Influence of Climatic Factors on the $\square^{13}$c Values of the C₃, C₄ And CAM Dicot Species (Vegetation) of the Centrospermeaealong Altitudinal Gradient in western Region of Kenya.

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ABSTRACT: *Species of the Centrospermeae occurring at different altitudes were analyzed for $\delta^{13}C$ values and assigned for graphical representation. The aridity of the study area was evident as defined using the Klimadiagramm. Climatic data was studied and represented on graphs for interpretation. The frequency of$\delta^{13}C$ values of the species at different altitudes, namely 500m a.s.l., 1000m a.s.l., 1500m a.s.l., 2000m a.s.l., 2500m a.s.l., 3000m a.s.l., 3500m a.s.l. and 4000m a.s.l., are presented on graphs. The data show that$\delta^{13}C$ values is a good predictor of spatial diversity and shift of the species along the altitudinal gradient of environmental factors.There is phenomenal trend such that $\delta^{13}C$ values distribution along altitudinal differentiation the values of -10.60‰, to -16.65‰, -17.75‰ to -18.87‰, and -18.89‰ to -32.42‰ correspond to the species at low altitudes (0m a.s.l. – 1500m a.s.l.), intermediate altitude (1,550m a.s.l.-1,700m a.s.l.) and high altitude (1,800m a.s.l. – 4200m a.s.l.0, respectively. The inverse correlation between temperature and rainfall defines the causal climatic factors affecting C_3 and C_4 species along the altitudinal gradient. The occurrence of the transition zone between temperature and rainfall mirror that between the relative abundance of the C_3 and C_4 species along the altitude. This floristic data predict NAD-ME, NADP-ME AND PEP-CK types of monocot-dicot transition along the altitude with respect to bioproductivity in the tropics.*

Keywords:*Climatic factors, C_3, C_4 and CAM Centrospermeae species, vegetation, $\delta^{13}C$ values, Klimadiagramm*

I. INTRODUCTION

The $\delta^{13}C$ values are commonly used to distinguish between the C₃ and C₄ plants(Bender, 1971[1]; Smith and Epstein, 1971[2]; Smith, Oliver and McMillan, 1976[3]; Caldwell, White, Moore and Camp, 1977[4]; Epstein, Lauenroth, Burke and Coffin, 1997[5]). During the photosynthesis process, plants promote isotopic fraction of carbon dioxide has isotopes as ^{13}C and ^{12}C in the atmosphere. Plants species show a tendency to favour the lighter isotope (^{12}C) with respect to the heavier isotope (^{13}C). Thus, plants will have less ^{13}C in comparison to the atmospheric carbon dioxide. The degree of this isotopic discrimination is different between C₄ and C₃ species. The C₄ species category tends to have less negative values compared to the C₃ species category.

This carbon isotopic differentiation has been used to in the distinguishing the C₃ and C₄species (Hatch, 1976)[6]. The abundance of the isotopic composition (stable carbon isotopes) is influenced by environmental factors (isotopic composition of the atmospheric carbon dioxide and its concentration) and physiological factor (the carbon dioxide concentration inside the leaf intercellular space that controls the $\delta^{13}C$ values). Although the C₄ photosynthesis appears to be an adaptation to hot, high light intensity and arid environments (Mooney, 1974)[7], concern has been expressed that isotopic differences between C₃ and C₄ plants may represent, in part, phenotypic plasticity rather than true genetic difference (Moore, 1983)[8]. Earlier evidence show that the difference is indeed genetic (Bjorkman, Nobs and Berry, 1971[9]; Hatch and Boardman, 1972[10]) but concern for the influence of the environment variables on the $\delta^{13}C$ values exist (Buchmann, Brooks, Rapp and Ehleringer, 1996[11]; Williams and Ehleringer, 1996[12]; Hatch, 2002[13]). It has been shown that the changes in temperature or light intensity at which plants grew resulted in changes in isotopic ratios [6]. As a consequence, isotopic fractionation may occur at several sites (Park and Epstein, 1960[14]; Tieszen,1978[15]) such as diffusion of CO_2 into the leaf, diffusion from the atmosphere into the chloroplast, carboxylase catalyzed fixation (Whelan, Sackett and Benedict 1973[16]; Farquhar, 1983[17];Farquhar, Ehleringer and

Hubick,1989[18]; Anderson and Avron, 1976[19]) and subsequent metabolic changes to some degree but the results were not reproducible and predictable with the Xanthium data[19].

Carbon isotope composition of the C_4 grasses was shown to be influenced by light and water [11]. This can be used as an indicator of changes in the isotopic composition and concentration of atmospheric CO_2 especially for climate construction[11], because photosynthetic discrimination against ^{13}C remains constant in grasses in different climatic conditions in the arid areas. Carbon isotope discrimination studies in three semi-arid woodland species along the monsoon gradient and variations related to evaporative and relative gradient, stomatal conductance and transpiration potential werecarried out [2] and it was concluded that plants display a continuum of response to humidity ranging from predominantly leaf gas exchange to whole plant morphological adjustments. Thus, the effect of the environmental factors on the stable carbon isotope could be confirmed by similar studies in the Centrospermeae taxa in the semi-arid and /or arid ecosystem in Kenya. Therefore, the present study was to investigate the influence of the climatic factors on the δ^{13}C values among the C_3, C_4, and Crassulacean Acid Metabolism (CAM) species of the Centrospermeae along altitudinal gradient.

II.   Material And Methods

The belt transect, 30km wide between 34°30'E and 36°30'E, ran from South-West of Mt. Elgon to Mt. Kulal. It was about 365km long rising from 670m a.s.l. at Lodwar to 4200m a.s.l. at Mt. Elgon through 250m a.s.l. in Kerio Valley up to 2415m a.s.l. to 2293m a.s.l. Mt. Kulal then 950m a.s.l. Samburu game reserve and 900m a.s.l. at Kapedo-Nginyang. The sampling sites were randomly selected in a wide broad range of vegetation types from semi-arid to near snowline.

Ten-year climatic data was sourced from meteorological stations in the area of study. Further, one-year data of climatic variable was taken in the field. The stations included Rohet (1538m a.s.l.), Lokori (830m a.s.l.), Kitale (2084m a.s.l.), Chemolingot (950m a.s.l.), Perkerra (1067m a.s.l.), Chewoyet (2134m a.s.l.),Lodwar (506m a.s.l.), Nginyang (908m a.s.l.),Lokichogio (1050m a.s.l.) and Marigat (250m a.s.l.). Also, the field climatic data was measured. The mean of the eleven year climatic data was then calculated. Mean annual values of the climatic parameters collected included: mean annual temperature, mean annual maximum temperature and mean annual minimum temperature, mean annual relative humidity, mean annual potential rates of evaporation, mean annual radiation, mean annual rainfall and altitude measured by thermometer, dew-point hygrometer, 1litre pan water drying per metre in a day, thermopile Pyranometer, rain gauge and altimeter, respectively. The Klimadiagramm of Walter and Lieth (1973)[20] were used to describe the climate of the study sites.

