



Influence of soil chemical composition on U, ^{226}Ra and ^{210}Pb uptake in leaves and fruits of *Quercus ilex* L.

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ABSTRACT

To determine their transfer factors, activity concentrations of natural radionuclides were measured in the leaves and acorns of holm oak (*Quercus ilex* L.) trees collected from seven locations with different soil properties and radionuclide activity concentrations. The chemical and mineralogical compositions of the soils were also analysed to investigate the effect these had on radionuclide absorption by the trees. Soil chemistry showed significant effects on radionuclide incorporation into *Quercus ilex* L. tissues. A significant relationship was established between activity concentrations and soil content of Ca and P with ^{238}U and ^{226}Ra in the leaves and acorns of *Quercus ilex* L. Differentiated transfer was found for ^{40}K , which showed greater transfer to the leaves than the other radionuclides. The activity concentration of U and ^{226}Ra was higher in the fruits than in the leaves, with the opposite effect being observed for ^{40}K . The risk of U and ^{226}Ra transfer into the food chain through acorn consumption by livestock is predicted to increase in soils poor in Ca and rich in P.

1. Introduction

Holm oak (*Quercus ilex* L.) is a sclerophyllous broadleaved tree species, paradigmatic of the Mediterranean region. It is adapted to the water stress caused by the annual summer drought typical of the region, sharing many of these adaptations with other Mediterranean species (Barbeta and Peñuelas, 2016). Moreover, this species is able to thrive in nutrient-poor soils (Silla and Escudero, 2004) as well as in the presence of toxic metals (Prasad and Freitas, 2000), and high activity concentrations of radionuclides from natural radioactive series, such as U, Ra and Pb which are significantly higher than global averages (Bettencourt et al., 1988; Carvalho et al., 2007; UNSCEAR, 2000). Moreover, holm oak trees can be found in areas that are highly polluted by natural radionuclides, either in the form of pure mineral or zones treated by acid leaching, such as slag heaps, which prevent typical colonisation and growth due to their lack of nutrients and abundance of toxic elements

(Blanco Rodríguez et al., 2010; Charro and Moyano, 2017; Martínez-Ruiz et al., 2001). For these reasons, *Quercus ilex* L. (QI) has been studied for the biomonitoring of trace elements, and it has been proved to accumulate them both in foliar tissue and superficial deposits, as well as demonstrating significant correlations between soil and foliar concentrations of these elements (Alfani et al., 1996; Maisto et al., 2004).

In this study, we selected QI because of certain characteristics: it accumulates toxic metals, it can grow on waste dumps with a high natural radioactivity content; and it is used as feed for pigs, whereby pollutants could be transferred to humans through the food chain (López-Hidalgo et al., 2021). For this reason, it is necessary to study the potential transfer of natural radionuclides in areas where their activity concentrations are higher than the natural background in the distribution area of the holm oak (e.g., areas with abandoned mining activities). This last point is important from a radiation protection point of view as Ra ingestion is 90% through food (Sam and Eriksson, 1995) and U is

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carcinogenic when the intake exceeds $0.05 \text{ mg U kg}^{-1} \text{ body mass d}^{-1}$ (Chen et al., 2021).

The uptake of radionuclides by plants is a process based on 5 main factors (Mortvedt, 1994; Yan et al., 2021): i) the amount of organic matter or clays; ii) the pH of the soil; iii) the chemical composition of the soil; iv) the chemical species of the radionuclides; and v) the presence of fungi and bacteria. UO_2^{2+} is a chemical form that is taken up by plants in the pH range 5.0–5.5 (Gil-Pacheco et al., 2021). In the case that U forms hydroxide, phosphate, or carbonate complexes, its bioavailability is reduced (Ebbs et al., 1998). Ra and Pb are mainly associated with organic matter, and their transfer to plants is therefore via this pathway (Avadhani et al., 2005; Vandenhove and Van Hees, 2007). The order of radionuclide uptake in plants is as follows: Pb and Po > Ra > U > Th (Gil-Pacheco et al., 2020). In addition, ^{40}K is also a radionuclide of interest, since levels of up to 4.425 mg g^{-1} (corresponding to an activity concentration of 140 Bq kg^{-1} of ^{40}K) have been measured in the fruit of certain tree species (Nikolic et al., 2006).

Studies on the incorporation of radionuclides in plants therefore require not only knowledge of the transfer factors (TF) of the different radionuclides but also their correlation with the chemical characteristics of soil. In this sense, any anthropogenic alteration of soil chemistry may cause a variation in the incorporation rate of radionuclides into plants. In order to study this phenomenon, plots with different anthropic influence were selected; from (semi-)natural soils to one in which acid leaching of uranium ores was performed. The number of samples was not large due to the low population of QI in the study areas.

Based on all the above, the hypothesis of this study was that the uptake of radionuclides in QI leaves and fruits is more related to acid

leaching processes in the soil than to their activity concentration. We tested our hypothesis by studying the chemical, mineralogical, and radiological characteristics of different types of soils. Three types of soils were selected: those characteristic of the Mediterranean forest; those present in sandy mining areas; and those altered by mining processes such as the acid washing of minerals. The objectives of our study were to: i) study the chemical and mineralogical characteristics of soils using X-ray fluorescence and X-ray diffraction; ii) determine the activity concentration through gamma spectrometry and radiochemical separations of the natural radionuclides in the soil in addition to QI leaves and fruits, and use these results to calculate the transfer factors as well as the bioavailability and bioavailability coefficients; iii) correlate and study the behaviour of chemical and radiological parameters based on existing plant radionuclide transfer models.

2. Experimental

2.1. Description of the study areas

Three study areas were used in this work (Fig. 1): i) in the vicinity and in the interior of a leaching bed from an abandoned uranium mine in the province of Salamanca; ii) a tailings dump from an abandoned copper mine in the Community of Madrid; and iii) two QI forests located in the Community of Madrid, in the towns of Boadilla del Monte and Tres Cantos. Appendix 1 of the Supporting Information (hereafter S.I.) presents the maps and satellite images with their locations, also Table A1.1 presents the study areas together with the location of the QI forests selected in this study.

