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D9.14 – Published dataset on transfer in Mediterranean ecosystems

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Abstract

The quantification of transfer parameters to human foodstuffs is essential to predict ingestion doses. Such parameters are a key input for the development and validation of radioecological models and decision support systems. In the literature, there are many data about human foodchain transfer factors, but these are mainly for temperate ecosystems. Data are sparse for Mediterranean ecosystems which are important within southern European. Seasonal variations in temperature and rainfall, among other variables, may mean transfer parameters in Mediterranean ecosystems differ to those for temperate ecosystems. Data are lacking for some foodstuffs that are typical of, and important for, the European Mediterranean area (e.g. wine, olive oil, etc.). We have collected and analysed typical Mediterranean foodstuffs from Spain to derive transfer parameter values. This deliverable is a description of the methodology used the resultant dataset (Guillen et al., 2019, available from https://doi.org/10.5285/48d5395e-e9fb-45ed-b69f-1ea0d2d36be6). Two sampling schemes were used: i) in the main production regions of key foodstuffs and ii) in a managed dehesa (Mediterranean semi-natural grassland). Analysed foodstuffs were: i) cereals, ii) grapes (and wine), iii) olive (including oil), iv) lamb, v) beef, vi) pork, and vii) dairy products (milk, cheese, kefir and yogurt). The corresponding soil, grass, and animal feedstuffs were also sampled. Information about animal diet composition was provided by the producers so that radionuclide and stable element concentrations in total diet could be calculated. As anthropogenic radionuclide concentrations in Spain can be generally considered as low level (main contributor global fallout), composite samples and long measurement times were used for the determination of gamma-emitting radionuclides (^{226}Ra, ^{137}Cs, ^{228}Ra and ^{40}K). Analogue stable element, heavy metals and major element concentrations in the collected samples (Cs, Sr, K, Na, Ca, Mg, P, Pb, U and Th) were determined by ICP-MS. Due to the small amount of sample required, we were able to obtain information about transfer to individual animals for stable elements. Transfer factor, F_v, and concentration ratios, CR, were calculated according to IAEA TRS472. As a result of this deliverable, the information about transfer parameters in Mediterranean ecosystems is increased by 124 and 196 values per radionuclide / stable element for plant and animal products respectively.

Content

This dataset constitutes Deliverable D9.14 "Published dataset on transfer in Mediterranean ecosystems" of CONFIDENCE project. The published dataset is available from the following link: https://doi.org/10.5285/48d5395e-e9fb-45ed-b69f-1ea0d2d36be6.

1. Material and Methods

1.1. Sample sites

Foodstuffs were collected in Spain using two sampling schemes:

i) main production regions, sampling preferentially in areas where the main production of a given food occurs according to public information from the Spanish Ministry of Agriculture; and
ii) local level, sampling in a dehesa managed by the regional government in Extremadura. The dehesa is a typical Mediterranean semi-natural grassland with disperse tree cover, mainly holm oaks (*Quercus ilex*), which is also used for crops. Figure 1 shows the approximate location of the different sampling sites.

Foodstuff samples can be classified into the following groups:

a. Cereals: wheat (Extremadura, Castilla-León, and dehesa), triticale (a wheat-rye cross) (Extremadura and dehesa); oats (dehesa); and barley (Castilla-León and dehesa).
b. Grapevine: Andalusia and Extremadura, different grape varieties sampled (plant, grapes and wine sampled).
c. Olive: Andalusia and Extremadura, different olive varieties sampled (olives and olive oil sampled).
d. Lamb: dehesa, four flocks with different feeding regimes.
e. Beef: dehesa, three herds with different feeding regimes.
f. Pork: Extremadura, Iberian pig.
g. Dairy products: sheep milk (Castilla-León and dehesa, five flocks with different feeding regimes); sheep cheese (dehesa, two flocks with different feeding regimes); goat milk (dehesa, two flocks with different feeding regimes); goat cheese (dehesa, two flocks with different feeding regimes); cow milk (Galicia); cow cheese (Galicia); cow yogurt (Galicia); cow kefir (fermented milk drink) (Galicia).

At each sampling location, soil (0-10 cm), wild grass, and different animal feeds (where appropriate) were also collected.