Twenty-five quadrats placed at 10m interval random sites along the belt transect were used. Plant species were collected, especially healthy leaves, vegetative organs and flowering organs from different sites and enclosed in a wet toweling jar(s). Enclosed containers were used to transport the plant material to laboratory for further studies. Species were identified in the field. Difficulty species were taken to East African Herbarium or Chiromo Campus, University of Nairobi, Nairobi, Kenya for further verification and identification. Nomenclature followed Clayton (1974)[21], Beentje (1994)[22], Blundell (1992)[23], Lotschert and Beese (1994)[24], Agnew (1974)[25], Olembo, Fedha and Ngaira (1995)[26] system of identification and documentation. The vegetative parts, especially leaves of the species were dried under natural conditions 25°C - 30°C in the field for carbon isotope analytical studies.

Carbon isotope studies involved the following procedures. Air oven at 25°C-30°C circulation dried the leaves or vegetative organs until there was no further change in weight. Dried specimen were used for ^{13}C/^{12}C isotope analysis at Bayreuth Universitat, Germany. Dried leafy-milled grains of each of the species were analyzed using an elemental analyzer (HEREAUS CHN.O RAPID) for Dumas combustion of the sample, a FINNIGAN MAT Delta (δ) gas isotope mass spectrometer with dual inlet system, a method of Gebauer and Schulze (1987) [27]. Standard gas of carbon dioxide was calibrated with respect to international standard (CO_2 in Pee Dee belemnite) by use of reference substance NBS 16 to 20 for carbon isotopic ratio provided by International Atomic Energy Agency (IAEA), Vienna. The ^{13}C/^{12}C isotopic ratios (denoted as δ values or ^{13}C/^{12}C values) were calculated according to the following equation:

$$\delta_x = (R_{sample}/R_{standard}-1) \times 1000‰,$$

where δ_x, is the isotope ratio delta unis relative to the international standards, R_{sample} and $R_{standard}$ are the ^{13}C/^{12}C are the ratios of the samples and standards, respectively [27]. The δ^{13}C values were recorded for each species studied (Sikolia, Beck, Kinyamario, Onyango and Ouma, 2008) [28]and represented on graphs.

III.   RESULTS

Climatical factors measured included temperature, rainfall, relative humidity and radiation using thermometer, rain gauge, dew–point hygrometer and calibrated Eppley Black/White Pyranometer, respectively. Also, potential rates of evaporation was measured using a designated study model involving 1litre (mm^3) of water poured in a pan (1m^2) in randomized areas and allowed to evaporate between six-twelve hours

(Thornthwaite, 1948) [29]. Mean annual temperature and mean annual rainfall show negative correlation along the altitudinal gradient (Fig. i). Mean annual temperature, mean annual radiation and potential rates of evaporation show positive correlation along the altitudinal gradient (Fig. ii).
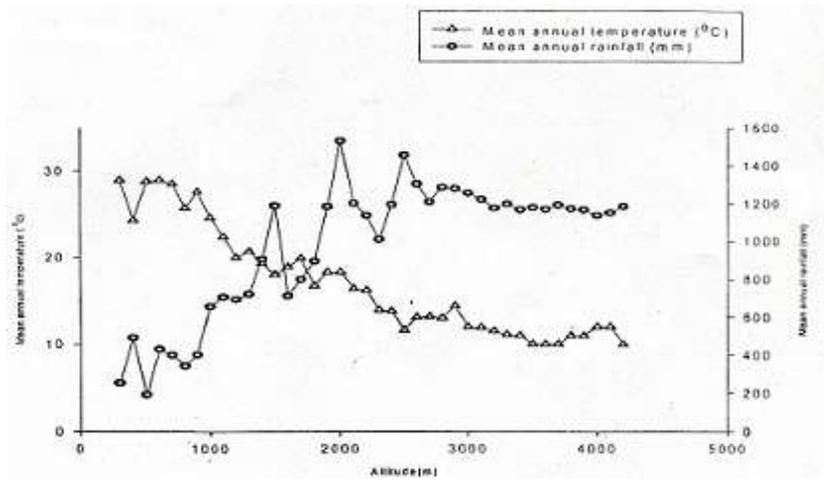
Figure 1. Mean annual temperature and mean annual rainfall along altitudinal transect gradient
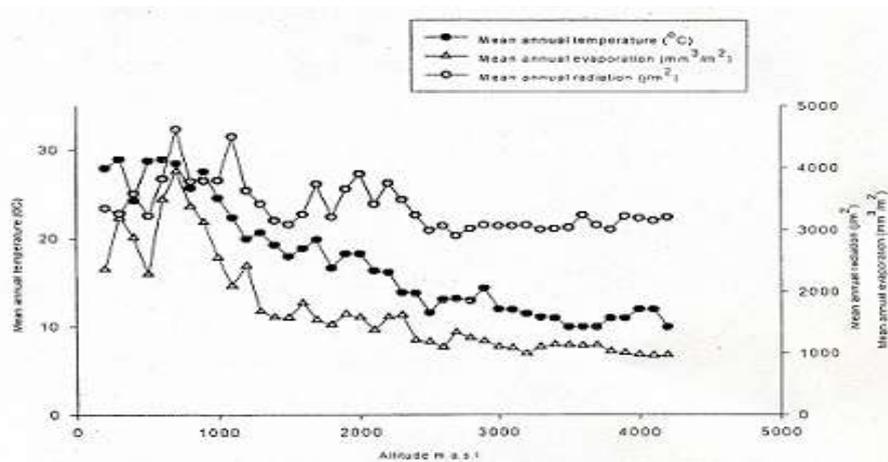
Figure 2. Mean annual temperature, mean annual radiation and mean annual potential rates of evaporation along the altitudinal gradient.