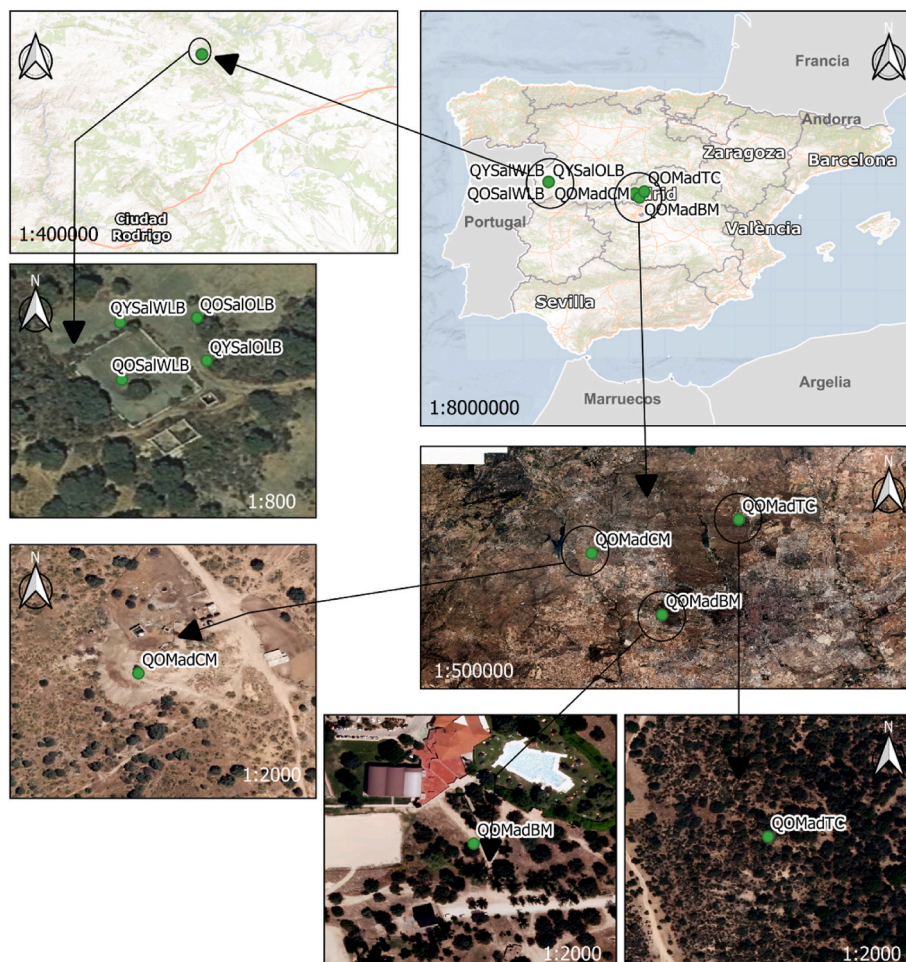


Fig. 1. Location of sampling points ((IGN), 2005, 2014).

The soil present in the leaching bed area in the province of Salamanca mainly comprises metasediments affected by the Variscan Orogeny and igneous rocks. The granites in this area contain calc-alkaline feldspars. They are coarse-grained and porphyritic with megacrystals of potassium feldspar and no facies differentiation. The main components of the granite are quartz, potassium feldspar, plagioclase, biotite and muscovite, with cordierite, andalusite, sillimanite, apatite, rutile, zircon and few opaques as accessories. The cordierite is always destabilised into aggregates of muscovite, biotite, and chlorite (Mellado et al., 2006). The peculiarity of this uranium mine is that U was leached using sulphuric acid (Charro and Moyano, 2017).

The area affected by the copper mine is located in the north-west of the Community of Madrid (Colmenarejo), which was abandoned in 1909. In this area, the tailings generated by the mining operation include: chalcocopyrite, arsenopyrite, malachite, azurite, siderite, fluorite, olivenite and calcantite (Pérez-Esteban et al., 2019).

The area sampled in Boadilla del Monte comprises a flat surface on sandy loam soils of Aragonian detrital arkoses, oligotrophic and slightly acidic, and which are predominantly classified as Cambisols, with the presence of some Luvisols and Regosols (Pérez Aparicio, 2016). Finally, the area located in Tres Cantos has deep sandy loam over arkosic sand sediments from eroded granite and gneiss. The soil in the woodland plot is slightly acidic, with a high base saturation and less than 5% organic matter in the topsoil (Aguillaume et al., 2017).

2.2. Sampling and preparation of soil, leaf and acorn samples

Even when holm oak trees can develop deep root system and a long tap root in xeric environments, most of the root biomass occurs in first 45 cm of soil depth. Soil sampling was performed at 20–50 cm depth so that the samples were representative of the nutrient absorption by the tree and this was consistent with the depth used in other comparable studies (Barescut et al., 2005). Moreover, the studied areas presented a high activity concentration of natural radionuclide, so depth profiles were not taken into account. For each of the sites, five different samples were combined into a composite sample for analysis.

In mining areas, all trees were sampled, due to their limited population. Leaf samples were selected according to availability, and acorns were collected directly from the trees. Each sample was taken from healthy specimens without apparent microbiological affection (bacterial, fungal or viral). For each tree, leaf and acorn samples from different parts of the tree were mixed into a composite sample for analysis, since the minimum sample size required by the analytical technique to reach the detection limits was 400 g. One leaf sample and one acorn sample were taken for each QI sampled. The leaf and acorn samples were dried for 48 h in a Selecta oven at 105 °C to a constant weight. The samples were then calcined at 450 °C in HOBERSAL muffles. A 0.5 g aliquot of the ash obtained was digested in an Ethos One microwave oven using a mixture of HNO₃ (9 mL) and HF (3 mL) acids. The programme used for this mineralisation consisted of a 20-min cycle at 1000 W and 220 °C. Once the sample had been mineralised, the excess HF was removed with 30 mL of 5% H₃BO₃. This process was carried out in a 5 min cycle at 1000 W and 220 °C. Designations for tree samples are “QY” and “QO”, corresponding to younger and older trees, respectively. The age of the holm oak trees were estimated by dividing the measured diameter at 1 m height by a growth factor of 0.2 mm y⁻¹, in accordance with previous studies (Farguell et al., 1994). The resultant ages of the sampled trees were 6.28 and 23.5 years for two of them (young trees, QY) and more than 100 years for the rest of them (old trees, QO).

2.3. Gamma spectrometry

Gamma spectrometry was used to determine the activity concentration of the soil samples as the activity was high and secular equilibrium in the three natural radioactive series could be ensured. The samples were measured on 3 HPGe detectors: two closed-end coaxials of

n-type material and one broad energy p-type material. Although the detectors had different configurations, the activity concentrations determined by them are equivalent for the type of samples analysed in this study. All three detectors were characterised by Canberra Industries to use LabSOCS (Laboratory Sourceless Calibration Software). LabSOCS allows detector counting efficiency to be calculated for different measurement geometries, sample compositions, and sample densities (Bronson, 2003). The electronic chain components (high-voltage supply, amplifier, analogue-to-digital converter and AIM communications module) were from Canberra Industries. The measurement geometry used in this work was a cylindrical container of 72 mm diameter and 30 mm height that was experimentally verified in a previous study (Suárez-Navarro et al., 2020). The following radionuclides were analysed (Be et al., 2016): ²³⁴Th (63.30 (2) keV), ²²⁶Ra (186.211 (13) keV), ²¹⁴Pb (351.932 (2) keV), ²¹⁴Bi (609.312 (7) keV; 1120.287 (10) keV; 1764.494 (14) keV), ²¹⁰Pb (46.539 (1) keV), ²¹²Pb (238.632 (2) keV), ²⁰⁸Tl (583.187 (2) keV), ²²⁸Ac (911.196 (6) keV), ²³⁵U (163.356 (3) keV; 205.16 (4) keV; 143.767 (3) keV), ²²⁷Th (235.971 (20) keV), ⁴⁰K (1460.822 (6) keV) and ¹³⁷Cs (661.657 (3) keV). Interference due to ²³⁵U at the 186 keV photopeak of ²²⁶Ra and ²²⁸Ac at the 1460 keV photopeak of ⁴⁰K were suppressed by the algorithm developed by (Suárez-Navarro et al., 2018). The samples were milled, sealed with parafilm and allowed to stand for 21 days to reach secular equilibrium between ²²²Rn and its short-lived progeny. The activity concentrations of ²¹²Pb and ²²⁷Th were determined by resolving the quartet at 238 keV following the method proposed by Harbottle and Evans (1997) (Harbottle and Evans, 1997). In order to improve the reliability of the analysis, the activity concentration of every sample was measured for 80,000 s. (i.e., approx. 1 day per sample). The uncertainties of the activity concentrations determined by gamma spectrometry have been evaluated taking into account the counting, calibration, background and sample preparation uncertainties with a coverage factor of k = 2. The gamma spectrometry technique used in this work is accredited by the Spanish National Accreditation Agency to Spanish, European and international standard UNE-EN ISO/EC 17025:2005 (UNE, 2005).

