and application. The findings hereby obtained will go a long way in informing the right time for application tailored for various plant needs.

Materials and methods

Design of experiment

The research was guided a longitudinal design of study. This method was chosen for its suitability to meet the objectives of the study that involved examining the characteristics of *T. diversifolia* organic fertilizer at different stages of its formulation. Foliage powder samples were formulated and analyzed the preparation and application stage (separated by a grace period of 5 days). There was no variation in other variables for the 2 set of analytes. The difference in chemical composition, functional groups, morphology and particle size was then analyzed by spectroscopic methods.

Sampling was done in Maasai Mara University, Kenya (1.1041°S, 36.0893°E) while analysis was done at University of Johannesburg, South Africa (26.2041°S, 28.0473°E).

Sampling and preparation of *T. diversifolia* foliage samples

Fresh *T. diversifolia* leaves were collected with the help of a Maasai Mara University, Kenya botanist before assigning sample numbers. Sampling was strictly purposive with only fresh, succulent and green leaves being targeted. The samples were collected from around Narok, Kenya (co-ordinates 1.1041°S, 36.0893°E).

The samples were then washed with distilled water and chopped into small pieces of 5 to 10 mm. These pieces were shade-dried in abundant ventilation at 18-24 °C until they were brittle due to loss of moisture. The samples were then homogenized and the process repeated until all the samples were dry. An electronic blender (Binatone 350W Blender BLG-451) was then used to fluidize the samples into finer particles. Mechanical milling was thereafter employed and a 0.90 µm filter used to sieve out larger particles. The fertilizer particles were assessed for their physical properties before being divided into two equal batches. The first batch represented that used at preparation stage while the second batch (to be incubated for 5 days) represented that at application stage. The second (representing application stage) batch was stored in a cool and dry place away from direct light as is conventionally done. During the incubation period, the samples were regularly agitated to avoid any adhesion of the particles.

Characterization of the *T. diversifolia* fertilizer samples

Both sets of fertilizer analytes were subjected to similar characterizations (all by spectroscopy) as described below:

Functional groups were elucidated using Fourier Transform Infra-Red (FT-IR) spectroscopy (Perkin-Elmer Spectrum Version 10.4.2). The extracts were gradually concentrated to remove any moisture content available. The samples were then cast into pellets using standard KBr pellet before analysis.

Particle sizes of the samples were determined using Transmission Electron Microscope (JEM-2100 multipurpose, 200 kV analytical electron microscope equipped with Oxford SDD Detector for TEM: X-MaxN 80T EDS). The samples were dispersed in suitable aqueous suspension; (0.1% wt) then deposited on the surface of a copper grid coated with a thin carbon film. Subsequently, the samples were dried before

carrying out TEM analysis at an accelerating voltage of 100-120 kV.

Scanning Electron Microscopy, SEM (VEGA3 TEGSCAN) was used to impart a means to directly observe the morphological appearance of the samples. The images were captured using a Field Emission Scanning Electron Microscope (FESEM). Prior to imaging, the samples were coated using the gold sputtering method.

Elemental composition of the fertilizer samples was determined by Energy Dispersive Spectroscopy, EDS (FEI XL40 ESEM equipped with two EDAX Sapphire Si (Li) EDS detectors and MLA software). The machine was set according to standard methods and the dry powder samples introduced for analysis. All standard procedures were followed.

Results and discussion

Physical observation and characteristics of *T. diversifolia* fertilizer particles

The synthesized *T. diversifolia* organic fertilizer samples were green and had a fine texture indicating presence of very small particles (Hussein et al. 2019). The particles were relatively soluble in water with some of them dissolving as others remained suspended on the water surface. This indicated that the bulk of the particles were less dense than water. The partial solubility of *T. diversifolia* is attributed to presence of organic non-polar compounds. Solubility was however enhanced by agitation of the mixture after which majority of the particles dissolved. This property is quite desirable in many fertilizers as it enables easier applications of the fertilizers in formulations with polar carriers such as water (Bargaz et al. 2018). The fertilizer particles were granular shaped owing to irregular milling of the foliage samples. From the physical observation on this fertilizer, there was no significant justification for storage of the fertilizer as the parameters analyzed supported its applicability to plants.

Functional groups analysis

Fourier Transform Infrared (FT-IR) analysis ascertained there was effective sequestering or shift of functional groups present in the *T. diversifolia* fertilizer between the period of preparation and application stage (Fig. 1).

