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Temporal evolution of $^{137}Cs^+$, K^+ and Na^+ in fruits of South American tropical species

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HIGHLIGHTS

► Concentrations of ^{137}Cs, K and Na in fruits of lemon (*Citrus limon* B.) are presented.
► Concentrations of K and Na in fruits of coconut (*Cocos nucifera* L.) are also showed.
► We investigated the use of ^{137}Cs as a tracer for the plant absorption of macronutrients.
► A model was developed to simulate the temporal evolution of ^{137}Cs, K and Na by fruits.
► This model exhibited close agreement with our results along the fruit development.

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ABSTRACT

Concentrations of ^{137}Cs, K and Na in fruits of lemon (*Citrus limon* B.) and of K and Na in fruits of coconut (*Cocos nucifera* L.) trees were measured by both gamma spectrometry and neutron activation analysis, with the aim to understand the behaviour of monovalent inorganic cations in tropical plants as well as the plant ability to store these elements. Similar amounts of K^+ were incorporated by lemon and coconut trees during the growth and ripening processes of its fruits. The K concentration decreased exponentially during the growth of lemons and coconuts, ranging from 13 to 25 g kg^{-1} dry weight. The incorporation of Na^+ differed considerably between the plant species studied. The Na concentration increased linearly during the lemon growth period (0.04 to 0.70 g kg^{-1} d.w.) and decreased exponentially during the coconut growth period (1.4 to 0.5 g kg^{-1} d.w.). Even though radiocaesium is not an essential element to plants, our results have shown that ^{137}Cs incorporation to vegetable tissues is positively correlated to K distribution within the studied tropical plant species, suggesting that the two elements might be assimilated in a similar way, going through the biological cycle together. A mathematical model was developed from the experimental data allowing simulating the incorporation process of monovalent inorganic cations by the fruits of such tropical species. The agreement between the theoretical approach and the experimental values is satisfactory along fruit development.

1. Introduction

Mineral nutrients have many functions in plants. They are important as constituents of plant tissues, catalysts in various reactions, osmotic regulators, constituents of buffer systems, and regulators of membrane permeability. Deficiencies of these elements can alter physiological processes and reduce plant growth, often before visible symptoms appear. On the other hand, some of these minerals can be very toxic if present in larger quantities. Certain mineral nutrients

also tend to counterbalance the effect of others (Pallardy, 2008; Barker and Pilbean, 2007; Epstein and Bloom, 2005; Mengel and Kirkiby, 2001; Marschner, 1995). A few of these mineral nutrients are present as monovalent inorganic cations, such as the stable alkali elements (sodium and potassium), with similar physicochemical behaviour. The hydrated sodium ion (Na^+) has a radius of 0.36 nm, whereas that of potassium ion (K^+) is 0.33 nm (Marschner, 1995). Potassium, which is highly mobile in plants, is involved in enzyme activation, protein synthesis, osmoregulation, stomatal opening and closing, photosynthesis, and cell expansion. Sodium may also occur in large quantities in some plants, but despite this element can sometimes increase plant growth and may be required for specific plants, it

is not generally regarded as essential or necessary for all higher plant species (Pallardy, 2008; Mengel and Kirkiby, 2001). Most higher plants have developed high selectivity in the uptake of potassium in comparison with that of sodium, and this is particularly obvious in transport to shoot (Marschner, 1995). Therefore, K and Na can have very different roles and hence it follows that they can be treated very differently by mechanisms involved in short- and long-range transport (Barker and Pilbean, 2007). In this way, there are many complicated interactions among various mineral nutrients, with one element modifying absorption and utilization of others, making it a challenging study (Pallardy, 2008; Barker and Pilbean, 2007; Epstein and Bloom, 2005; Mengel and Kirkiby, 2001; Marschner, 1995).