Further, mean annual temperature, mean annual rainfall and mean annual evaporation along altitudinal gradient show positive correlation (Fig.3) and similar trend is shown with mean annual radiation and mean annual evaporation (Fig. 4).
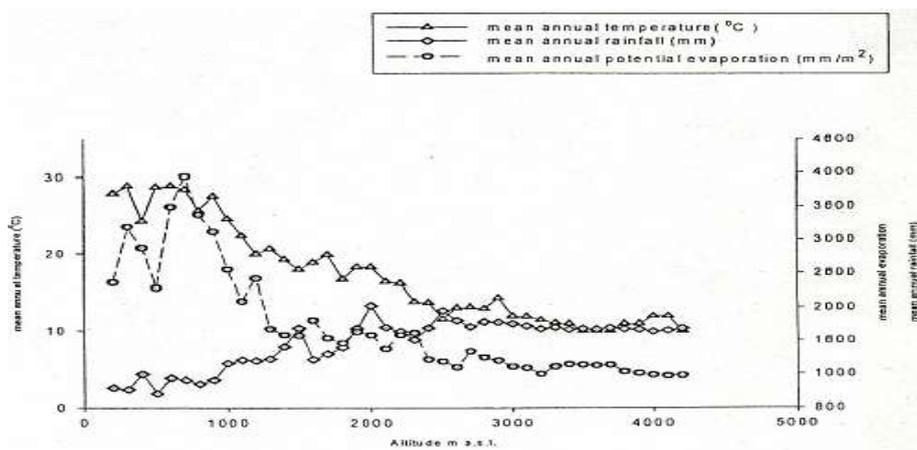
Figure 3. Mean annual temperature, mean annual rainfall and mean annual evaporation along altitudinal gradient.
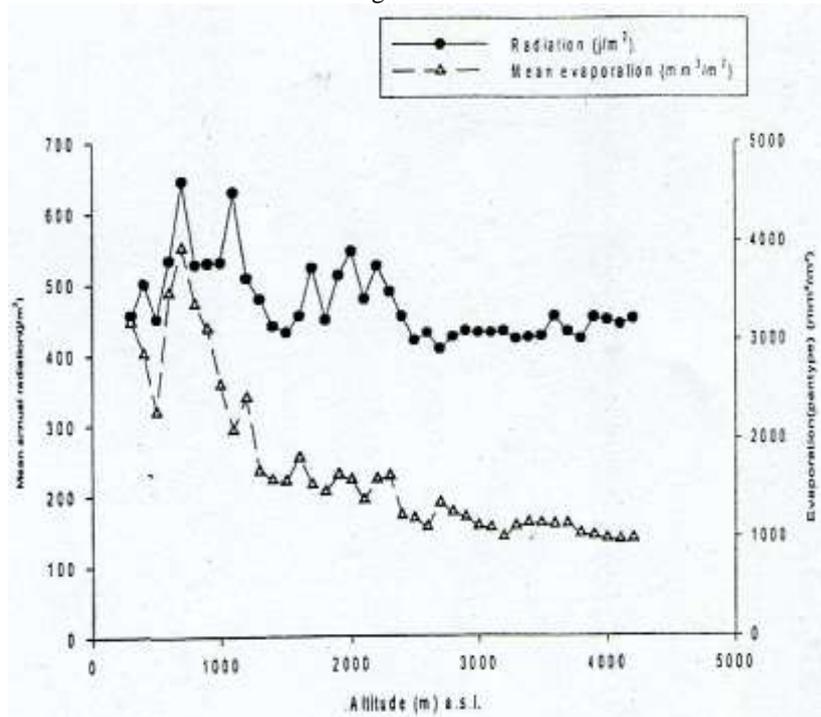
Figure 4. Mean annual radiation and mean annual evaporation along altitudinal gradient

Klimadiagramm (Figs. 5 and 6) were used to describe the extreme climatic conditions of the study area. Thus, the area of study is aridity in nature with varying climatic conditions.

Figure 5. Klimadiagramm graph of Rohet research area showing the extensions of wet seasons (hatched) and the dry seasons (dotted)

Figure 6. Klimadiagramm of Lokori research area showing the extensions of the dry seasons (dotted)
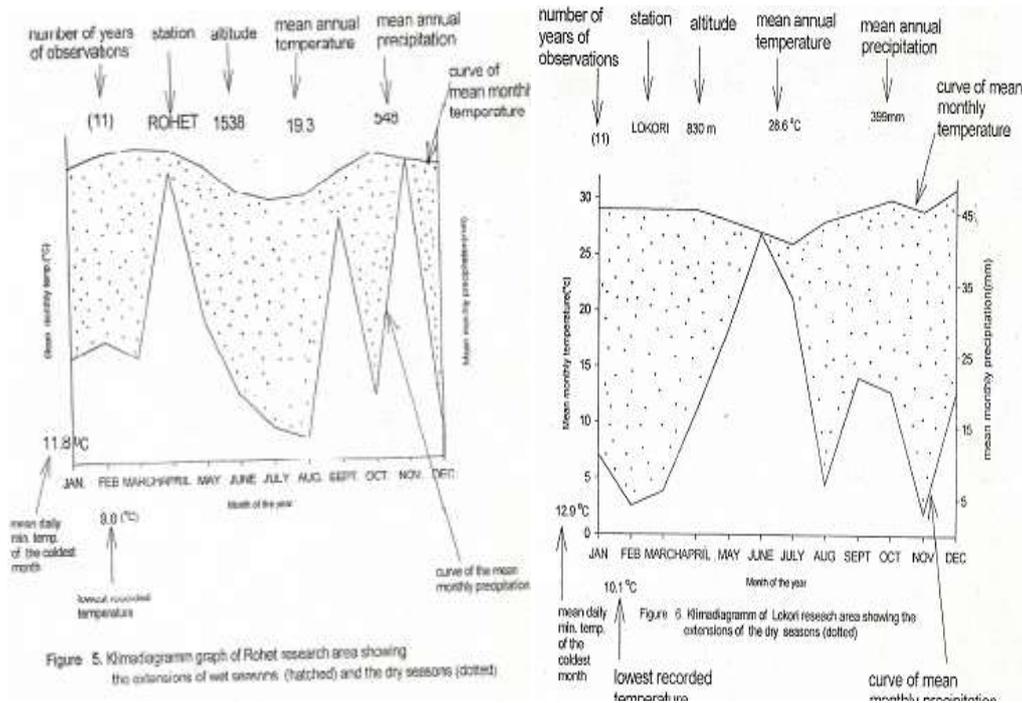
The δ^{13} C values were used to distinguish between C₃ and C₄ plant species and their corresponding δ^{13} C values provided [28]. The C₃ species constituted approximately 71.8% of the total species. These had δ^{13} C value of the total species had values between -18.89‰ while an exceptionally high δ^{13}C value -32.42‰ was recorded in *Chenopodiumbotryoides*. The plant, *C. botryoides*, thrives in highly moist regions of Baringo and