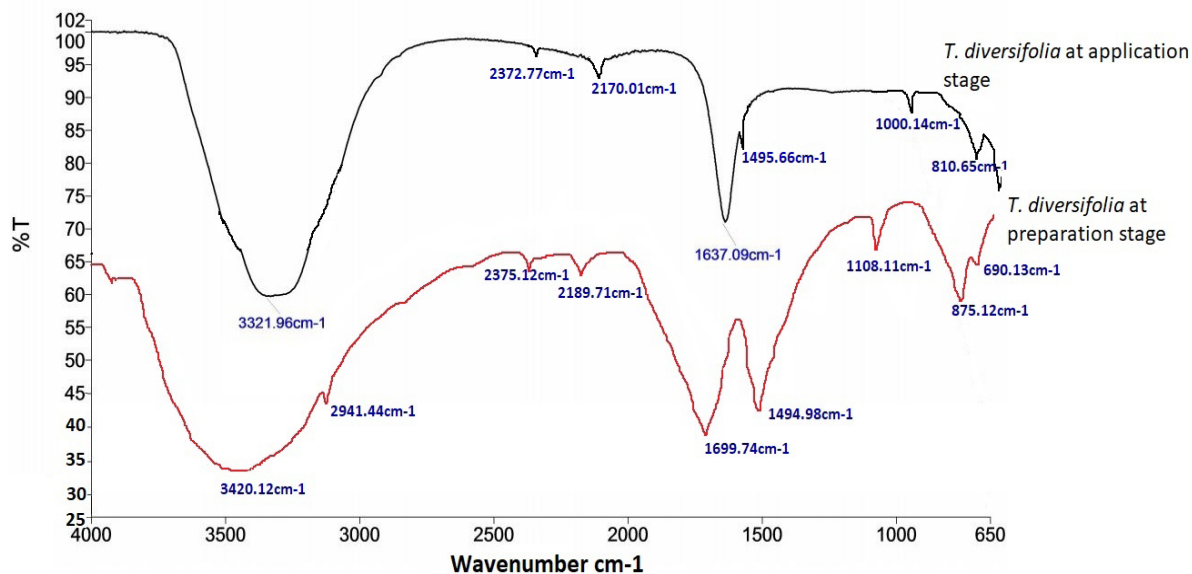


Fig. 1 Variations in functional groups present in *T. diversifolia* leaf sample at preparation and application stage

The broad peak at 3420 cm⁻¹ in the fertilizer sample at preparation stage is attributable to carboxylic acids O-H stretch. This peak shifted to 3321 cm⁻¹ in the fertilizer sample at application stage (5 days later). This can be as a result of chemical reactions that preceded the sample preparation during the storage period. The peak at 2941.44 cm⁻¹ which is associated with sp³ C-C stretch of plant biomass is seen to disappear at application stage. The disappearance is correlated with shifting of the O-H peak at 3321.96 cm⁻¹. Additionally, over time, carbon chains in the biomass are degraded by heat minimizing the C-C bonds. The peaks at 2375.32 cm⁻¹ (nitrides) and 2189.71 cm⁻¹ (azides) are seen to shift to 2372.77 and 2170.01 cm⁻¹. The intensity of these peaks is also reduced between the preparation and application stage. Nitrides and azides resulting from protein matter in the biomass degrade with time. Some of it is converted to ammonia gas which is quite volatile. The carboxylic acid carbonyl peak at 1699.74 cm⁻¹ shifted to 1637.09 cm⁻¹ as a result of adsorbed water by the fertilizer. There was a peak at 1494.98 cm⁻¹ associated with lignin, hemicellulose and pectin present in the *T. diversifolia* biomass. The peaks at 1108.11 cm⁻¹ that shifted to 1000.14 cm⁻¹ are as a result of C-O for sp³ hybridization (Moosavineja et al. 2019). From the changes in functional groups observed, there is need for the farmers to improvise better curing or storage conditions that minimize changes in the fertilizer properties.