In contrast to K^+ and Na^+, Cs^+ is not an essential nor beneficial element to plants. However, radioecological studies have shown that ^{137}Cs, a univalent cationic species with a similar size and water mantle to K^+ and Na^+, has comparable behaviour to K^+ in the biogeochemical pathway (IAEA, 2010). In the environment, radiocaesium is mainly present in soil solution as a free hydrated cation ($^{137}Cs^+$) with little or no tendency to form soluble complexes. The hydrated radiocaesium ion has a radius of 0.31 nm (Marschner, 1995). Similarly to K^+ and Na^+, $^{137}Cs^+$ can be absorbed by plant roots from soil solution and translocated to the above-ground plant parts (Delvaux et al., 2000; White and Broadley, 2000; Zhu and Smolders, 2000). Additionally, radiocaesium uptake can be influenced by the root distribution within the soil profile, soil type, soil humidity status and characteristic uptake rate of fruit species as well as by specific activity of the roots (Atkinson and Webster, 2001). Nevertheless, previous studies performed by our group have shown that the distribution of ^{137}Cs varies appreciably in different tissues and organs of a same tropical species. Deciduous trees generally accumulate more ^{137}Cs than evergreen trees. The concentration of $^{137}Cs^+$ in woody fruit trees usually varies as follows: fruits > leaves > small branches > large branches > stems (Carvalho et al., 2006; Mosquera et al., 2006; Sanches et al., 2008; Anjos et al., 2007, 2009a, 2009b; Velasco et al., 2010).

The same behaviour has been observed during the incorporation of mineral nutrients. According to Pallardy (2008), the amounts of these elements in woody plants vary with species and genotype, site, season, and in different parts of the same plant. Total nutrient contents of forests vary in the following order: tropical > temperate broadleaf > temperate conifer > boreal forests. Deciduous trees generally accumulate more minerals than evergreen trees. The concentration of minerals in trees usually varies as follows: leaves > small branches > large branches > stems.

These findings suggest, then, the possibility of using alkali metals to predict the ^{137}Cs behaviour in soil–plant systems. From a radioecological viewpoint, the assessment of radionuclide fruit contamination is important to estimate dose rates associated with potential releases of radionuclides in the environment and its consequences to man. This issue on the other hand, is interesting from a physiological point of view, since measurements of ^{137}Cs in plants can be used to understand the transport and accumulation of monovalent inorganic cations by plants.

This paper presents experimental and theoretical approaches to study and assess the accumulation of radiocaesium, potassium and sodium in fruits growing in tropical and subtropical environments. Their distributions were studied in more detail, covering the whole development period of this specific organ. This organ was chosen, since it is well known that the concentrations of Cs, K and Na in the soil remain steady during fruit growth, therefore, fluctuations are not expected to occur in its levels during the fruit development due to variations of their concentrations in the soil. In addition, investigations that may result in determining ways to stimulate growth of fruits are of great practical and scientific interest. For this purpose, we discuss the dynamics of growth, and the temporal evolution of $^{137}Cs^+$, K^+ and Na^+ incorporation in fruits from two tropical trees: lemon (*Citrus limon* B.) and coconut (*Cocos nucifera* L.).

2. Material and methods

2.1. The collection and preparation of samples

Two experimental sites were selected to perform this work. The first site is a small farm located in the Niterói region, southeastern coast of Rio de Janeiro State, Brazil (22°56′50″S, 43°01′23″W), where ^{137}Cs concentrations in soil can be considered negligible. The second site is located in Goiânia region, in central Brazil (16°40′14″ S, 49°16′17″W), where in 1987, there was a radiological accident involving a source of ^{137}Cs and, at present, ^{137}Cs concentrations are higher than average (IAEA, 1988; Anjos et al., 2002, 2007; Carvalho et al., 2006; Mosquera et al., 2006).

In order to estimate the incorporation of monovalent inorganic cations during the process of growth and maturation of the edible parts in plants, fruit samples at various stages on its development were taken from lemon and coconut trees.

Lemon plant (Rutaceae family) is a species that can grow in both dry and humid atmospheres, preferring areas with abundant sunlight and annual rainfall from 25 to 125 cm (Morton, 1987). The lemon tree can reach 3 to 4 m in height. Its leaves are elliptic, 6–11 cm long and present slender wings on the petioles. They are light-green when young and become dark-green when old. The mildly fragrant flowers can be single or in two or more clustered in the leaf axils. The fruits are oval with a nipple-like protuberance at the apex, 5 to 7 cm long. As lemon trees bear fruit twice a year, there were two sampling, the first going from March to May 2009 and the second from September to December 2009. Five 6–8 year old individuals of lemon trees were used for this study. Two lemon trees were growing on ^{137}Cs contaminated soil of Goiânia region, and the other three on uncontaminated soil of the Niteroi farm. From each lemon plant, 5 lemon fruits were collected at each sampling time.