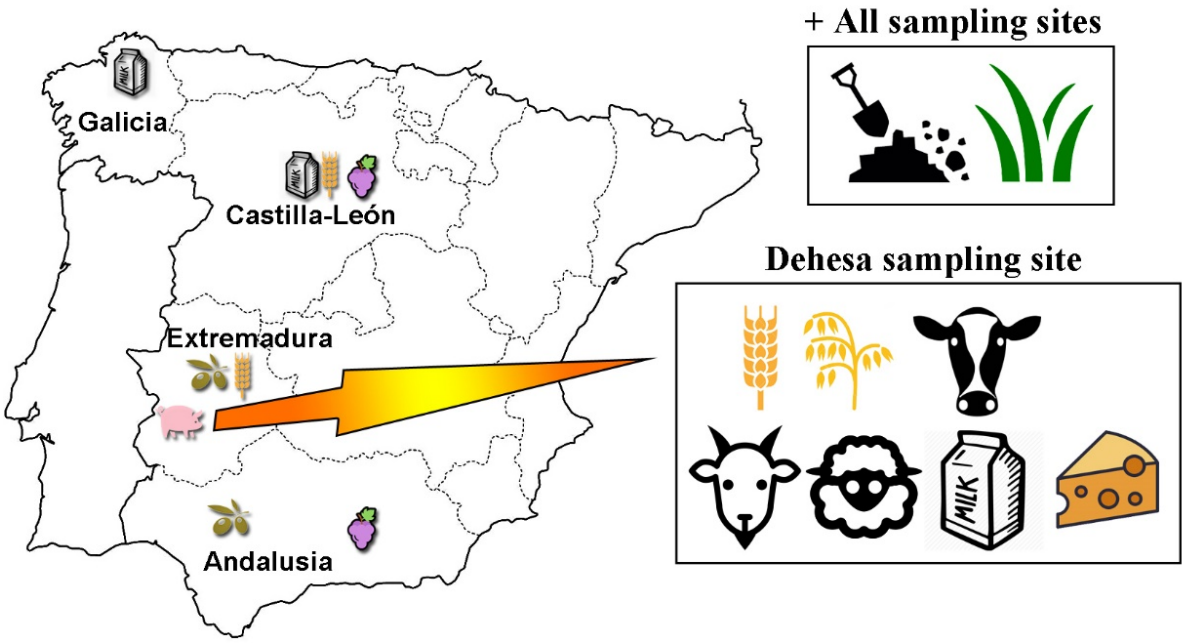

Fig. 1. Approximate location of sampling sites in Spain

1.2. Sample collection

The location of the sampling sites were recorded using hand held GPS (latitude and longitude WGS84 co-ordinate system used).

1.2.1. Soil

Soil samples, 0 – 10 cm, were collected at each sampling site. At least 6 soil samples (0 – 10 cm) were collected from the same area as the food product, and combined to make a composite sample.

1.2.2. Wild grass

Wild grass was collected by hand cutting about 1 cm above the soil surface to avoid soil contamination. The dominant grass species in samples were identified.

1.2.3. Cereals

Whole cereal plants were sampled and then separated into roots, stem, and grain. Stems were cut about 1 cm above soil in order to minimise any adhered soil. Roots were washed with distilled water to remove soil particles. Grain samples were separated from the husk. Wheat (*Triticum aestivum*) was collected in Torreorgaz (Cáceres, Extremadura), Piñel de Abajo (Valladolid, Castilla-León), Barcina de los Montes (Burgos, Castilla-León) and Valdesequera dehesa (Badajoz, Extremadura). Triticale (*Triticosecale*) was collected in Torreorgaz and Valdesequera. Oats (*Avena sativa*) was collected in Valdesequera. Barley (*Hordeum vulgare*) was collected in Piñel de Abajo and Valdesequera.

1.2.4. Grapevine

Mature grapes, leaves and branches were collected from grapevine (*Vitis vinifera*) collected in Ronda (Málaga, Andalusia), variety Pinot noir, and Piñel de Abajo (Valladolid, Castilla-León), variety Tempranillo. Wine from Ronda was obtained from the wine cellar at the sampling site; whereas wine from Piñel de Abajo was made in the laboratory from crushed and filtered grapes.

1.2.5. Olive

Mature olives, leaves and branches from olive trees (*Olea europaea*) were collected in Torreorgaz (Cáceres, Extremadura), mixture of varieties Verdial and Manzanilla cacereña, and in Rociana del Condado (Huelva, Andalusia), variety Picual. Olive oil from Torreorgaz was obtained from an oil mill using collected olives; whereas olive oil from Rociana del Condado was obtained from the oil cooperative where the olives were sent.