Surface morphology

Scanning electron microscopy (SEM) analysis indicated difference in surface features of the fertilizers from *T. diversifolia* both at preparation and application stage (Fig. 2). From Fig. 2(a) (at preparation stage), well dispersed dark grey and light grey surface of the fertilizer particles were observed. The morphological properties depicted less agglomerations of the fertilizers with few clumps that can be as a result of reagents used during sample preparation prior to analysis. Small pores are seen on the surfaces that are well distributed on the SEM image. The pores are very important in fertilizers as they enhance better aeration of soil and circulation of water (through capillary) to be absorbed by the plants as well as passage of the plant roots into the soil (Torbert and Wood 1992). From Fig. 2(b) (at application stage), the fertilizers started to whiten with increased agglomerations as observed from the SEM image. Whitening of the surface can be as a result of loss of volatile nutrients, and speciation of metal ions. The agglomerations can be associated with moisture adsorbed by the fertilizer from the environment that made the particles to come together (Wuana and Okieimen 2011). The pores on the surface are seen to decrease with the increase in diameter as a result of the formation of big clumps of the fertilizer. This is because during their incubation period the fertilizer particles adhere onto each other. This is attributed to presence of moisture content that

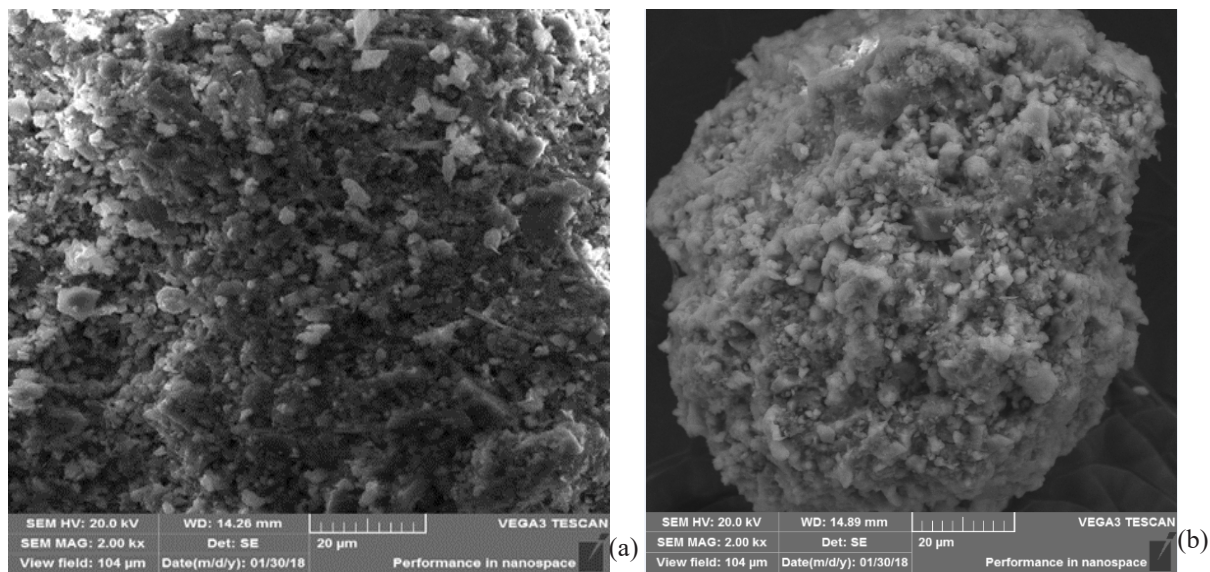


Fig. 2 SEM images of *T. diversifolia* fertilizer at preparation stage (a), and at application stage (b). The two images portray a change in the properties of the fertilizer over time

enhances molecular interactions of the fertilizer particles leading to formation of clumps (Olad et al. 2018). The surface of the clumps thus exhibits less pores compared to those of *T. diversifolia* fertilizer at preparation stage. Fig. 2 illustrates the surface and morphological appearance of the fertilizer at both stages of analysis.

From the SEM images, freshly prepared samples were quite adhesive to each other. This feature disappeared when the samples were analyzed after the incubation period (application stage). This phenomenon is attributed to loss of water in the samples at application stage. Water is known to aid in gelling fertilizer particles together (Shavit et al. 2003). The morphological results observed indicate that there is need for close and regular monitoring of *T. diversifolia* fertilizer under curing or under storage conditions. This can ensure that agglomeration, which can reduce the availability of nutrients uptake by plants is reduced (White and Brown 2010).