The coconut plant (Arecaceae Family) thrives on sandy soils and is highly tolerant to salinity. For optimal growth, areas with abundant sunlight, regular rainfall (150 cm to 250 cm annually) and high humidity (70–80%) are required (Morton, 1987). The plant can reach around 10 m in height. The leaves are 4 to 6 m long pinnate and 60 to 90 cm long pinnae. Flowering occurs continuously. The fruits are produced in clusters near the growing tip. The fruits are varied in shape, but generally are globose or oblong, up to 20 cm diameter. As the coconut tree bears fruit throughout the year, they were sampled from March to September 2009. Five 15–18 year old individual coconut trees, growing on uncontaminated soil, were used for this study. From each coconut plant, 3 coconut fruits were collected at each sampling time.

Sample preparation was carried out at the Laboratory of Radioecology (LARA) of the Physics Institute of Federal Fluminense University. Samples of whole fruits, including its skin, were washed with distilled water, dried in stove at 105 °C, and finally were ground to powder. Afterwards, all samples that were dry-weighed were packed into cylindrical plastic containers and sealed.

About 2.0 to 3.0 g dry weight of fruit sample was used for the measurements of ^{137}Cs concentration by gamma-ray spectrometry. However, determination of ^{40}K activity concentration would require about 30 to 50 g d.w of fruit sample in order to compensate the count rate decrease due to the lower detection efficiency of this radionuclide compared to ^{137}Cs. Therefore, gamma spectrometry technique became unfeasible to determine the ^{40}K activity concentration since single fruit lemon sample mass was not enough. This problem did not occur for coconut samples. Therefore, measurements of ^{137}Cs in lemons and ^{40}K in coconuts were performed by gamma spectrometry. Measurements of K in lemons and Na in lemons and coconuts were performed by Neutron Activation Analysis (NAA).

Since Neutron Activation Analysis is a more expensive technique compared to conventional gamma spectrometry, a larger number of measurements were performed with the latter. Thus, the total number

of analyzed samples was limited by the amount of sample material available and the cost of measurements.

2.2. Determination of ^{137}Cs and ^{40}K

Gamma-ray analysis was carried out at the Laboratory of Radioecology (LARA) of the Physics Institute of Federal Fluminense University, using a 60% efficiency high-purity Germanium detector (HPGe), in order to obtain the activity concentrations of ^{137}Cs and ^{40}K. Energy spectra measurements from soil and fruit samples were accumulated for 4 h and 24 h, respectively. ^{137}Cs and ^{40}K activities in soil and fruit samples were calculated from the net full energy peaks (661.7 keV gamma-ray line for ^{137}Cs and 1460.8 keV for ^{40}K) and the measured detector efficiency. The ^{40}K calibration was performed using a reference material (RGK-1) obtained from the International Atomic Energy Agency. Radiocaesium standards were made by mixing uncontaminated sawdust with a ^{137}Cs solution prepared by the Brazilian National Nuclear Energy Commission (CNEN) and its results were inter-compared with two laboratories in Brazil. Technical details of sample preparation and analysis can be obtained in Mosquera et al. (2006) and Carvalho et al. (2006).

Radioecological studies express the radionuclide concentrations in the traditional units of Bq kg^{-1} d.w. Mass units, however, provide insight and information about the status of monovalent inorganic cations as a nutrient. Typically units for expressing mass in environmental media for soil and plant organs are g kg^{-1}. These mass units can also be expressed as parts per million (ppm), which is equivalent to mg/kg. The activity concentration (AC) in Bq kg^{-1} d.w. may be converted to content (C) in g kg^{-1} d.w., by the following equation:

$$C(g/kg) = 7.56 \times 10^{-17} A T_{1/2} AC (Bq/kg) \qquad (1)$$

where A is the radionuclide atomic weight in g mol^{-1}, and $T_{1/2}$ is the radionuclide half-life in years.

Specific activity standard deviations for each individual measurement of fruit and soil samples ranged from 3 to 7% for ^{137}Cs and around 10% for ^{40}K. The detection limit was estimated to be 0.7 Bq kg^{-1} d.w. for ^{137}Cs and 6.3 Bq kg^{-1} d.w. for ^{40}K.

2.3. Determination of K and Na

Potassium and sodium contents in fruit samples were quantified by NAA performed at the IEA-R1 research reactor at Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN-SP). In this technique, samples are bombarded with neutrons, resulting in the production of radioactive isotopes and gamma rays emitted are then analyzed by gamma-ray spectrometry.

In order to determine the K and Na concentrations (in g kg^{-1} d.w.), Cadmium Ratio Technique was used for the measurements of both thermal and epithermal flux distributions. In this technique, Au foils (<1 mg), both bare and Cd covered, were irradiated together with the fruit sample in the IEA-R1 nuclear reactor (2–4 MW, pool type) allowing the simultaneous activation of these materials under the exact same irradiation conditions. Using this procedure, gamma-ray activity induced in both Au foils (neutrons detectors) and fruit samples was obtained.