1.2.6. Lamb

Lamb (*Ovis aries*, pure Merino breed) foodstuffs were collected in Valdesequera dehesa (Badajoz, Extremadura), from flocks with four different feeding regimes: hay + cereal based concentrate feed; vetch-oats silage + cereal based concentrate feed; algae based feed + cereal based concentrate feed; and wine by-products silage + cereal based concentrate feed. Animal feedstuffs were also sampled, and information about diet composition was provided by the producers. Meat, bone, liver and kidney samples were collected at the slaughterhouse; sample size depended on the number of individuals culled. Information (e.g. individual identification number) on the individual animals was obtained.

1.2.7. Beef

Beef (*Bos taurus*, Retinto breed) foodstuffs were collected in Valdesequera dehesa (Badajoz, Extremadura), from herds with three different feeding regimes: straw + cereal based concentrate cattle feed; vetch-oats silage + cereal based concentrate cattle feed; and sunflower silage + cereal based concentrate cattle feed. Animal feedstuffs were also sampled. Meat, bone, liver and kidney samples were collected at the slaughterhouse, and sample size depended on the number of individuals culled. Information on individual individuals was obtained from the producers.

1.2.8. Pork

Pork (*Sus scrofa domesticus*, black Iberian pig breed) foodstuffs were collected in Higuera la Real (Badajoz, Extremadura). In the last stage of growth (called “montanera” in Spanish), the pigs were free ranging in a dehesa, with holm oak acorns (*Quercus ilex*) as their main feed. Animal feed was sampled in the same way as in the Valdesequera dehesa for beef. Meat, bone, liver and kidney samples were collected at the slaughterhouse. No information about individuals (e.g. identification number) apart from where they originated from was available when samples were collected.

1.2.9. Dairy products

Dairy products from sheep, goats and cows were sampled. Sheep (*Ovis aries*) milk were collected in Barcina de los Montes (Burgos, Castilla-León), Churra breed, and in Valdesequera dehesa (Badajoz, Extremadura), pure Merino breed with five different feeding regimes: hay + cereal based concentrate feed; vetch-oats silage + cereal based concentrate feed; algae based fodder + cereal based concentrate feed; wine by-products silage + cereal based concentrate feed; and tomato by-products silage + cereal based concentrate feed. Sheep cheese was collected in Barcina de los Montes and in Valdesequera (with two different feeding regimes: hay + cereal based concentrate feed and wine by-products silage + cereal based concentrate feed). Goat (*Capra hircus*), Verata breed, milk and cheese were collected in Valdesequera dehesa with two different cereal based concentrate feedings: hay + cereal based concentrate feed and tomato by-products silage + cereal based concentrate feed. Cow (*Bos taurus* Holstein Frisian breed) milk, cheese, yogurt and kefir were collected in Xanceda (Coruña, Galicia). Animal feedstuffs were also sampled from the different sites. Information about animal diet composition was provided by producers.

2. Sample analyses

2.1. Sample preparation for ICP-MS

Soil samples were sieved, and elements greater than 2 mm were discarded. Soil samples were homogenized and oven dried.

Cereal, leaves, branches, wild grass, animal feed and bone samples were oven-dried until of a constant mass. Grapes, wine, olives, dairy products, meat, liver and kidney samples were freeze-dried at -80 ºC (Telstar LyoQuest). Olive oil was analysed without any prior preparation.

2.2. Microwave assisted acid digestion of samples

Soil samples, about 0.5 g dry mass, were acid digested by means of a microwave oven (Ethos Pro Milestone Ltd) with HNO_3 ultrapure (distilled from HNO_3 analytical grade using a DST-1000 acid purification system (Savillex)), HCl and HF (9:3:6 mL) at 180 ºC for 30 min. After digestion, samples were evaporated in Teflon vessels, then HCl and boric acid were added to remove fluorides, and evaporated. Finally, HNO_3 was added, so that medium was 5 % HNO_3 and filtered.

About 0.5 g dry mass of foodstuff and animal feed samples, , were acid digested using the same microwave oven, with HNO_3 and H_2O_2 (6:1 mL) at 200 ºC for 30 min. Then, water grade 1 (ultrapure) was added so that the resultant medium was 5 % HNO_3.