Mt. Elgon. The frequencies of the carbon discrimination values were analyzed at different altitudes. δ^{13}C values of -10.0‰ to -17.7‰ occurred frequently between 500m a.s.l. (Fig.7).
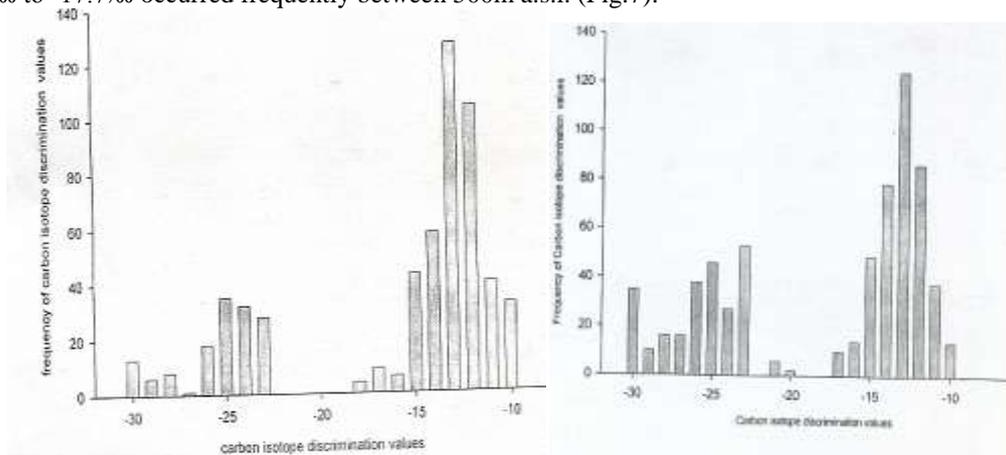
Figure 7. Frequency distribution of carbon isotope discrimination valuesfor different Centrospermeae species collected at 500m a.s.l. sites along the elevation transect.
Figure 8. Frequency distribution of carbon isotope discriminationfor different Centrospermeae species collected at 1000m a.s.l. sitesthe elevation transect.

to 1,000m a.s.l. (Fig. 8) decreasing at 2,000m a.s.l. (Fig. 9) to 2,500m a.s.l. (Fig.10) almost rare at 3,000m a.s.l. (Fig. 11) and absent at 3,500m a.s.l. (Fig. 12 and 13) and 4000m a.s.l. (Fig. 14).
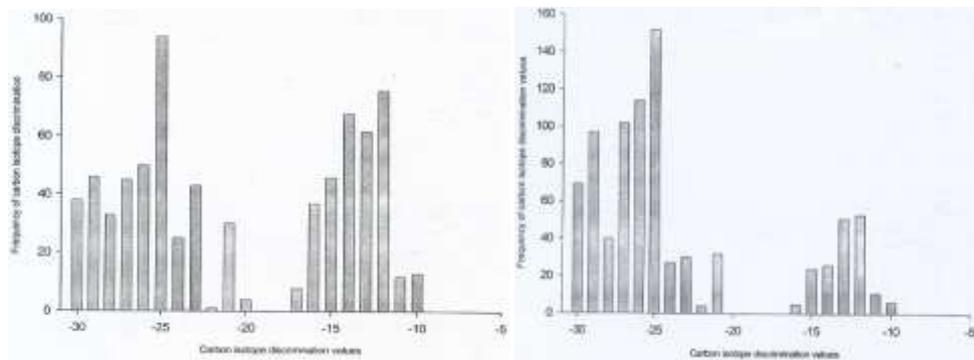
Figure 9. Frequency distribution of carbon isotope discrimination values for different Centrospermeae species collected at 1500m a.s.l. sites along the elevation transect.
Figure 10. Frequency distribution of carbon isotope discrimination values
for different Centrospermeae species collected at 2000m a.s.l. sitesalong the elevation transect.
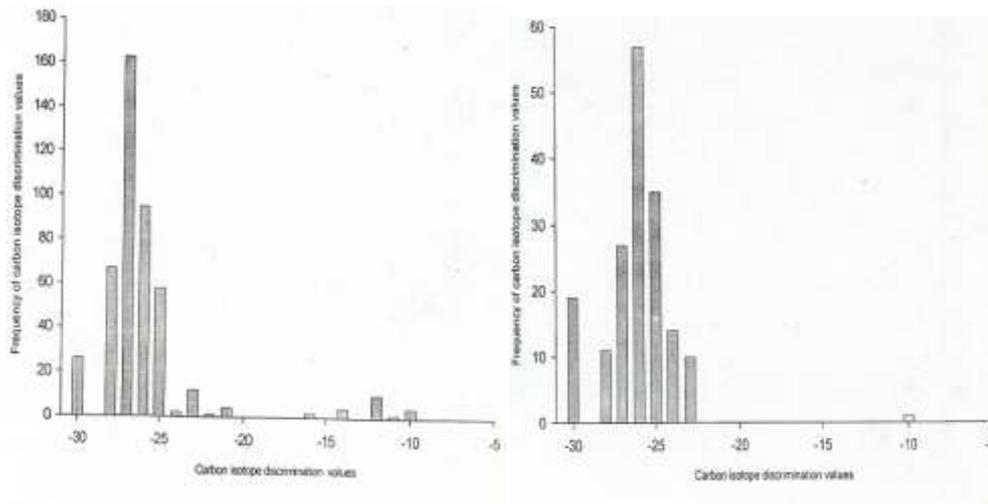
Figure 11. Frequency distribution of carbon isotope discrimination valuesfor different Centrospermeae species collected at 2500m a.s.l. sites along the elevation transect.
Figure 12. Frequency distribution of carbon isotope discrimination values
for different Centrospermeae species collected at 3000m a.s.l. sitesalong the elevation transect.
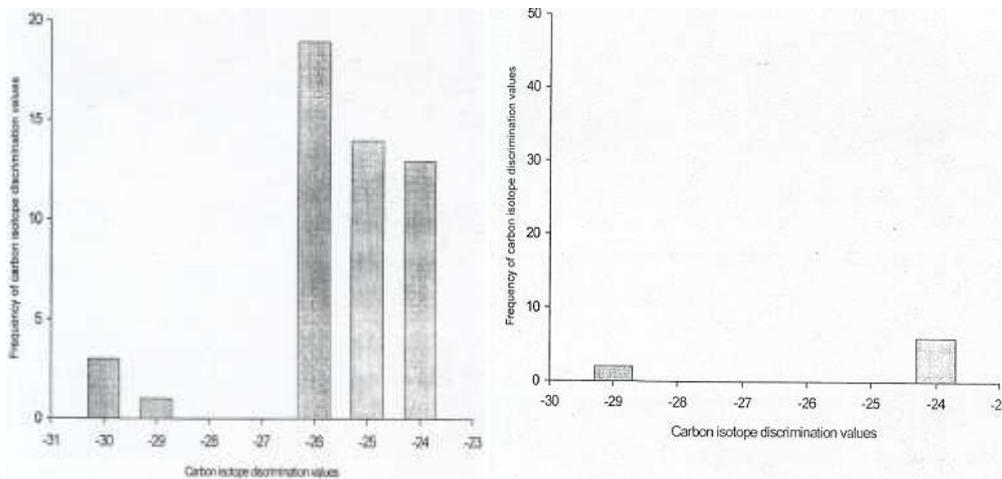
Figure 13. Frequency distribution of carbon isotope discrimination values for different Centrospermeae species collected at 3500m a.s.l. sites along the elevation transect.
Figure 14. Frequency distribution of carbon isotope discrimination values
for different Centrospermeae species collected at 3500m a.s.l. sitesalong the elevation transect.