Fruit samples from 20 to 150 mg of each species were accurately weighed and sealed into individual pre-cleaned polyethylene bag. Then, lemon samples were irradiated in a pneumatic station in the nuclear reactor using irradiation times of 30 s, decay time of 60 s and counting time of 300 s. Coconut samples were irradiated for 60 s, decay time of 1800 s and counting time of 600 s. Each sample was analysed in triplicate and the results are the median value. Gamma spectrometry was performed using a 60% efficiency high-purity Germanium detector (HPGe). Potassium and sodium concentrations were calculated from the net full energy peaks (1525 keV

gamma-ray line from ^{42}K and 1368 keV from ^{24}Na). For quality control certified standard reference material NIST 1573a Tomato Leaves was measured. In addition, inter-comparison between NAA and conventional gamma spectrometry results for K contents in fruits samples were very satisfactory. The standard deviations ranged from 5 to 10% for the sodium and potassium contents.

3. Results

In this work temporal evolution of sodium, potassium and caesium was studied, covering the whole development period of a specific organ: fruit. From Figs. 1, 2 and 3 fruit development is observed through time (in days) starting on the flowering day (zero time) for each fruit. Fig. 1 shows the K and Na concentrations in fruits from coconut trees through time, growing on uncontaminated soils. During ripening process of coconuts, K is consumed in larger quantities and is present in fruit tissues in quantities from 15 g kg^{-1} to 25 g kg^{-1}, while the Na concentration ranges from 0.5 to 1.4 g kg^{-1}. These results indicate that the concentrations of K and Na are age-dependent for fruits. Moreover, the accumulation of Na in fruit tissues is proportional to K concentration, decreasing with increasing age of fruits.

Fig. 2 shows the concentrations of K and Na in fruits from lemon trees through time, growing on uncontaminated soils. Since lemon and coconut trees were grown in the same ground, where the concentrations of K were similar, the results suggest that similar amounts of K$^+$ were incorporated by the lemon and coconut trees during the growth and ripening processes of its fruits. K concentration ranged from 13 to 25 g kg^{-1} d.w., decreasing exponentially with fruit growth. In contrast, the accumulation of Na increased with fruit growth. Na concentration ranged from 0.04 to 0.70 g kg^{-1} d.w. A comparison between Figs. 1 and 2 indicates that coconut fruits can accumulate higher amounts of Na for periods of less than 160 days.

Simultaneous measurements of Na and K concentrations in woody fruit species are scarce. However, it is possible to find a few publications in the literature which show that the distributions of Na and K in fruits from woody plants are similar to the ones observed in lemons. Azuma et al. (2010) have shown that the Na concentration tends to increase during the fruit development in pepper trees while Carvalho et al. (2006) have shown that the K concentration decreases.
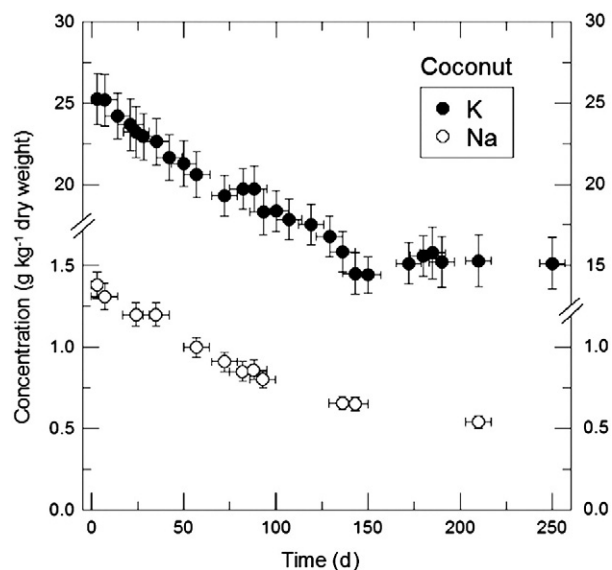