2.3. ICP-MS analyses

Concentrations of stable elements (Cs, Sr, U, Th, Pb, K, Ca, Na, Mg and P) were determined using a NexIon 350X ICP-MS Perkin-Elmer with mass-analyser quadrupole. The sample introduction system consisted of a nebulizer tip coupled to a cyclonic spray chamber. Sample transport from the autosampler (ESI 2DX) to the nebulizer was established by a peristaltic pump. Working parameters were as follows: argon flow 16 L/min; auxiliary gas flow, 1.2 L/min; nebulising flow 1 L/min; radiofrequency power 1600 W; dwell time 50 s; sweeps per reading 20; integration time 1000 ms; replicates 3. To remove polyatomic interferences, the instrument was set using kinetic energy discrimination (KED mode), with a collision cell gas of helium at a flow of 0.5, 2 and 3 mL/min.

All standards and sample solutions were prepared by using water grade 1 (ultrapure) and HNO_3. Quantitative analyses of Cs, Sr, U, Th, Pb, K, Ca, Na, Mg and P concentration were performed using external calibration and internal standardisation. Standards for Cs, Sr, U, Th, K, Ca, Na, Mg and P were prepared from single standard solutions of 1000 mg/L (Scharlau and Perkin Elmer); a Pb standard solution was prepared from a multi-element standard solution of 10 mg/L (Scharlau). For calibration of Cs, Sr, U, Th and Pb standards of concentrations 0.1, 1 and 100 µg/L were used; for K, Ca, Na, Mg and P standards of concentrations 100, 1000, 2000, 5000 and 10000 µg/L were used. An aliquot from a commercial multi-element internal standard solution of 10 mg/L (Perkin Elmer) was added as an internal standard to the blanks, calibration standards, and samples in a concentration of 9.5 µg/L of scandium (^{45}Sc), indium (^{115}In), terbium (^{159}Tb), holmium (^{165}Ho) and bismuth (^{209}Bi) to control plasma fluctuations and to correct for ion signal instability; the mean value of recoveries was 106%. Calibration coefficients of correlation (r) were >0.999 for each isotope measure.

Quantification limits (used as detection limits) were calculated as three times the standard deviation of the 10 reagent blanks, and were as follows: Cs=0.0005 µg/L, Sr=0.03 µg/L, U=0.0004 µg/L, Th=0.009 µg/L, Pb=0.002 µg/L, K=10 µg/L, Na=5 µg/L, Ca=30 µg/L, Mg=0.5 µg/L and P=9 µg/L.

Combined uncertainty (k = 2) was calculated taking into account: duplicate samples; certified reference materials (surface water SPS-SW2 (Spectrapure Standards), organic material (Cabbage) IAEA-359 (IAEA), and soil IAEA-SOIL-7 (IAEA)); and measurement relative standard deviation (counting uncertainty).

Daily verification of the instrument was performed using a 1 µg Be, Ce, Fe, In, Li, Mg, Pb, U L^{-1} mixture in 1 % HNO_3 (Perkin Elmer) to check the correct instrument response; the oxides (CeO-156/Ce-140) and double-charge ions (Ce2+-70/Ce-140) formation levels were < 0.015 and 0.003, respectively, and the background intensity at mass 220 was < 1 cps.

2.4. Sample preparation for gamma spectrometry

Composite soil samples were sieved, and the fraction greater than 2 mm was discarded. Soil samples were then homogenized, oven dried at 100 ºC and encapsulated in 112 cm^3 Petri type dishes.

Since low activity concentrations of anthropogenic radionuclides in food and animal feed samples were expected (global fallout main contributor), composite samples were analysed for those samples with enough collected material. Composite samples were dried and then calcined at 400 ºC to reduce volume and achieve lower detection levels. Wine samples were dried and encapsulated in Petri dishes. Olive oil samples were measured fresh in 1 L Marinelli beakers.