Carbon discrimination values of -17.79‰ to -18.75‰ occurred at 500m a.s.l. only (Fig. 7); whereas, -20.0‰ to -21.0‰ to -30.5‰ showed an upward trend from 500m a.s.l. to 3,500m a.s.l. along the altitude. Increase in negativity reflects increase in the occurrence of the plants along the altitudinal gradient (Figs. 7,8,9,10,11,12,13, 14) which is associated with low temperatures, high potential rates of evaporation, high precipitation and high relative humidity(Figs. 1, 2,3, 4). $\delta^{13}C$ values of -23.0‰ to -27.0‰ exhibited an increased frequency upto 3,000m a.s.l. but $\delta^{13}C$ values of -28.0‰ to -30.1‰ showed constancy in the frequency peak from 500m a.s.l. to 3,500m a.s.l. altitude (Figs. 7,8,9,10,11,12,13, 14).Concomitantly, the degree of $\delta^{13}C$ negativity increased with the altitudinal gradient. This is associated with the vegetation dominance shift from the C_4 dicot species (showing $\delta^{13}C$ negativity) to the C_3 dicot species of the Centrospermeae group. This observation in the shift of the dicot species along the gradient is a reflection of the climatic variables (and/or environmental) attributes and/or influence on the distribution of the C_3 photosynthetic and C_4 photosynthetic systems that partition vegetation paradigm shift and to the extent primary productivity.

Low altitude is characterized by high temperature, high rate of evaporation and low moisture and high relative humidity (Figs.1, 2, 3, 4, 5, 6). These indices define measures of aridity where low $\delta^{13}C$ values occurred in the ecotype of study. Furthermore, $\delta^{13}C$ value of C_3 dicot species such as -20.0‰ to -21.0‰ occurred in areas of low aridity and in an ecotypic area inhabited by C_4 dicot species whose $\delta^{13}C$ value range from -15.0‰ and -17.78‰. The δ^{13} C values describe interphasic transition zone of the C_3 or C_4 types which is a proportion

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influenced by climatic attributes at a given altitudinal gradient (Fig. 14) and of biotype specific. There is a high occurrence of the C_3 dicot species at high altitudes as shown by a related proportion of increased negativity of the $\delta^{13}C$ values (Fig. 15).
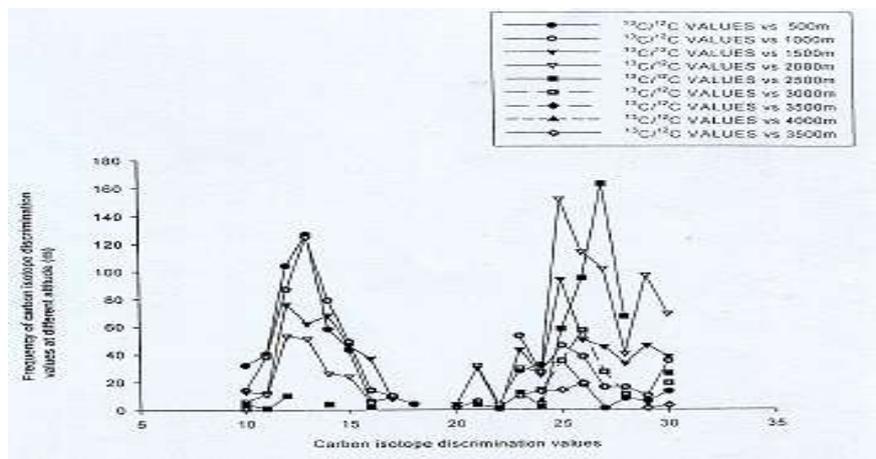
Figure 15. Distribution curves of carbon isotope discrimination values for different dicots species of the Centrospermeae along the elevation transect. Measurements from the Centrospermeae species subdivided into C_3 species (170), C_4species (67), CAM species and C_3-C_4 intermediate (3) species.

This is in contrast with the degree of $\delta^{13}C$ value for the C_4 dicot species at the same altitude (Fig. 14). There are two main frequency peaks of $\delta^{13}C$ values at -13.0‰ and -27.0‰ (Fig 15) along the altitude. This implies that a high proportion of the C_4 dicot species and C_3 dicot species tend to show mean $\delta^{13}C$ values of -13.0‰ and -27.0‰ along the altitude in the families of the Centrospermeae, respectively (Fig. 15).

The intensity for the species to attain the mean $\delta^{13}C$ values was shown by the nature of stratification of the frequency peaks tending towards their mean $\delta^{13}C$ value peaks (Fig. 15). The intensity in the stratification (or degree of occurrence of species) is more pronounced in the C_3 dicot species for $\delta^{13}C$ values tending towards -27.0‰ as shown by two main peaks at -25.0‰ and -27.0‰ (Fig. 15). At this altitudinal range, the influence of climatic conditions tends to normalize, leaving the ability of the species to fix CO_2 as a differentiating factor in the carbon invested and therein $\delta^{13}C$ value index.

IV. Discussion

The C_4 species which constituted approximately 28.2% of the total species had $\delta^{13}C$ values ranged from $-10.6‰$ to -16.55‰, with a high cluster around -12.0‰ to 13.0‰. [1]reported -10‰ to -20.0‰ $\delta^{13}C$ values for C_4 species and -22‰ to -33‰ $\delta^{13}C$ value for the C_3 species. A less negative $\delta^{13}C$ value was associated with low moisture index, high temperatures, high radiation and low relative humidity areas, especially in Turkana and Baringo [2009]. A similar trend is exhibited under the saline ecotypic area in Turkana (near Lake Turkana), Baringo (Perkerra irrigation scheme).This suggests that C_4 photosynthesis is an adaptation to hot, bright, arid/semi-arid and saline. Low light intensity during the growth life cycle, leads to a higher ration of stomatal conductance to photosynthesis (higher ci/ca ratio) in leaves from the forests leading to more negative $\delta^{13}C$ values [18]. The $\delta^{13}C$ values of the atmospheric carbon dioxide inside forests is more negative -8.0‰ to -9.0‰) (Medina, Montes, Cuevas and Rokzandic, 1986[30]; Sternberg, Mulkey and Wright1989[31]; Grace, Lloyd, Mcintyere, Miranda, Meir, Miranda, Moncrieddd, Massheder, Wright and Gash 1995[32]; Lloyd, Kruijt, Hollinger, Grace, Francey, Wond, Kelliher, Miranda, Farquhar, Gash, Vygodskaya, Wright, Miranda and Schulze 1996[33]; Pearcy and Calkin, 1983[34]; Kruijt, Lloyd, Grace, Mcintyer, Farquhar, Miranda and McCracken,1996[35]). This also contributes to decrease in the value C_3 leaves from the forest trees although to a lesser extent than the light factor (Sternberg, 1997)[36]. Isotopic fraction variation suggests that it is either C_3 or C_4 species dependent whereas small change in the $\delta^{13}C$ (isotopic fraction) is due to fluctuation in environmental factors. The most differential variables were temperature, precipitation [37] and assumed a positive and negative linear trend respectively,in the present study. The effect of temperature has also been reported (Troughton and Card, 1975[38]; Ehleringer, Cerling and Hellicker, 1997[39]).