Fig. 1. Concentrations of K and Na in coconut fruits, during its ripening process on uncontaminated soil.
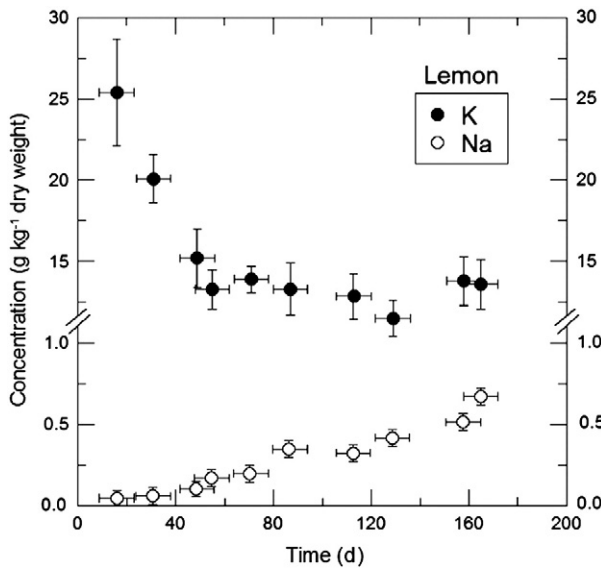
Fig. 2. Concentrations of K and Na in lemon fruits, during its ripening process on uncontaminated soil.

This antagonistic behaviour of Na confirms that its beneficial effects on fruit growth differ considerably between plant species. The required Na concentration to cause a beneficial effect during ripening process of lemons is low. In this way, coconut tree can be considered a "high or medium Na species", where the favourable effect of Na is important on plant growth. In this species, Na contributes to the osmotic potential of the cell and thus has a positive effect on the water regime of plant (Mengel and Kirkiby, 2001).

Fig. 3 shows the temporal evolution of ^{137}Cs concentrations in lemons fruits, growing on caesium contaminated soil (Goiânia site). In these plants, incorporation of K and Na has a similar pattern to plants growing on uncontaminated soil (Fig. 2). Like K, ^{137}Cs distribution is age-dependent and decreases when fruit age increases. This result confirms that caesium and potassium exhibit similar behaviour during the development of the edible parts of citrus trees.
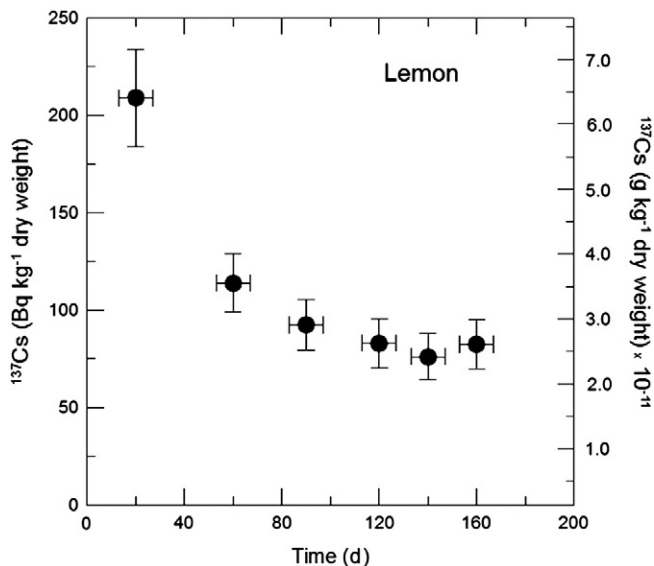

Fig. 3. Concentrations of ^{137}Cs in lemon fruits, during its ripening process on Goiânia soil.

Unfortunately, there are no coconut plants growing in Goiânia region. However, results obtained by Robison et al. (2009) support the hypothesis that cesium and potassium behave similarly during the growth process of coconuts. Robison et al. (2009) analyzed the concentration of ^{137}Cs and K in palm species: coconut tree (*C. nucifera* L.). Similarly to that observed for woody plants, their concentrations were higher in young (spike, spathe, frond, and fruit) than in old compartments (trunk). The concentrations of ^{137}Cs in the fruits, spikes and spathe were about 5.5–6.2 times higher than in the trunk. In the fronds they were about 2.1 times higher. However, the mean ^{137}Cs/K ratio for each compartment was essentially the same. Robison et al. (2009) have also reported that concentration of ^{137}Cs in fronds declines exponentially with the age and retrieval of this ion by the plant is observed, just as for K.

During analysis of the mineral composition of coconuts, Santoso et al. (1996) found that concentrations of K in young and mature fruits were 4.47 and 0.68% d.w., respectively. Concentrations of Na were found to be 0.10 and 0.02% d.w., respectively. These results suggest that the Na/K ratio for fruit compartment is essentially the same.