2.5. Gamma spectrometry determination of radionuclides

After approximately 21 days to assure secular equilibrium, γ-spectrometric analysis were carried out using HP(Ge) detectors, with efficiencies in the range 25 – 45 %. The radionuclides analysed were ^{137}Cs, ^{214}Pb and ^{214}Bi (in equilibrium with ^{226}Ra), ^{228}Ac (in equilibrium with ^{232}Th series) and ^{40}K. The ^{226}Ra activity concentration in samples was calculated as the mean value of ^{214}Pb and ^{214}Bi activity concentrations. The ^{228}Ac activity concentration in samples was considered to be in equilibrium with ^{228}Ra in food- and feedstuff samples, and with ^{228}Ra and ^{232}Th in soil samples. The HP(Ge) detectors were calibrated using a multi-radionuclide gamma cocktail from NPL and using the same geometries and matrices as the samples. The overall quality control of these radiochemical procedures is guaranteed by the accreditation of the laboratory to carry out radioactivity assays in environmental samples according to UNE-EN ISO/IEC 17025 (ISO, 2010a). Reference materials provided by the IAEA (IAEA 385 and IAEA Soil6) were used to verify the quality of the measurement; estimated activity levels were within the recommended intervals for the reference materials. Uncertainties were calculated according to ISO11929 (ISO, 2010b).

2.6. Determination of physico-chemical parameters of soils

Soil pH was determined in water. 500 mL of distilled water was added to each 5 g of soil sample and stirred for 15 min. After settling for 10 min, pH was measured with a calibrated pH-meter.

Soil conductivity was determined by adding 250 g of soil 1 L of distilled water. The resultant solution was stirred for 15 min, then, sieved through a paper filter (2-4 µm pore size). Conductivity was measured with a calibrated electrical conductivity meter.

Soil texture was determined using 20 g of soil, previously sieved to 2 mm. Organic matter content was removed with H_2O_2 and the sample stirred for two hours in distilled water. Then, it was put into a 1 L test tube and left to stand for 12 h. The first 20 mL of supernatant were taken to calculate clay content. The remainder was sieved through 0.21 mm and 0.053 mm sieves to determine coarse and fine sand, respectively. Silt was calculated as the subtraction of all previous fractions (100 % - % clay - % coarse sand - % fine sand).

3. Calculation of foodstuff transfer parameters

Transfer parameters were calculated according to IAEA TRS472 (IAEA, 2010). The transfer factor, Fv, for the uptake of radionuclides and stable elements from soil by plants is defined as the ratio of dry weight concentration in the plant to the dry weight concentration in the soil layer (0 – 10 cm).

$$F_v = \frac{Plant\ concentration\ (Bq/kg\ dm\ \ or\ mg/kg\ dm)}{Soil\ (0-10\ cm)\ concentration\ (Bq/kg\ dm\ \ or\ mg/kg\ dm)}$$

The transfer factor to liquid foodstuffs of plant origin (i.e. wine and olive oil), F_v, is defined as the ratio of activity or stable element concentration in liquid foodstuff (per L) and the dry weight concentration in the soil layer (0 – 10 cm).

$$F_v(kg\ dm/L) = \frac{Liquid\ foodstuff\ concentration\ (Bq/L\ \ or\ mg/L)}{Soil\ (0-10\ cm)\ concentration\ (Bq/kg\ dm\ \ or\ mg/kg\ dm)}$$

The transfer factor to food products derived from animals was calculated as the concentration ratio, CR, defined as the ratio of the radionuclide or stable element concentration in food product (fresh weight) and its concentration in the total diet (dry matter). When animal diet consisted of several feedstuffs, information about proportions in animal diet was provided by producers enabling the estimation of an overall diet concentration. Wild grass intake by cattle, sheep, goat and pigs was calculated by subtracting the mass diet (e.g. cereal based concentrates) given from the required daily dry matter intake rate of dry matter taken from the literature (Bach et al., 2010; Calsamiglia et al., 2009; de Blas et al., 2013; Jarrige G, 1988; Ferret et al., 2008).

If a radionuclide or stable element concentration was not detectable in all components of animal diet, the following approach was used: i) using detection limits to estimate the feed concentration if those feeds below detection limits contributed to < 30 % to the whole diet; ii) if their contribution was > 30 % of the dry mater intake rate, detection limits were used to calculate feed concentration, but reported as a 'less than' value. In the latter case, transfer parameters were not determined.

$$CR = \frac{Food\ concentration\ (Bq/kg\ fm\ \ or\ mg/kg\ fm)}{Feed\ concentration\ (Bq/kg\ dm\ \ or\ mg/kg\ dm)}$$

For milk (liquid), the concentration ratio was defined as the ratio of the radionuclide or stable element concentration in milk (per L) and its concentration in the whole diet (dry matter).

$$CR\ (kg\ dm/L) = \frac{Milk\ concentration\ (Bq/L\ \ or\ mg/L)}{Feed\ concentration\ (Bq/kg\ dm\ \ or\ mg/kg\ dm)}$$

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Disclaimer

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