2.4. Radiochemical determination of isotopic U and ²²⁶Ra

The activity concentrations of isotopic uranium (²³⁸U, ²³⁵U and ²³⁴U) and ²²⁶Ra in the leaf and acorn samples were determined by radiochemical separations from the solution obtained following the procedure described in Section 2.2. ²²⁶Ra was determined using a modification of the method described by (Suarez-Navarro et al., 2015) in which the Ra is preconcentrated by precipitating [Pb–Ba–Ra]SO₄. The Pb was removed using an EDTA solution in a strong ammoniacal media. The Sr that was potentially present was then separated by precipitating BaCr₂O₇ (Blackburn and Al-Masri, 1992). The BaCr₂O₇ precipitate was converted to BaCl₂ by dissolving HCl with diethyl ether (6:1) (Ames et al., 1949). Then the BaCl₂ was dissolved in H₂O and precipitated as BaSO₄ using 1 M H₂SO₄. Finally, the BaSO₄ was vacuum filtered on a 0.45 µm filter which was dried under an infrared lamp. The filter was measured in a solid scintillation counter on a stainless steel plate with a ZnS(Ag) disk for 60,000s. The activity concentration, its associated uncertainty and detection limit were determined using the equations presented in S.I. Appendix 2. Isotopic U was separated using the liquid-liquid extraction method in ethyl acetate from a strongly saline solution of Al(NO₃)₃ with tartaric acid [33]. The samples were measured in a Canberra Industries Alpha Analyst device with 12 vacuum chambers containing the implanted silicon semiconductor detector for 500,000s. The uncertainty of the activity concentrations determined for the isotopic uranium and ²²⁶Ra included the uncertainties associated with sample preparation, counting, chemical yield (tracer or carrier), calibration and blank, with a coverage factor of k = 2. The expressions used to determine the activity concentration are given in S.I. Appendix 2.

2.5. Determination of the chemical and mineralogical composition of the soil samples

X-ray diffraction (XRD) was used to determine the crystalline phases present in the soil samples using a Malvern-PANalytical X'Pert Pro diffractometer operating at 45 kV and 40 mA. The data was collected using a configuration in the angular range of $10^\circ < 2\theta < 1200^\circ$ with a step size of 0.017° . The phases were identified using the comparison method in the HighScore Plus software package (Malvern-PANalytical), the Inorganic Crystal Structure Database (ICSD), and the Crystallography Open Database.

For the x-ray fluorescence analysis (WDXRF) an aliquot of each soil sample was pulverised to $74\ \mu\text{m}$ with a 200 mesh screen. The analysis was performed with a Malvern-PANalytical AXIOS automated spectrometer.

2.6. Determination of the pH and exchangeable fraction of soil

The soil pH was determined according to UNE-ISO 10390 (UNE-ISO, 2012); 5 mL of soil was placed in a graduated cylinder and mixed with 25 mL of H_2O . The emulsion was hermetically sealed in a separate funnel and agitated for 1 h in a vibroagitator. The emulsion was left to stand for 1 h and then transferred to a beaker where the pH was measured immediately with a Crison Basic 20+ pH meter.

The transfer factor (TF) was determined using the following expression:

$$TF = \frac{[A_{\text{plant}}]}{[A_{\text{soil}}]} \quad (1)$$

where $[A_{\text{plant}}]$ is the activity concentration of the selected radionuclide in the studied plant portion (Bq kg^{-1} , dry weight), and $[A_{\text{soil}}]$ is the activity concentration of the selected radionuclide in the soil sample (Bq kg^{-1} , dry weight).

The available fraction was determined using the $\text{CH}_3\text{COONH}_4$ 1 M method at pH 7.0 by taking 2 g of soil and extracting U and Ra with 20 mL of extractant for 5 min (Duquène et al., 2010; Sheppard and Evenden, 1992). Although this method has been commonly used to determine exchangeable cations in soils, it has also been used for radionuclides such as U and Ra in plant uptake studies (Baeza et al., 1999; Sheppard and Evenden, 1992). The bioavailable fraction was determined through the following expression ($BC_{\text{plant/soil}}$) (Neves et al., 2008):

$$BC_{\text{plant/soil}} = \frac{[A_{\text{plant}}]}{Ex_Y \cdot [A_{\text{soil}}]} \quad (2)$$

where Ex_Y is the distribution constant between soil and $\text{CH}_3\text{COONH}_4$ (where Y is equal to U or Ra). The product of $Ex_Y \cdot [A_{\text{soil}}]$ corresponds to the available soil factor. This relationship quantifies the possible accumulation of an element in leaves (Maisto et al., 2004).

The uncertainties associated with the transfer factor and bioaccumulation coefficient were determined using the Kragten method (Kragten, 1994, 1995).

2.7. Statistics

The statistical analyses carried out in this work were performed using the MS Excel Real Statistics add-in (www.real-statistics.com) and the statistical software package Statgraphics Centurion XVII (version 17.0.16). The statistical tests performed were cluster analysis and simple linear regression models. Cluster analysis was used to identify possible groupings of the different chemical elements analysed in the three types of soil samples (uranium mines, copper mines and oak forests). Although the number of observations was limited to seven, this analysis was still possible (Miller and Miller, 2005). The observed groupings were then used to search for linear models between the chemical elements and the

radionuclides belonging to the natural radioactive series.

3. Results

3.1. Chemical, mineralogical and radiological composition of the studied soils

Table 1 shows the elemental composition percentages of the 7 soil samples determined by XRF for the most representative elements. The complete results of the XRF analysis are presented in SI Table A2.1. The mineralogical composition obtained using XRD is also presented in Table 2. Concentrations of Na and Ca were higher in leach-affected soil samples than in non-impacted soil samples. Concentrations of Ti, V, Fe, Se and V were also higher in areas where minerals were extracted. As shown in Table 2, soil samples from the uranium mining areas showed a higher percentage of muscovite phase.