Both observations of Santoso et al. (1996) and our results support that sodium and potassium behave similarly during the growth process of coconuts. Based on this information, we could suggest that Na$^+$ ions are similarly incorporated to both Cs$^+$ and K$^+$ ions in edible parts of palm species.

Based on these findings, we have developed a theoretical model aiming to describe the temporal evolution of ^{137}Cs, K and Na incorporations during the growth and ripening of tropical and subtropical fruits, when the concentration of these elements behaves in similar ways.

The period of time required for fruit growth varies widely among species and genotypes reaching from a few weeks to months, from anthesis until fruit ripening. According to Pallardy (2008) and Carini et al. (2001), fruit growth curves can be of two general types. The first is a simple sigmoid type in which there is initially an exponentially increase in size followed by a growth slowdown in a sigmoid fashion. This type of curve is characteristic of some tropical and subtropical species, such as orange, banana, avocado, mango, coconut and lemon. The precise shape of the growth curve differs somewhat among plant varieties. The second type is a more complex growth curve, involving two periods of fast growth with a period of slow or suspended growth between them. This is common in stone fruits (such as peach, apricot plum and coffee), as well as some nonstone fruits (such as grape and currant).

Our model assumes that the dry mass of fruit grows according to a logistic model, taking into account two main assumptions:

1. The growth curve of fruit dry matter has a sigmoidal shape, following the differential equation:

$$\frac{dM(t)}{dt} = \alpha M(t)(M_m - M(t)) \tag{2}$$

where M is the dry matter (g), t the time in days, α the maximum growth rate (d^{-1}), and M_m the maximum value of $M(t)$. Its solution is:

$$M(t) = \frac{M_0 M_m}{(M_m - M_0)\exp(-\alpha t) + M_0} \tag{3}$$

where, $M_0 = M(0)$.

2. Taking into account that the concentration of ^{137}Cs, K and Na in coconuts and the concentration of ^{137}Cs and K in lemons decrease exponentially, the following differential equation can be used:

$$\frac{dC_i}{dt} = -\beta\left(C_i - C_i^{mim}\right) \tag{4}$$

where C_i is the concentration of ion i in the fruit (g kg^{-1}) at time t (d); $i = {}^{137}$Cs, K, Na; β is the exponential decrease parameter (d^{-1}); and C_i^{min} is the minimum value of $C_i(t)$ (g kg^{-1}). Its solution is:

$$C_i(t) = C_{Fr} \exp(-\beta t) + C_i^{mim} \tag{5}$$

where $C_{Fr} = C_i(0) - C_i^{min}$.

From the multiplication of Eqs. (3) and (5), the amount of ion i incorporated in the fruit as a function of time can be expressed by:

$$i(t) = M(t) \; x \; C_i(t). \tag{6}$$

Fig. 4 shows for the fruit development of lemons, the growth curve of dry matter (Fig. 4.a), ^{137}Cs and K concentrations (Fig. 4.b) and ^{137}Cs and K contents (Fig. 4c). The growth curve of dry matter in lemons was fitted using Eq. (3). The experimental data of K were fitted using Eq. (5). Fig. 4c was obtained from the direct application of Eq. (6). The agreement between the model and experiment is reasonable. When the parameters obtained to fit K are confronted to the incorporation of ^{137}Cs, the experimental values are perfectly matched by theoretical potassium curves (Fig. 4b and c). Moreover, from Fig. 4c it follows that the greater is the weight of fruit, the greater is its potassium content. On the other hand, the increase of potassium content of the fruit is much smaller than the increase of its weight, due to the effect of growth dilution.

Potassium is the main intracellular cation, being dissolved in solution in the gaps of cell walls, cytosol, and organelles, such as chloroplasts and mitochondria and especially in vacuoles (Barker and Pilbean, 2007). Based on this distribution, it is possible to associate that the higher is K content of a tissue the more water it contains. If the concentrations of these elements in plant tissues, plant organs, or total plants are expressed on a fresh weight basis, differences in the concentration may be very dramatic. In order to avoid this problem, the mass and concentration values in our model are related to dry matter weight.

Similar results are found for the incorporation of K and Na during the coconut development (Fig. 5). Again, the growth curve of dry matter in coconuts (Fig. 5a) was fitted using Eq. (3). The experimental data of K were fitted using Eq. (5). Fig. 5c was obtained from the direct application of Eq. (6). When the parameters obtained to fit K are confronted to the incorporation of Na, the experimental values are perfectly matched by theoretical potassium curves (Fig. 5b and c), indicating that the agreement between the model and experiment is reasonable.