These $\delta^{13}C$values for the C_3 and C_4 species reflect change in the carbon fixed, water lost,oxygen used and prevailing light intensity. Carbon source include atmosphere CO_2, respired CO_2 and soil bicarbonate (HCO_3^-); and finally intercellular carbon dioxide concentration. Variation in $\delta^{13}C$ value may occur at several sites due to diffusion from the leaf atmosphere into chloroplast; carboxylase catalyzed fixation and subsequent metabolic changes. Environmental variables may affect any of these sites to some degree but most likely influence the

enzyme-catalyzed kinetic steps. Thus, the carboxylase reaction and subsequent metabolic fractions might be expected to be more strongly influenced by external selective forces (or variables), that have a sum effect. Similar natural environmental factor influences compared to greenhouse optimal growth conditions variations were reported in *Xanthium strumarium* species and its ecotypic hybrid species(Brazilian Hong Kong, Indian *X. strumarium*),*Larrea cuneifolia, L. divaricata, Abronia maritima, A. gracilis, A. umbrellata, Atriplex leucophylla and Calstegia soldanella* species [3].The $\delta^{13}C$ values variation were observed to be species dependent. This is because CO_2 availability through the external and internal structural organization to reach RUBISCO is species dependent. RUBISCO reacts with $^{12}CO_2$ more easily than $^{13}CO_2$ due to a kinetic isotope effect PEP carboxylase is less discriminating against $^{13}CO_2$ than RUBISCO.

Both C_3 and C_4 species prefix atmospheric CO_2 through the PEP carboxylase activity in mesophyll cells. Later, C_4 species fix all these CO_2 through the RUBISCO activity in the sheath cell compartment (gas tight). Therefore, C_4 species discriminates against $^{13}CO_2$ more than C_3species. Thus, C_4 species ends up with greater $\delta^{13}C$ value in the range of -14.0‰ than the C_3 species $\delta^{13}C$ value of -28.0‰. This means that carbon isotope composition of C_4 dicot species has the potential to be used as an indicator for variations in the isotopic composition and concentration in the atmospheric CO_2 especially for past climate construction. The assumption is that, C_4 species photosynthetic discrimination range against ^{13}C remains constant in a wide range of environmental conditions, including the past present climates (Lloyd and Farquhar, 1994[40]; White, Clais, Figgie, Kennedy and Markgraf, 1994[41]).C_4 grasses and shrubs have been used as experimental material for the carbon isotope composition of atmospheric CO_2 (Marino, McElroy, Salawitch and Spaulding, 1992[42])[11] and changes in atmospheric CO_2 concentration (Cerling, Wang and Quade,1993)[44].

Within the conventional C_3, C_4 and Crassulacean acid metabolism pathways in the present study, variation in the $\delta^{13}C$ values was observed. Farquhar, O'Leary and Berry (1982)[45] suggest that the causes of variation in the C_3 pathway may be associated with variations in the ratio of intercellular and atmospheric partial pressure of carbon dioxide and hence of changes in water use efficiency. A quantitative expressionwas developedrelating the ratio to the proportion bundle sheath cells which leaks back into the mesophyll cells, and to the ratio of intercellular and atmospheric partial pressure of carbon dioxide for C_4 species $\delta^{13}C$ value variation[17]. The results supported suggestions that leakage may be significant (Berry and Farquhar, 1978)[46] and variation in the $\delta^{13}C$ values in C_4 species may reflect variations in the amount of leakage (Hattersley, Watson and Osmond 1976)[47].

The general pattern of $\delta^{13}C$ values distribution along altitudinal differentiation shows that $\delta^{13}C$ values of -10.0‰ to -16.55‰, -17.15‰ to -18.87‰ and -18.89‰ to-32.42‰ occur at low altitudes (0m-1500m a.s.l.), intermediate (1550m-1700m a.s.l.) and high altitude (1800m-4200m a.s.l.), respectively(Sikolia,2005)[48]. These altitudinal distributions for dicot species is well within the range reported for C_3 and C_4 monocot species (Smith and Brown, 1973[49]; Tieszen, Senyimba, Imbamba and Troughton, 1979[50]).

Within the intermediate altitudinal range (1550m a.s.l.-1700m a.s.l.), species with $\delta^{13}C$ values of -10.6‰ to 16.55‰ and -18.89‰ to -32.42‰ occur and thrive in low percentage. The low altitudes (< 1550m a.s.l.) are associated with water drought, high temperatures and low relative humidity as aspects of aridity index where C_4 species are well adapted. In contrast, less water drought, low temperature, high relative humidity and high radiation are experienced at high altitudes (> 1700m a.s.l.) where a high percentage of C_3 dicot species thrive. This altitudinal trend based on the $\delta^{13}C$ values s fully supported by the distributional pattern on floristic information of the C_3 and C_4 species.

Data in the transition zone between C_3 dicot and C_4 dicot species of photosynthetic systems depicts a rather sharper interphase change along the altitudinal differentiation spectrum. Thus, less $\delta^{13}C$ value negativity is a potential aridity indicator and C_4 syndrome marker which contrast the C_3 syndrome occurring in moist and low temperature (less arid-like) areas. Similar studies in the Poaceae of the Northern Saharan desert (Winter, Troughton, Evenari, Lauchli and Luttge 1976) [51], open grassland vegetation in Kenya [50], and in Java (Hofstra, Aksornkoe, Atmowidjojo, Bannaq, Santosa, Sastrohoetomo and Thu, 1972 [52]) low altitudes tend to be less negative or show a tendency towards C_4 like morphological parameters and regions of understorey of closed forests vis-à-vis from tribes consistent with C_4 syndrome like Amaranthaceae.