Particle size analysis

TEM was used to determine the effects of stage on the particle sizes of *T. diversifolia* fertilizer. The fertilizer was analyzed for particle sizes at preparation stage and after storage period of 5 days (Fig. 3). From the TEM images, the particles of the fertilizer at preparation and application stage are seen to be spherical in shape with uniform distribution throughout the matrices of the fertilizers. However, the fertilizer samples displayed

several agglomerations with particle sizes of 112 nm at preparation stage and 133 nm at application stage. This means that during the incubation stage, and as a result of the moisture adsorbed by the fertilizer, it was able to agglomerate with unnoticeable changes in the shape of the particles. Several dark spots were observed and this might be as a result of the TEM sample preparation medium. The observed agglomerations result from molecular interactions of fertilizer particles over time (Olad et al. 2018).

From the TEM images some 'rod like shapes are observed and could be as a result of ostward ripening of the organic fertilizer (Singh et al. 2018), enhancing further agglomeration of the fertilizer samples. Further agglomeration is attributed to more adhesion between the fertilizer particles with each other (Pietsch 1997) during the incubation period. This is why regular agitation of *T. diversifolia* fertilizer is necessary. Regular monitoring of *T. diversifolia* fertilizer as it cures is pertinent to ensure agglomeration is controlled. If this is not done, the efficiency of the resulting fertilizer is likely to reduce as plant minerals are not freely available for plants to uptake (White and Brown 2010).

Elemental composition

EDS analysis carried out indicated variation of chemical constituents of the organic fertilizer both at preparation and application stage (Fig. 4). From the spectra, Si, C and O presented highest percentages whereas Fe, Al,

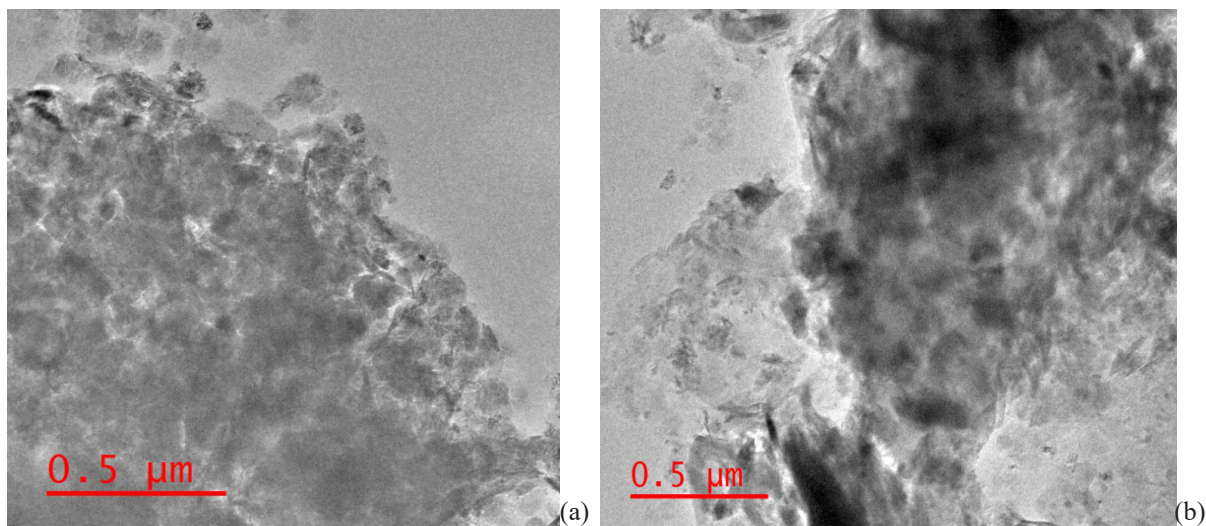


Fig. 3 The TEM images of the organic fertilizer at preparation (a) and application stage (b)

K, Ca, Na, Cu and Ti had less percentages. Si impurity is presumed to have come from sample collection site and preparation procedures. Variation in metallic composition of *T. diversifolia* leaves at preparation and application stage is observed with Ca and Fe presenting the highest variation. This variation can be as a result of Fe getting oxidized to other states of ionic iron (Moshe and Yona 2005). This can be attributed to increase in O percentages from 37.6% to 42.7% since oxygen is measured in form of its metal oxides. The same scenario is observed in variation of Ca levels in the fertilizer at preparation and application stage. Potassium which is the main macro-nutrient helps in enzyme activation, process of photosynthesis and production of Adenosine Triphosphate (ATP) as well as osmoregulation in plants (Wang et al. 2013). Its value in the fertilizer samples were seen to be much stable with slight changes in its percentage composi-