These results suggest, therefore, that the growth curves of lemons and coconuts can be well interpreted by a sigmoid type. The incorporation of K$^+$ and ^{137}Cs during the lemon growth is time dependent, where their concentrations decrease exponentially with the increase in fruit age. Although some uncertainty exists about the status of sodium as a nutrient, partly arising from the semantics of essentiality, Na$^+$ incorporation exhibits a similar behaviour to K$^+$ in coconut trees, since, in contrast to that observed for the growth of lemons, its concentration decreases exponentially with the fruit age. According to literature (Robison et al., 2009), the same behaviour is expected for the ^{137}Cs$^+$ incorporation in coconuts. However, this similarity and the experimental confirmation of an exponential behaviour of ^{137}Cs concentration in coconuts will be evaluated in further measurements.

4. Conclusions

The behaviour of ^{137}Cs, K and Na in tropical species was evaluated through the calculation of the temporal evolution of their incorporation in fruits of two tropical species. Despite the absorption and transport of ^{137}Cs, K and Na exhibiting considerable dependence on plant species, our results disclosed that ^{137}Cs has similar behaviour of K in
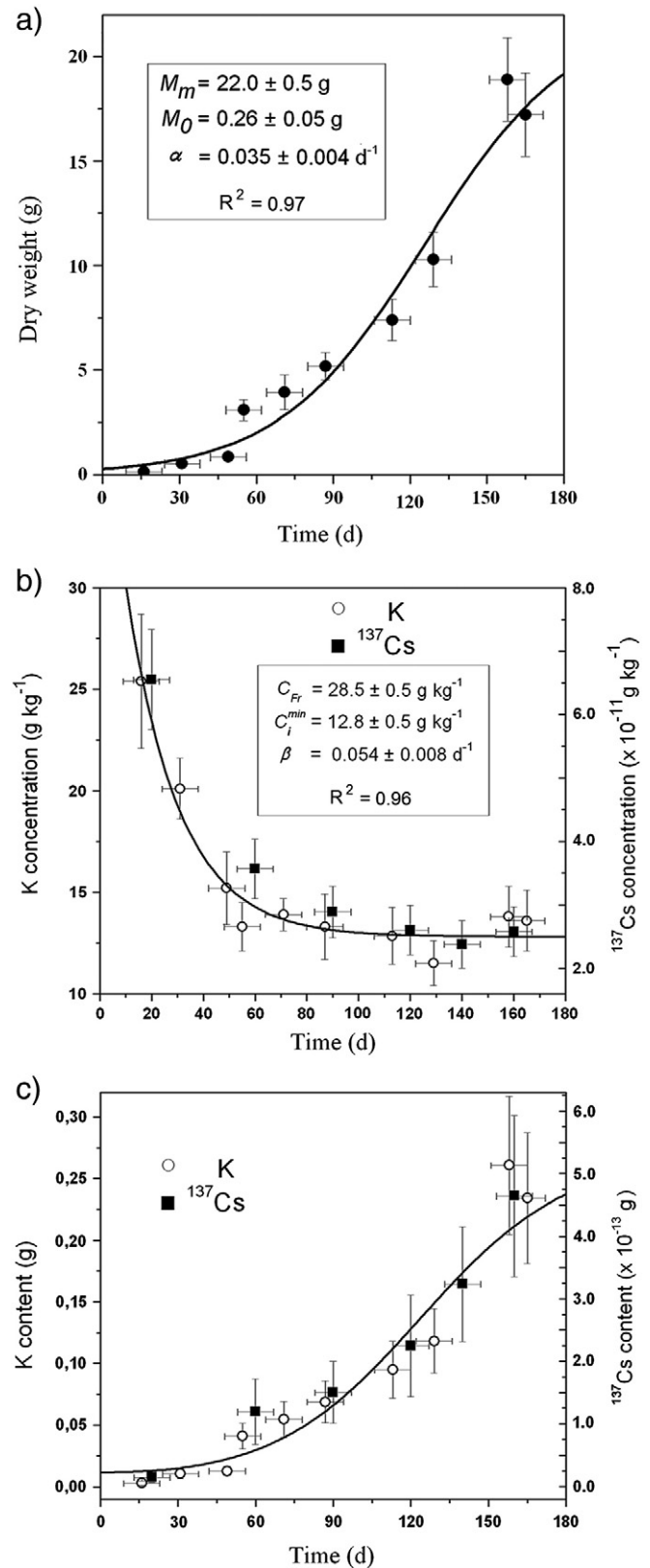