3.2. Chemical properties of soils

Table 3 shows the chemical properties of each of the soils: pH and exchangeable ^{238}U (Ex_U) and ^{226}Ra (Ex_{Ra}) in the soil determined using the ammonium acetate method at pH = 7. In the area altered by the acid leaching processes, the pH was below 5.5. Fig. 2 plots the relationships between Ex_U and Ex_{Ra} as a function of pH. In the case of U, the isotope ^{238}U was selected as its activity concentration is statistically equal to that of ^{234}U and ^{235}U (multiplied by the $^{238}\text{U}/^{235}\text{U}$ ratio of 21.429 ± 0.043 (Suárez-Navarro et al., 2021)). The linear fit obtained for ^{238}U indicates a low statistical relationship between Ex_U and pH ($p > 0.05$) and the correlation was -0.16 , and R^2 only represented 2.66% of the dispersion of the values. However, Ex_{Ra} is statistically related to pH in a linear model ($p < 0.05$), with a correlation of 0.825 and R^2 represented 68.10% of the dispersion.

3.3. Activity concentrations of natural radionuclides in soil samples as well as leaves and fruits of *Quercus ilex* L

The activity concentrations of the natural radionuclides belonging to the radioactive uranium, thorium and actinium series together with ^{40}K and ^{137}Cs are presented in Tables 3 and 4 for the soils close to the QI specimens, as well as their leaves and fruits.

3.4. Transfer factors and bioaccumulation coefficients, and their relation to soil properties

Transfer factors for ^{238}U , ^{234}U , ^{226}Ra , ^{210}Pb and ^{40}K were determined using Expression 1. The transfer factors as a function of soil activity concentration for ^{226}Ra and ^{210}Pb were fitted to a model $TF = a+b/AC$ (inverse of AC), where AC is the soil activity concentration and a and b the fitting coefficients (Fig. 3) (Sheppard and Sheppard, 1985). For ^{226}Ra , the ANOVA fit test gave a p-value < 0.05 . This means that the two variables were statistically significant. The correlation was 0.948, indicating a strong relationship between the variables, while the R^2 value indicated that the model explained 89.83% of the variability of the TF. In the case of ^{210}Pb , the points followed the same model as in the case of ^{226}Ra . The correlation between TF and CA was 0.927 indicating a strong relationship between the variables, while R^2 explained 89.92% of the variability of the TF. TF ^{238}U did not fit this model as p-value < 0.05 was obtained. Again, only the ^{238}U TF was re-used, for the reasons given in Section 3.2. Finally, in the case of ^{40}K the points also fitted the inverse AC model with correlation between the experimental values of 0.987, while R^2 explained 98.71% of the variability of the TF.

Bioaccumulation coefficients (Expression 2) were only ascertained for ^{238}U , ^{234}U and ^{226}Ra , as the available soil factor was only determined radiochemically for these three radionuclides (Table 5). The activity concentrations of the different radionuclides determined in the QI leaves and fruits were represented as a function of the percentages of all the

Table 1
Chemical composition of soils close to each *Quercus ilex* L.

Element (%)	QYSalOLB	QOSalOLB	QYSalWLB	QOSalWLB	QOMadCM	QOMadBM	QOMadTC
Na	0.36	0.28	0.42	0.37	1.2	1	1.2
Ca	0.17	0.24	0.024	0.013	1.5	0.58	1
Ti	0.49	0.55	0.51	0.49	0.33	0.16	0.18
V	0.024	–	0.018	0.024	–	–	–
Fe	5.1	3.9	4.8	4	3.2	1.1	1.5
Cu	0.0052	0.0044	0.0059	0.0074	0.17	0.0027	0.0036
As	–	–	0.013	–	0.01	–	–
Se	0.0038	–	0.0018	0.0029	–	–	–
Ba	0.14	0.051	0.16	0.14	0.043	0.051	0.039

Table 2
Mineralogical composition of soils in the vicinity of each *Quercus ilex* L.

Sample	Albite (%)	Anorthite (%)	Microcline (%)	Muscovite (%)	Orthoclase (%)	Quartz (%)
QYSalOLB	–	–	–	50.4	–	49.6
QOSalOLB	–	–	–	40.7	–	59.3
QYSalWLB	–	–	–	48.7	–	51.3
QOSalWLB	–	–	–	47.7	–	52.3
QOMadCM	–	29.4	25.4	–	–	45.2
QOMadBM	15.4	–	21.6	–	–	63
QOMadTC	39.4	–	–	12.6	20.4	27.6

Table 3
Activity concentration of gamma emitters in soils in the vicinity of the *Quercus ilex* L. specimens (Bq kg⁻¹).

Sample	Uranium serie						Actinium serie		Thorium serie			⁴⁰ K	¹³⁷ Cs
	²³⁴ Th	^{234m} Pa	²²⁶ Ra	²¹⁴ Pb	²¹⁴ Bi	²¹⁰ Pb	²³⁵ U	²²⁷ Th	²²⁸ Ac	²¹² Pb	²⁰⁸ Tl		
QYSalOLB	2050	2340	14200	13300	12080	12700	102	606	53.2	57.6	41.8	680	4.20
	±210	±160	±1200	±1000	±290	±1800	±10	±66	±2.4	±5.0	±2.7	±30	±0.49
QOSalOLB	618	579	847	657	589	710	26.7	30.6	51.2	57.9	39.2	547	10.54
	±64	±52	±86	±50	±15	±100	±2.1	±3.6	±2.0	±4.7	±2.5	±24	±0.70
QYSalWLB	1600	1790	11800	10230	9260	9900	75.9	347	45.6	54.5	35.9	787	<2.9
	±170	±100	±1000	±770	±220	±1400	±7.1	±38	±2.5	±4.6	±2.5	±35	
QOSalWLB	1990	2050	8450	6800	6240	6610	71.5	321	52.6	59.8	21.2	894	2.76
	±210	±130	±750	±510	±150	±940	±7.6	±35	±2.2	±5.0	±1.4	±43	±0.37
QOMadCM	59.9	<71.6	71.7	43.0	36.19	47.4	<2.7	<6.7	60.8	65.1	36.33	905	1.83
	±4.5		±6.3	±1.2	±0.75	±4.4			±1.2	±2.0	±0.93	±18	±0.13
QOMadBM	27.6	<70.0	39.5	30.8	28.5	36.3	<3.2	<5.9	37.9	41.0	26.5	1131	0.619
	±3.8		±5.2	±2.5	±1.1	±5.8			±1.9	±3.3	±1.7	±49	±0.080
QOMadTC	38.1	<92.9	52.2	33.6	33.7	56.8	<5.3	<8.2	31.3	32.6	11.30	1137	6.61
	±5.2		±7.3	±2.7	±1.5	±8.9			±1.4	±2.7	±0.80	±49	±0.52

The uncertainties are quoted for a coverage factor of k = 2.