The more the dicots were stressed by low light intensity, high temperature or limited water supply, the lower their $\delta^{13}C$ value and the higher their carbon isotope discrimination. This differentiation in the $\delta^{13}C$ value, as a result of suberized middle lamella; also reported in the *Panicum* species (Oshugi, Samejima, Chonan and Murata 1988[53]) [11]. This anatomical variation is compensated for by location of the chloroplasts in a centripetal position in the bundle sheath, thus increasing the CO_2 diffusion pathway to the mesophyll cell, and higher bundle sheath surface to volume ratio (Hattersley and Browning, 1981[54]; Henderson, Von Caemmerer and Farquhar,1992 [55]). Increasing stomatal closure as a result of increasing aridity is associated with decrease in carbon discrimination of leaves (Ehleringer and Cerling, 1995[56]; Liu, Phillips and Campbell, 1996[57]). The decline in carbon isotope discrimination in the more arid areas may be a function of both low species diversity (Basellaceae species), and a highly seasonal and unpredictable rainfall regime (Schulze, Ellis, Schulze,

Trimborn and Ziegler, 1996) [58], decreased humidity[12] and soil water availability (Ehleringer and Cooper, 1988)[59], which vary along the altitude. Thus, altitudinal results show that leaf $\delta^{13}C$ value may follow quite different patterns to those observed globally (Körner and Larcher, 1988)[60].

Along the soil moisture gradient from relatively wetter areas to the relatively drier ecological regions (low altitudes), leaf stable carbon isotope ratios increased in all the species, indicating that water use efficiency increased as soil availability (precipitation) decreased. The results in differential $\delta^{13}C$ values depended on the ability of the individual species to compete for soil moisture and carbon dioxide availability. This leads to the observed $\delta^{13}C$ values along the climatic gradient of integrative nature and natural disturbance factors like human activities and fire.

Generally, the differing C_3 and $C_4\delta^{13}C$ values can be related to the species dependent water-use efficiency and habitat. The water–use efficiency can contribute directly and in a predictable manner to their long term growth and survival. This relationship can occur under the unpredictable and fluctuating drought conditions that characterize the semi deserts or deserts. The conditions are punctuated with salinity affecting water diffusion into plant root. Finally, the influence of climatic factors on the composition species results in competition of species in its habitat. This affects the association, vegetation zonation, longevity, relative frequency/abundance of species, diversity of the species and their $\delta^{13}C$ values. Concomitantly$\delta^{13}C$ values become a predictor of spatial diversity and shift of the species along the altitudinal gradient of environmental factors interplay. The most limiting climatic variable in an ecosystem dictates the trend of negativity or positivity of the $\delta^{13}C$ values, with the rest of factors influencing it from a supportive dimension, given that the plasticity and flexibility of the adaptation of the species remain constant or are not influential. Beerling and Woodward(1993)[61] reported that leaf $\delta^{13}C$ value declined in response to CO_2 increases for the past 200 years, which probably reflects the anthropogenic increase of atmospheric ^{12}C as a result of fossil fuel burning and deforestation; and high temperatures being associated with high $\delta^{13}C$ value. This is consistence with the observations from a global latitudinal (and altitudinal) survey of leaf $\delta^{13}C$grown at low temperatures [61].

The influence of climatic variables on the $\delta^{13}C$ value variation reported in this study, confirms the view of the analysis of quaternary fossil leaves which indicates that leaf $\delta^{13}C$ values could either reflect palaeo-temperatures directly or indirectly through rate of respiration in the soil as a function of temperature, atmospheric CO_2 change which together offer the possibility of determining the comparative timing of both changes. Stomatal and morphological adjustments concur in an integrated functional dimension to meet the transpiration demands across broad climatic gradients. It's therefore, not surprising to have differential influence of factors leading to $\delta^{13}C$ value variation within a species and or amongst species. These influences achieve sustainable photosynthetic productivity and constant water use efficiency in the species. Similar observations have been reported (Comstock and Ehleringer, 1988[62], 1992[63]). Therefore, plants can display a continuum of responses (or a wholly functional adjustment) to climatic conditions ranging from predominantly leaf gas exchange to whole plant morphological adjustments, and the expressions of these responses could determine carbon invested (or gained) giving varied $\delta^{13}C$ values [59] and survival along broad environmental variable gradients. This confirms the observation of correlation between $\delta^{13}C$ values along the climatic factor(s) and altitudinal gradients. By extension, whether stomatal or morphological adjustments predominate may depend as much on phylogenetic constraint as on trade-offs that may exist between leaf level and whole plant level flexibility.

The unexpected observation that more negative $\delta^{13}C$ value at high altitudes where carbon dioxide concentration would favour C_4 species unlike C_3 species could be attributed to the Pyruvate Phosphate (PP) Dikinase enzyme which requires ADP and not ATP and is subject to dark/light regulation and optimal temperature unlike low temperatures at the high altitudes. The mesophyll enzyme PP Dikinase which exhibit different states of activation becomes non-functional to attain the standard photosynthetic reaction in C_4 species. This leads to reduced or increased biochemical non-functional requirements of the PP Dikinase enzyme for continuous metabolic pathway. This PP Dikinase enzyme is notabiochemical functional requirement inthe C_3species which utilizes malate dehydrogenase (or malic enzyme) in the PEP case of the Calvin cycle. Therefore the C_3 species survive well in the cold temperatures compared to the warm conditions of the C_4 species (occur upto 3,000m). The present results imply that CO_2 assimilation would decrease with increasing altitude for lack of normal biochemical functional of the metabolic mechanisms in the C_4 species for their growth and survival in high altitudinal habitats controlled by low temperatures (upto negative degrees centigrade); also reported by Sakai and Larcher (1987)[64], Rada, González, Azócar, Briceńos, and Jaumez (1992)[65], Cabrera, Rada and Cavieres (1998)[66]. Therefore, more negative $\delta^{13}C$ values (C_3species) occur at high altitudes in contrast with low altitude mostly dominated by the C_4 species showing less $\delta^{13}C$ values.

The C_3 andC_4 photosynthetic pathways adaptation are defined by low altitudinal ranges/maxima $\delta^{13}C$value peaks in response to the environmental gradients, at 750m a.s.l. – 1000m a.s.l. and1750 a.s.l. – 2500m a.s.l. for the C_3 and C_4 dicot species, respectively[37] [48]. These are major determinants for the success of species diversity in different habitants and micro environments partitioning competition effectively to attain

maximum efficiency in species productivity (carbon gain). The cold sensitivity exceptions for C_4 photosynthesis associated with low [60] have also been reported in *Z. japonica* but with increased rates in *Z. anglica* species which indicated that PEPC is co-limiting and decreased in activity (in *Z. japonica*) even greater than decrease in PCK [64]. Thus, PEPC is partly responsible for cold sensitivity in *Z.japonica*, but PCK would be a candidate particularly in PCK Carboxykinase type C_4 species exhibiting low PP Dikinase activity.