Fig. 4. Experimental data and logistic model fitting of fruit dry matter growth (a); ^{137}Cs and K concentrations (b) and; incorporation of ^{137}Cs and K (c), during ripening process for lemon fruits. The solid curves represent the theoretical approaches.

citrus species and that Na has a similar behaviour of K in palm species, in which its concentrations were age-dependent and decreased with increasing age of fruits. A very simple three compartment model
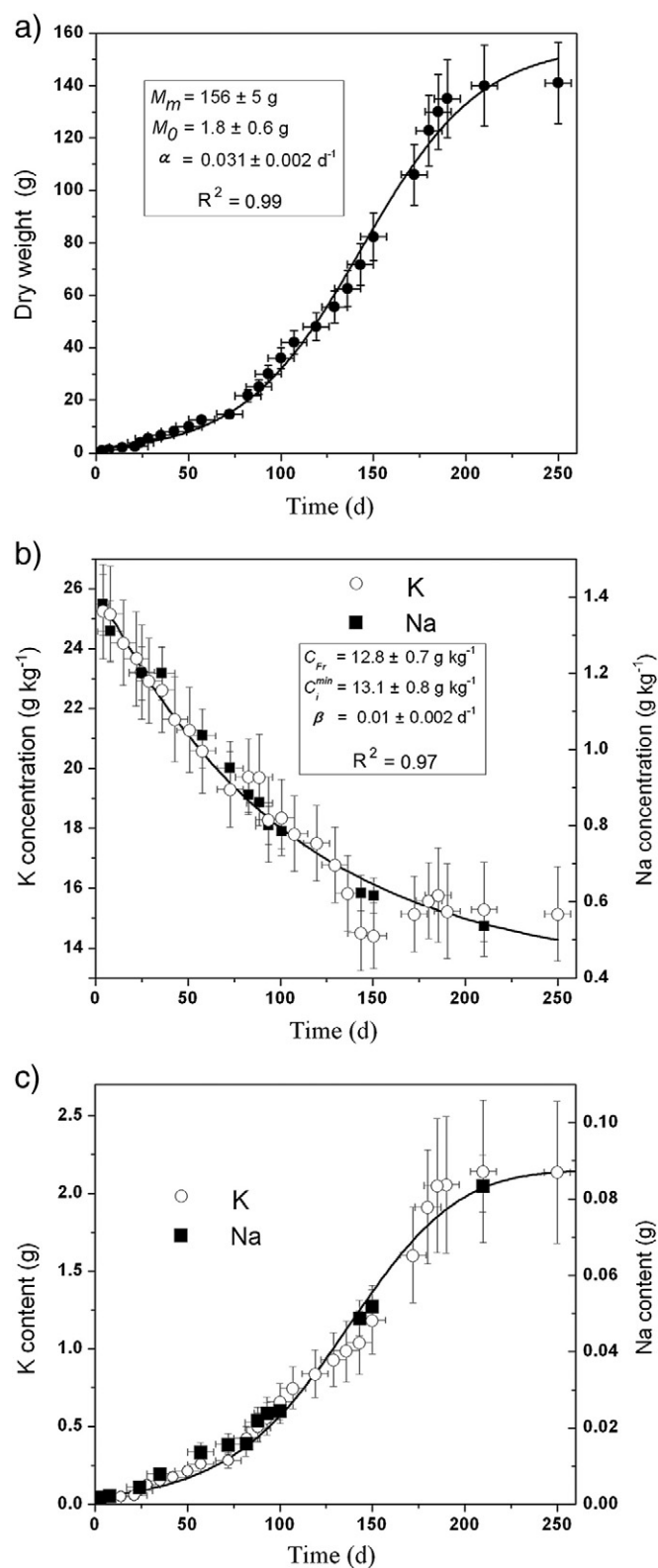
was proposed, which was calibrated with K experimental data. The agreement between the model and the experiment values is reasonable.

The availability of a theoretical approach to simulate the input of ^{137}Cs and K into the edible parts of plants is important, given that the assessment of these elements allows the understanding of forest and agricultural ecosystem evolution when submitted to radioactive pollutants.

Acknowledgements

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Fig. 5. Experimental data and logistic model fitting of fruit dry matter growth (a); K and Na concentrations (b) and; incorporation of K and Na (c), during ripening process for coconuts fruits. The solid curves represent the theoretical approaches.