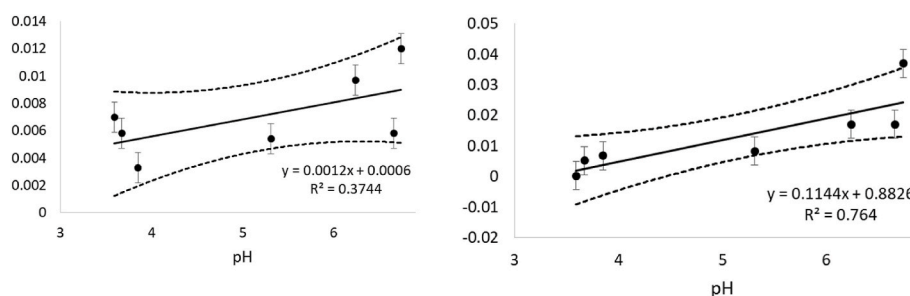


Fig. 2. Exchangeable ²³⁸U (EX_U) and ²²⁶Ra (EX_{Ra}) as a function of pH for the 7 soils studied. The data for the graphs were taken from Table A5.1 in the supplementary information.

elements in the studied soils (Table 1 and SI Table A2.1.). Of these, statistically significant relationships were found only for U and Ra in leaves and fruits with respect to Ca and P present in soils. The activity concentrations of ²³⁸U and ²²⁶Ra in the leaves and fruits of QI were modelled as a function of the concentrations of Ca and P in the soil in which QI grew, in order to investigate the relationship between them (Fig. 4). The model fitted to the experimental values was AC=(a+b/

Ca)², providing a p-value of less than α = 0.05 in all fits, indicating a statistically significant relationship between the AC and the percentage of Ca. R² values ranged from 76.4% to 99.9% and correlations were between 0.874 and 0.999, the highest being those obtained for fruits. The linear model for the activity concentration of ²³⁸U activity in leaves and fruits (Table 4) as a function of the percentage of P in the soil (Table 1) was also determined. In the case of leaves, the model was AC =

Table 4
Activity concentrations of gamma emitters in the leaves and fruits of *Quercus ilex* L. (Bq kg⁻¹).

Sample		Uranium series		Actinium series					Thorium series			⁴⁰ K	¹³⁷ Cs		
		²³⁸ U (d)	²³⁴ U (d)	²³⁴ Th	²²⁶ Ra (d)	²¹⁴ Pb	²¹⁴ Bi	²¹⁰ Pb	²³⁵ U	²³⁵ U (e)	²²⁸ Ac			²¹² Pb	²⁰⁸ Tl
QYSalOLB	L	0.265 ±0.046	0.322 ±0.048	<3.7	19.0 ±3.6	7.98 ±0.40	6.37 ±0.32	19.8 ±1.9	<1.2	<0.037	<2.0	1.13 ±0.18	0.388 ±0.078	209 ±10	<0.4
	F	0.186 ±0.032	0.226 ±0.034	<11.3	<18.9	8.8 ±1.0	7.89 ±0.84	<8.7	<5.2	<0.026	<4.5	1.52 ±0.36	<1.4	304 ±14	<1.2
QOSalOLB	L	19.82 ±0.49	17.36 ±0.43	16.35 ±0.85	29.7 ±1.3	11.38 ±0.29	9.96 ±0.29	31.17 ±0.85	<1.0	0.772 ±0.038	<1.3	1.37 ±0.13	0.75 ±0.15	196.1 ±3.9	<0.3
	F	0.190 ±0.033	0.230 ±0.035	<1.6	9.0 ±1.0	6.72 ±0.55	6.53 ±0.36	3.05 ±0.43	<0.6	<0.027	1.59 ±0.16	0.216 ±0.060	<0.2	265 ±18	0.776 ±0.059
QYSalWLB	L	8.07 ±0.30	7.37 ±0.28	8.9 ±2.1	34.2 ±6.0	16.5 ±1.2	13.76 ±0.80	35.0 ±3.7	<3.3	16.5 ±2.0	<3.1	1.53 ±0.16	1.27 ±0.54	188 ±11	<0.8
	F	3.09 ±0.12	2.83 ±0.11	<24.6	67.3 ±7.3	36.5 ±2.0	29.2 ±1.9	<24.1	<12.7	0.119 ±0.011	<17.5	3.93 ±0.65	<4.5	468 ±23	<4.3
QOSalWLB	L	123.8 ±3.5	105.5 ±3.0	137.2 ±3.8	514 ±20	342.5 ±6.6	297.0 ±2.6	55.3 ±3.0	5.11 ±0.53	5.57 ±0.35	4.25 ±0.55	3.55 ±0.17	1.15 ±0.17	167.4 ±4.6	<1.2
	F	8.79 ±0.32	8.04 ±0.30	11.78 ±0.77	269 ±10	203.1 ±3.9	192.6 ±1.7	29.2 ±1.6	<2.2	0.338 ±0.031	3.29 ±0.33	1.62 ±0.10	<0.5	250.1 ±5.3	<0.4
QOMadCM	L	0.611 ±0.055	0.469 ±0.052	<3.6	<6.2	<1.3	<1.5	32.2 ±4.9	<1.8	<0.034	2.63 ±0.64	1.42 ±0.23	<0.8	179 ±13	<0.7
	F	0.076 ±0.023	<0.073	<1.8	<3.0	0.61 ±0.13	<0.4	<1.6	<0.9	<0.019	<0.6	<0.2	<0.2	237.8 ±3.7	<0.2
QOMadBM	L	1.48 ±0.10	1.64 ±0.11	<4.1	10.8 ±1.8	3.83 ±0.40	3.01 ±0.31	24.5 ±1.2	<2.1	<0.056	<1.8	1.40 ±0.27	0.85 ±0.16	135.2 ±9.0	<0.3
	F	<0.057	<0.059	<1.4	1.51 ±0.46	0.860 ±0.093	0.920 ±0.072	<1.3	<0.6	<0.016	<0.4	0.112 ±0.017	<0.1	135.9 ±5.2	0.075 ±0.015
QOMadTC	L	0.126 ±0.028	0.123 ±0.029	<1.5	5.8 ±2.9	5.25 ±0.55	5.51 ±0.74	48.8 ±7.1	<1.3	6.75 ±0.86	<3.1	1.56 ±0.21	0.31 ±0.21	136 ±13	<0.3
	F	<0.061	<0.062	<1.4	<2.9	1.37 ±0.16	1.10 ±0.16	<1.4	<0.8	<0.017	<0.8	<0.2	<0.2	214 ±20	<0.2

The uncertainties are quoted for a coverage factor of k = 2.

^a The activity concentrations of ²³⁸U, ²³⁵U, ²³⁴U and ²²⁶Ra were determined by radiochemical methods (see section 2.4).

exp(a+b/P) with a p-value of less than 0.05, providing a correlation of 0.896 and an R² that explained 78.0% of the dispersion. Furthermore, the fruit model was AC = 1/(a+b·P) with a p-value of less than α = 0.05, with a correlation of 0.924 and an R² that explained 85.4% of the dispersion between AC and P. The proposed models were chosen among the alternative models generated with StatGraphics (see section 2.7), selecting those with the highest values for correlation coefficient and a higher representativeness of the dispersion (R²). The axes have been appropriately transformed to obtain a linear function in order to better visualise the trend of the experimental values.