tion from 3.8% to 3.5%. The slight variation can be presumed to be as a result of accuracy of the EDS analyzer and environmental factors such as loss to the environment in volatile organic matter present. Volatile organic compounds (VOCs) volatilize into the environment over the incubation period alongside any ions (e.g potassium) present in the process reducing their levels (Baligar and Bennett 1986). Sodium (Na) percentage was observed to increase within the storage period from 2.1% to 2.8%. The increase is presumed to be as a result of conversion of sodium ions that were formed during the preparation stage to sodium metal. The increment in sodium levels during the incubation process implies the fertilizer at application stage has more ability to aid in plant metabolism and enhances the synthesis of chlorophyll (Maathuis 2014). Incubation process thus favors plant metabolism and chlorophyll formation.

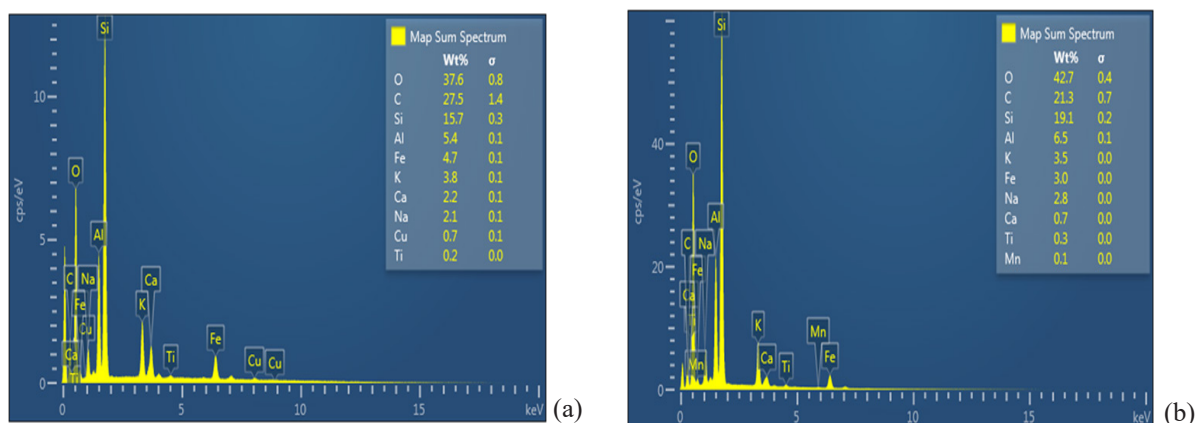


Fig. 4 Chemical composition of *T. diversifolia* organic fertilizer at preparation (a) and application stage (b). The EDS spectra illustrate there is a decrease in most of the micro-nutrients over time

The changes in chemical composition of *T. diversifolia* at preparation as a fertilizer and application stage are illustrated in Fig. 4.

Presence of Titanium (Ti) with the least percentage might have resulted from absorption of Ti present in the soil within growing areas. Carvajal and Alcaraz (1998) reported presence of the two major forms titanium, i.e., ilmenite (ferrous titanate) or rutile (titanium dioxide) in plants resulting from absorption from soils rich in these compounds. From both the EDS spectrum, absence of N which is the main macro-nutrient in *T. diversifolia* leaves can be as a result of complete utilization of nitrogen by the plant before the leaves fall off. Nitrogen is also susceptible to volatilization into the atmosphere, especially in the form of ammoniacal nitrogen (Otto et al. 2017). Elemental copper (Cu) which plays an important role in enzyme activation in plants (Printz et al. 2016) and in metabolism of carbohydrates and proteins (Esipenko and Marsakova 1990) was observed to be stable with a percentage weight of 0.7% at preparation and application stage. This implied negligible ionization of the metal to other forms as well as less volatility of the metal with other VOCs in *T. diversifolia*. From the elemental composition analysis carried out, curing and storing have favor increment of some elements while reducing others.

Conclusion

T. diversifolia leaf organic fertilizer samples were found to differ in functional groups, morphology, particle size and chemical composition between preparation and application stage (5 days apart). There was reduction or complete loss of carboxylic groups and amine groups over the storage period. *T. diversifolia* organic fertilizer samples were quite porous during preparation stage. However, this feature changed as the particles clustered together over time. The leaf particle sizes also grew from 112 nm to 133 nm between the time of preparation to application time. There was a slight variation in oxygen, potassium, sodium, titanium and calcium present between these two periods.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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