Studies show that there is monocot-dicot photosynthetic pathway stratification at a given altitudinal range where the C_4 dicot syndrome overlaps C_4 monocot syndrome with decreased proportion along the altitudinal gradient (Sikolia, Onyango, Beck and Kinyamario, 2009) [66]. This differentiation phenomena is likely to be replicated through transition occurrence of the C_4 subtypes of the NAD-ME, NADP-ME and PEP-CK types species of the monocots and dicots along the altitudinal gradient with corresponding vegetation change and productivity implications along different gradients of climatic, soil moisture index and altitude in the temperate and tropical ecosystems. This stratification is influenced by climatic factors, which in turn, determines the occurrence of the C_3, CAM, C_3-C_4 and C_4 species and their corresponding $\delta^{13}C$ values along the altitudinal gradient. This results the observed spatial differentiation of the C_3, C_4 and CAM photosynthetic pathways at low altitudinal ranges/maxima $\delta^{13}C$ value peaks in response to the climatic factorial gradients, at 750m a.s.l. – 1000m a.s.l. and 1750 a.s.l. – 2500m a.s.l. for the C_3 and C_4 dicot species, respectively. Studies have shown the transition zone between C_3 and C_4 dicot species is rather abrupt and occurs at 1500m a.s.l. – 1700m a.s.l. along the altitudinal gradient, less behind that documented for monocots in Java [52] and Kenya[50] at 2000m a.s.l. – 2300m a.s.l. altitude [66]. This transition zone was defined by climatic parameters as mean annual minimum temperature ($^{\circ}C$), mean annual maximum temperature ($^{\circ}C$), mean annual rainfall (mm), mean annual potential evaporation (mmm^{-2}), mean annual radiation (cal cm^{-2}) and mean annual relative humidity (%) of 15.7 $^{\circ}C$, 25.6 $^{\circ}C$, 700-950mm, $1575mmm^{-2}$-$1650mmm^{-2}$, 450 jm^{-2} and 50%-55%, respectively [37][66]. This climatic paradigm informs the transition zone between of the between C_3 and C_4 dicot species $\delta^{13}C$ values along the altitudinal gradient. The mean annual temperature of 20.7 $^{\circ}C$ – 25.6 $^{\circ}C$ is the temperature for the transition shift from the C_3 to C_4 dominance regardless of the elevation. This paradigm is predicted for the $\delta^{13}C$ values in the order of occurrence for the monocot-dicot C_4 subtypes, namely, NAD-ME, NADP-ME and PEP-CK species along the altitudinal gradient. Thus, the sequence of photosynthetic pathway stratification should be able to define vegetation change and productivity over time and space in the ecosystems.

The use of carbon ratios is becoming a useful scientific technique (Troughton, Card and Hendy, 1975)[68] and tool in studying different biological questions in the environment and related conditions. Here, it has been used to establish the different photosynthetic types, their abundance and distribution along the altitudinal gradient in the semi-arid and arid ecosystems in Western Kenya. Further, it has been applied to identify the C_3 and C_4 species in the Centrospermeae taxa. The $\delta^{13}C$ value have been used in partitioning the photosynthetic types [1], forensic studies as fingerprints of biological agents (Horita and Vass,2003[69]; Kreuzer- Martin, Chesson, Lott, Dorigan and Ehleringer,2004[70]; Sharp, Atudorei, Panarello, Fernandez and Douthitt, 2003[71]; Ehleringer, Cerling and Dearing,2005[72]), studying flow of organic carbon as different dietary inputs (Hobson, 1999)[73], reconstructing palaeodiets (Macko Lubec, Teschler-Nicola, Andrusevich and Engel, 1999)[74], constructing nutritional relationship between herbivores/primary producers (Tieszen and Imbamba, 1980[75]) and detecting the point of origin of illicit drugs (Ehleringer, Cerling and Dearing, 2002[76]; Carter, Titterton, Murray and Sleeman,2002[77]). Further, the carbon discriminations values are used in solving the question of adulteration of food and beverages (Brooks, Buchmann, Phillips and Lott, 2002)[78], following carbon transport across the ecosystems (Conte and Weber, 2002)[79] and studies of root segregation of the C_3 and C_4 species (Eleki, Cruse and Albrecht,2005)[80]. In addition, has been applied to establish whether species in semi-arid and arid source water from the same depth level (Schwinning, Starr and Ehleringer, 2005)[81]. The above examples confirm the contributions of $\delta^{13}C$ value study in the science world.

Recently, he *Juniperus* of Rancho La Brea fossil collection has been used to show that glacial and modern trees were operating at similar leaf intercellular CO_2 concentration values (Ward *et al*, 2005)[82]. As result, glacial trees were operating at leaf inter-cellular concentration values much closer to the CO_2 compensation point for C_3 photosynthesis than modern trees. This indicates that glacial trees were undergoing carbon starvation and C_3 productivity was greatly diminished in Southern California during the glacial period [82]. The dynamics of change is ongoing process whose consequences demands further researches to understand their effects on vegetation change and productivity for the benefit of the future generation.

V.   Conclusions

Low negative value of $\delta^{13}C$ value is a potential aridity indicator and C_4 syndrome marker which contrast the C_3 syndrome occurring in more moist and low temperature area. The more the dicots are stressed by low light intensity, high temperature or limited amount of precipitation, the lower the $\delta^{13}C$ value and the higher their carbon isotope discrimination. $\delta^{13}C$ values are a good predictor for spatial diversity and shift of species along the altitudinal gradient of climatic factors interplay. The general pattern of $\delta^{13}C$ values distribution along

altitudinal differentiation show that the values of -10.60‰, to -16.65‰, -17.75‰ to -18.87‰, and -18.89‰ to -32.42‰ correspond to the species at low altitudes (0m a.s.l. – 1500m a.s.l.), intermediate altitude (1,550m a.s.l.-1,700m a.s.l.) and high altitude (1,800m a.s.l. – 4200m a.s.l., respectively. The altitudes are associated with aridity stress, high temperatures and low relative humidity. Thus, there is spatial differentiation of the C_3, C_4 and CAM photosynthetic pathways at low altitudinal ranges/maxima $\delta^{13}C$ value peaks in response to the climatic factorialgradients, at 750m a.s.l. – 1000m a.s.l. and1750 a.s.l. – 2500m a.s.l. for the C_3 and C_4 dicot species, respectively. This differentiation phenomena is likely to be replicated through transition placement of the C_4 subtypes NAD-ME, NADP-ME and PEP-CKspecies of the monocots and dicots along the altitudinal gradient with corresponding vegetation change and productivity implications modified by environmental factors. Biomass data (dry weight) of the C_3 and C_4 monocot-dicot is required to confirm this phenomenon associated with floristic composition.

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