4. Discussion

The results obtained in this study support our hypothesis that soil chemical alteration is more relevant than the soil activity concentration itself in the incorporation of radionuclides by QI. The origin and characteristics of each soil studied are related to the pH and chemical composition obtained through XRF. The soils sampled at the copper mine (QOMadCM) and the two holm oak groves (QOMadBM and QOMadTC) had an average pH of 6.5, while the pH of those obtained from the Salamanca mine (QYSalOLB, QOSalOLB, QYSalWLB and QOSalWLB) were all lower than 5.31. In the case of the QOSalOLB soil, the pH of 5.31 indicates that the soil is not influenced by the *in-situ* leaching being carried out. This pH is in line with that found in previous studies of the holm oak grove adjacent to the outside of the leaching bed (Charro and Moyano, 2017). On the other hand, the elements differentiating the soils from the uranium mine, copper mine and holm oak groves were: Na, Ca, Ti, V, Fe, Cu, As, Se and Ba (see S.I. Appendix 6). The percentage of Na was lower in the soils from the uranium mine. However, the percentages of Na obtained in the other samples were comparable to the average values in the soils from the uranium mine. (Towett et al., 2013). The decrease in Na can be explained by the leaching reactions and the interaction with clays under these conditions. (Seidel, 1979). Ca was also lower in the uranium mine soils, indicating a

loss at the beginning of the leaching process (Satybaldiyev et al., 2015), as well as in the subsequent H₂SO₄ treatment of the by-products in the regeneration of the reagents (Na₂CO₃ and NaHCO₃ or Glauber's salt and CaCO₃) (Suri et al., 2014). The higher percentages of Ti and Fe found in the uranium and copper mines are typical in these mining areas (Angileri et al., 2020; Liao et al., 2016) confirming their acid attack resistance in the leaching process, as in previous studies (Bowell et al., 2011). The presence of V in the three most acidic samples from the Salamanca area indicates that one of the U-containing mineral forms is carnotite (K₂(UO₂)₂(VO₄)·3·H₂O) (Ghorbani and Montenegro, 2016) which is typical of Spanish sites (Arribas Moreno, 1974). The percentage of Cu is in line with the soils originating from the copper mine, due to the presence in this area of chalcopryrite (CuFeS₂), malachite (Cu₂CO₃(OH)₂), azurite (Cu₃(CO₃)₂(OH)₂), olivenite (Cu₂AsO₄OH) and chalcantithite (CuSO₄·5H₂O) (Pérez-Esteban et al., 2019). The As found in the four soils from the uranium mine is in line with previous studies on the soils of the Salamanca mines (García-Sánchez and Alvarez-Ayuso, 2003) and, in the case of the copper mine, may be due to the mineral arsenopyrite (Navas et al., 2021; Pérez-Esteban et al., 2019). The presence of Se in the three acidic samples from the uranium mine, where a greater influence of the leaching processes was observed, was due to the fact that Se is redeposited by redox processes in the sandstones after the leaching of the slurry (Uhrig et al., 1996). The Se levels are comparable to those reported by Bullock and Parnell (2017), with a mean value of 57 mg kg⁻¹ (Bullock and Parnell, 2017). Finally, the higher percentage of Ba obtained in the acidic samples from the uranium mine is in line with the values obtained for V as it is associated with uraninite and uranium vanadate (Tsui, 1984).

The mineralogical phases determined through XRD were consistent with the study areas where the soils were sampled (Table 2). The soils from the two holm oak groves in Madrid consisted of albite (NaAlSi₃O₈), a feldspar typical of the soils in the Madrid region (Díaz Martínez et al., 2012). The presence of anorthite feldspar (CaAl₂Si₂O₈) is related to the higher percentage of Ca found in the soil from the copper mine (Table 1)

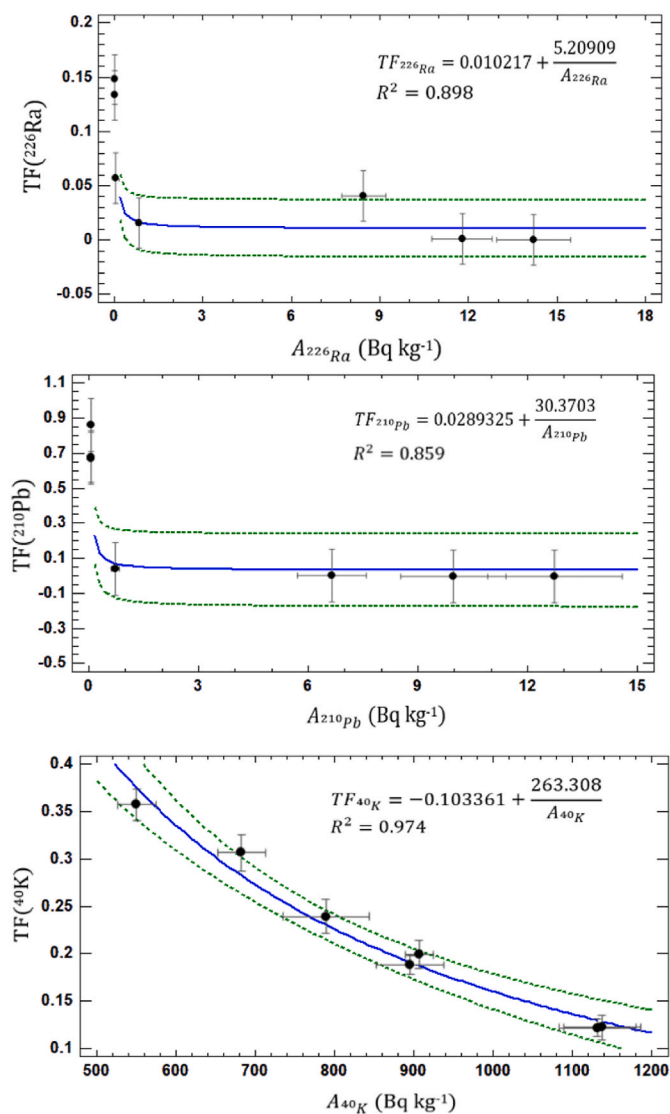


Fig. 3. Transfer factors obtained from the leaves of *Quercus ilex* L. as a function of soil activity concentration for ²²⁶Ra, ²¹⁰Pb and ⁴⁰K. The error bars on each point represent the uncertainty in the activity concentration of ²²⁶Ra, ²¹⁰Pb and ⁴⁰K, as well as the uncertainty associated with the model used to fit the experimental data, assuming a coverage factor of k = 2.

(Hidalgo Signes et al., 2016). Microcline feldspar and muscovite mica are also present in the soils of the Madrid region (Fesharaki et al., 2018; Hidalgo Signes et al., 2016). On the other hand, muscovite was found in a higher proportion in the soils of the uranium mine, in line with the findings of other authors in the province of Salamanca (Roda Robles et al., 2018).

Possible U-bearing mineral phases such as carnotite, uraninite, and uranium vanadate would account for less than 5% in the uranium mine soils, so their presence cannot be confirmed. The percentages of Cu- and As-containing minerals in the copper mine were less than 5% as the soil sampled was in the vicinity of the mine dump. These minerals cannot be identified using the XRD technique, as the detection limit of the diffractometer is generally 5%.

The activity concentrations obtained for the uranium mine soils were above the Spanish range of reference values for soils, while the others were within this range (²³⁸U between 6 and 250 Bq kg⁻¹, ²³²Th between 2 and 210 Bq kg⁻¹ and ⁴⁰K between 25 and 1650 Bq kg⁻¹). The activity concentrations were also higher than the permitted values for agricultural use for U and Ra, these being 288 Bq kg⁻¹ and 185 Bq kg⁻¹,

Table 5

Bioaccumulation coefficients for leaves and fruits of *Quercus ilex* L.

Reference	Type	²³⁸ U	²³⁴ U	²²⁶ Ra
QYSalOLB	L	0.00200 ± 0.00046	0.00284 ± 0.00060	0.215 ± 0.038
	F	0.00141 ± 0.00032	0.00199 ± 0.00042	0.125 ± 0.028
	L	0.66 ± 0.10	0.646 ± 0.099	0.202 ± 0.036
QOSalOLB	F	0.0063 ± 0.0014	0.0086 ± 0.0018	0.102 ± 0.019
	L	0.166 ± 0.026	0.187 ± 0.029	0.0197 ± 0.0035
QYSalWLB	F	0.0636 ± 0.0098	0.072 ± 0.011	0.0230 ± 0.0046
	L	1.17 ± 0.18	1.13 ± 0.17	0.74 ± 0.13
QOSalWLB	F	0.083 ± 0.013	0.086 ± 0.013	0.59 ± 0.13
	L	0.243 ± 0.044	0.214 ± 0.044	0.414 ± 0.080
QOMadCM	F	0.030 ± 0.010	-	0.189 ± 0.037
	L	1.03 ± 0.29	-	0.77 ± 0.20
QOMadBM	F	-	-	0.171 ± 0.050
	L	0.071 ± 0.024	0.085 ± 0.031	1.68 ± 0.49
QOMadTC	F	-	-	0.36 ± 0.10
	L	-	-	-

The uncertainties are quoted for a coverage factor of k = 2.

respectively (Abreu et al., 2014). As shown in Table 3, the activity concentration of ¹³⁷Cs reveal low levels of ground disturbance as the values are equivalent to fallout (4 Bq kg⁻¹) (Ahmad et al., 2019; Ritchie and McHenry, 1990). The activity concentration ratios obtained at the Salamanca uranium mine for ²³⁸U (618–2049 Bq kg⁻¹), ²²⁶Ra (847–14209 Bq kg⁻¹), ²³²Th (54.9–59.8 Bq kg⁻¹) and ²¹⁰Pb (707–12713 Bq kg⁻¹) were equivalent to those found in previous studies of the same area (Charro and Moyano, 2017). These results evidence the leaching activities carried out in the Salamanca mine area as the ²²⁶Ra/²³⁸U ratios are higher than 1. The activity concentration of ²²⁷Th multiplied by the ratio of the ²³⁸U/²³⁵U is equal to 21.429 ± 0.043 (Suárez-Navarro et al., 2021), equivalent to the activity concentration of ²²⁶Ra and confirming that 76–86% of the uranium has been leached. (samples QYSalOLB, QYSalWLB and QOSalWLB) (Carvalho et al., 2007). The activity concentration of ²¹⁰Pb was equivalent to that of ²²⁶Ra and its progeny, and no ²¹⁰Pb deposition process was detected due to the high activity concentration of ²²²Rn exhaled from the soil in the study area due to the high activity concentration of ²²⁶Ra.

The Ex_{Ra} obtained with the acetate method increased as a function of pH. This Ex_{Ra} is related to the exchangeable and carbonate fraction (CaCO₃ and MgCO₃) of the soil (Tessier et al., 1979). However, the Ex_U did not vary as a function of pH, in contrast to the relationship found by Manoj et al. for an exchange with H₂O (Manoj et al., 2020). Our results indicate an association of between 3.0 and 6.5% for U and between 0.2 and 18.6% for Ra. These percentages are comparable to those obtained in previous studies (Sam and Eriksson, 1995).

The activity concentrations obtained from both young and old holm oak leaves coincide with those obtained in a previous study carried out in one of the study areas (Charro and Moyano, 2017). The leaf activity concentrations of all radionuclides were higher than those of fruits, as was found for U (Arribas and Herrero-Payo, 1979) and Ra (Gerzabek et al., 1998) in previous studies. A cumulative effect was also observed in the QI sampled at the uranium mine. This pattern coincides with that observed by Blanco Rodríguez et al. in old and young leaves of different types of trees, including QI, but from the same individual (Blanco Rodríguez et al., 2010). The activity concentration of ⁴⁰K was higher in fruits than in leaves, which is in line with the observations of other authors (Tewari et al., 2021).

The activity concentration of U and Ra activity in the leaves and fruits of QI was lower when the percentage of Ca in the soil increased (Fig. 4). Likewise, the activity concentration of U activity decreased in the leaves as the percentage of P in the soil increased, the opposite behaviour being observed in the case of fruit. Although Ca prevents the uptake of Ra because of its chemical similarity (Linsalata, 1994), its subsequent passage from the roots to the leaves is easier. Subsequently,

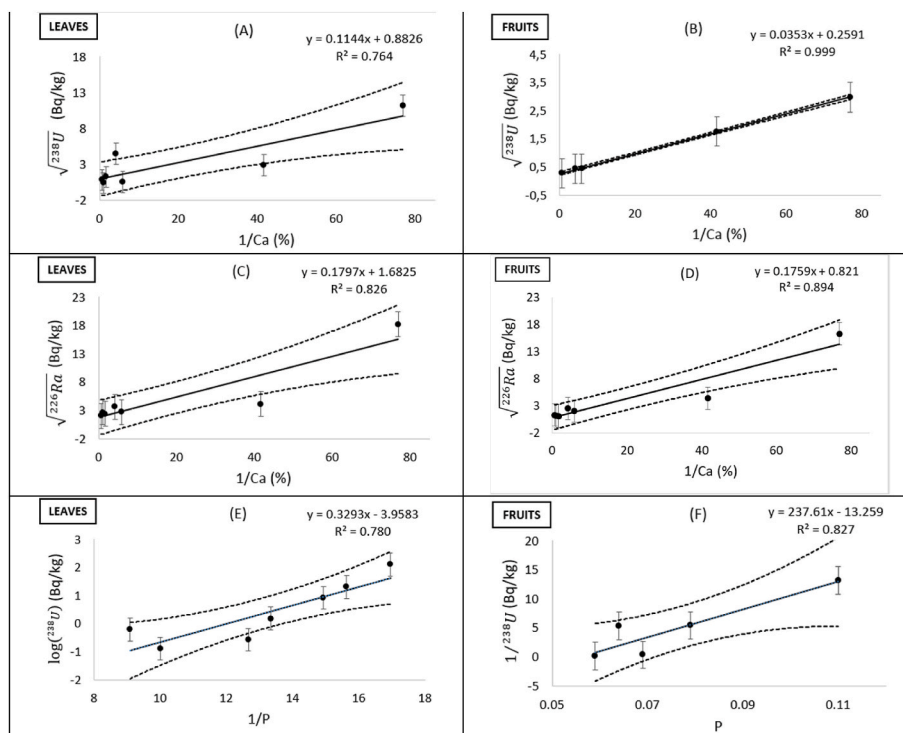


Fig. 4. Relationship between the percentage of Ca and P in the soil and the activity concentration of ${}^{238}\text{U}$ and ${}^{226}\text{Ra}$ in the leaves and fruits of *Quercus ilex* L.: a) ${}^{238}\text{U}$ leaves; b) ${}^{226}\text{Ra}$ leaves; c) ${}^{238}\text{U}$ fruits; d) ${}^{226}\text{Ra}$ fruits; e) ${}^{238}\text{U}$ leaves and f) ${}^{238}\text{U}$ fruits.

Ra is fixed in the leaves through the formation of insoluble compounds (Smith, 1971). U absorption is reduced by the presence of Ca^{2+} either through a competitive mechanism or the formation of a Ca-U-CO_3 complex that would reduce its bioavailability (El Hayek et al., 2018). On the other hand, the U uptake observed in the leaves is as expected, contrary to that found in the fruits (Rufyikiri et al., 2006). P also prevents the absorption of U through the formation of insoluble compounds when the pH is increased (Hewamanna et al., 1988). This effect is equivalent for Ra although our results do not confirm this (Blanco Rodríguez et al., 2002). The results obtained represent scientific evidence that could, however, be expanded with a greater number of samples and a wider range of activity concentrations, although the authors consider the range used to be significant.

The TFs obtained were ${}^{210}\text{Pb} > {}^{226}\text{Ra} > {}^{238}\text{U}$ as in previous studies (Blanco Rodríguez et al., 2010; Charro and Moyano, 2017). Furthermore, the TFs of ${}^{226}\text{Ra}$ and ${}^{210}\text{Pb}$ as a function of soil activity concentration were fitted to the soil-plant uptake model proposed by Sheppard et al. (Fig. 2) (Sheppard and Sheppard, 1985). However, U did not fit this model as R^2 was below 50%. According to the model, ${}^{226}\text{Ra}$ and ${}^{210}\text{Pb}$ would have an essential effect up to 850 Bq kg^{-1} and 700 Bq kg^{-1} , respectively, which could be due to a tolerance behaviour (Gil-Pacheco et al., 2021). At higher activity concentrations, both ${}^{226}\text{Ra}$ and ${}^{210}\text{Pb}$ would have a tolerance effect. The tolerance observed for ${}^{210}\text{Pb}$ coincides with the correlation found by Alfani et al. (1996) between the (stable) Pb in the leaves and that present in the soil. The kurtosis and standardised bias for ${}^{210}\text{Pb}$ were typical of a normal distribution, as the values were less than 2 (see box-and-whisker plot in S.I. Appendix 4). This finding could indicate a saturation effect for ${}^{210}\text{Pb}$ in the leaves of the QI, as the activity concentration in the leaves is independent of the activity concentration in the soil. The ${}^{40}\text{K}$ also followed the pattern found for ${}^{226}\text{Ra}$ and ${}^{210}\text{Pb}$, but in this case an essential effect is observed in the range of activity concentrations in all samples.

The $\text{BC}_{\text{plant/soil}}$ results for ${}^{226}\text{Ra}$ and ${}^{238}\text{U}$ using the ammonium acetate method indicate that there is no cumulative, indicator or exclusionary effect according to the models proposed by Baker under our

study conditions (Baker, 1981). $\text{BC}_{\text{plant/soil}}$ values were less than 1 except in the case of the leaves from QOSalWLB, where the values were greater than 1 (Reimann et al., 2001). However, this value is statistically indistinguishable from 1.

The exposure to soils with a high activity concentration in natural radionuclides could imply a risk for animal welfare due to an increased oxidative stress (Jović et al., 2009), but not because of a chromosomal-level damage, since the absorbed dose is below $100 \mu\text{Gy h}^{-1}$ (Fister and Jović, 2014; Real et al., 2004). On the other hand, the risk of ${}^{226}\text{Ra}$ transfer to the food chain through acorns, or fodder and flour made from them, would increase when the holm oaks grow on soils poor in Ca^{2+} . The main acorn consumers are in first place the pigs, but fodder is also used to feed cattle and sheep, and even poultry during winter season (Avilés et al., 2001; Mekki et al., 2019, 2022). The consumption of Ra and Po can cause the accumulation of these elements in liver and kidneys, and can be transfer to the milk and, therefore, to the food chain. However, the transfer of natural radionuclides –such as U, Th, Ra and Po– to the muscle tissue is lower (Carvalho et al., 2014). For example, uranium transfer factors to sheep meat through grass foraging are in the range of $2.5 \cdot 10^{-5}$ to $2.4 \cdot 10^{-4}$ (Fellows et al., 1998). According to these previous studies and the results of this research, ${}^{226}\text{Ra}$ could enter the food chain through dairy products when animals are fed with acorns (or their products) from trees growing in soils with high activity concentration of Ra and low concentration of Ca^{2+} .

5. Conclusions

This study provides preliminary conclusions on the absorption of radionuclides by *Quercus ilex* (QI) and its relationship with soil chemical composition. However, the number of QIs that met the conditions of our study was limited. Therefore, future research with a greater sample would be desirable to confirm and further develop the following conclusions.

Ca and P were the soil elements that most affect the transfer of U and Ra to specimens of QI, being even more related to the uptake than the

activity concentrations of these radionuclides in the soil. Ca might compete with U and Ra, decreasing their incorporation into the leaves and, to a lesser extent, the fruits of QI. This effect was greater in the soil sample most affected by the uranium ore washing process as there was a decrease in Ca levels in the soil. ^{210}Pb accumulated in the leaves of the QI regardless of its activity concentration in the soil. U, Ra, and Pb accumulated more in the leaves than in the fruits of the QI specimens sampled. The activity concentration of ^{40}K was higher in fruits than in leaves. ^{40}K showed essential behaviour while ^{226}Ra and ^{210}Pb exhibited tolerant behaviour. Therefore, the potential transfer of ^{226}Ra to the food chain through the milk of animals fed with fodder including acorns increases with a decreased concentration of Ca in the soil where acorns were collected. However, the risk of ^{226}Ra transfer to the food chain through meat is more unlikely since the transfer factors to muscle tissue are lower.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvrad.2023.107187>.

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