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# Inter-cultivar variation in soil-to-plant transfer of radiocaesium and radiostrontium in *Brassica oleracea*

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A B S T R A C T

Radiocaesium and radiostrontium enter the human food chain primarily via soil-plant transfer. However, uptake of these radionuclides can differ significantly within species (between cultivars). The aim of this study was to assess inter-cultivar variation in soil-to-plant transfer of radiocaesium and radiostrontium in a leafy crop species, *Brassica oleracea.* This study comprised four independent experiments: two pot experiments in a controlled environment artificially contaminated with radiocaesium, and two field experiments in an area contaminated with radiocaesium and radiostrontium in the Chernobyl Exclusion Zone. Radiocaesium concentration ratios varied 35-fold among 27 cultivars grown in pots in a controlled environment. These 27 cultivars were then grown with a further 44 and 43 other cultivars in the Chernobyl Exclusion Zone in 2003 and 2004, respectively. In the field-grown cultivars radiocaesium concentration ratios varied by up to 35-fold and radiostrontium concentration ratios varied by up to 23-fold.

In three of these experiments (one pot experiment, two field experiments) one out of the 27 cultivars was found to have a consistently lower radiocaesium concentration ratio than the other cultivars. The two field experiments showed that, five out of the 66 cultivars common to both experiments had consistently lower radiocaesium concentration ratios, and two cultivars had consistently lower radiostrontium concentration ratios. One cultivar had consistently lower radiocaesium and radiostrontium concentration ratios.

The identification of cultivars that have consistently lower radiocaesium and/or radiostrontium concentration ratios suggests that cultivar selection or substitution may be an effective remediation strategy in radiologically contaminated areas. Future research should focus on plant species that are known to be the largest contributors to human dose.



## 1. Introduction

Radiocaesium and radiostrontium enter the human food chain primarily via soil-plant transfer (Ehlken and Kirchner, 2002). However, the concentration of these elements taken up by plants varies at the family (Broadley and Willey, 1997; Willey and Fawcett, 2006), species (Broadley and White, 2012) and cultivar scale (Payne et al., 2004; Penrose et al., 2015). Cultivar substitution, where a cultivar with higher uptake of an element is substituted for a cultivar with lower uptake of that element, is a possible method for reducing transfer of radionuclides to humans (White et al., 2003;

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Penrose et al., 2015). However, little is known about the extent and nature of variation in radiocaesium and radiostrontium uptake between cultivars (Beresford et al., 2006), and thus cultivar substitution has not been widely adopted as a remediation strategy. The authors are only aware of one study that has reported the use of cultivar selection as a remediation strategy; 2–3 fold reduction in $^{137}Cs$ and $^{90}Sr$ uptake by rapeseed was achieved by selecting varieties with lower Cs and Sr uptake (Fesenko et al., 2007). A recent review of inter-cultivar variation, considering a range of plant species, showed that caesium (Cs) and strontium (Sr) concentrations vary about 2-fold between the cultivars with the lowest and highest concentration ratios, although the literature reviewed predominately reported studies with fewer than 20 cultivars (Penrose et al., 2015).

The aim of this study is to assess variation in soil-to-plant transfer of radiocaesium and radiostrontium into a leafy crop species, *Brassica oleracea*. This species belongs to the Brassicaceae (mustard) family, which includes other important crop types such as *Brassica napus* (oil seed rape, swede), *Brassica rapa* (pak choi, turnip) and the widely-studied plant species *Arabidopsis thaliana*. Domesticated *B. oleracea* has a number of familiar crop types, including cauliflower and broccoli, cabbage, Brussels sprout, kohlrabi and kale which display distinctive morphological variation (Kennard et al., 1994). *B. oleracea* is an important vegetable and fodder species: global production of 'cauliflowers and broccoli' exceeded 22 million tonnes in 2013 (FAOSTAT, 2015); >420,000 tonnes of *B. oleracea* were produced in the UK alone in 2013/2014 (DEFRA, 2014).

There are numerous published studies on both inorganic and organic compositional traits in *B. oleracea*. For example, for leaf calcium (Ca) and magnesium (Mg) concentrations, which as Group II elements are likely to display similarities to radiostrontium in terms of uptake and accumulation, there was about 2-fold variation in shoot concentrations among 74 field-grown cultivars (Broadley et al., 2008). For potassium (K), which as a Group I element is likely to display similarities to radiocaesium, albeit to a lesser degree than among Group II elements (Broadley and White, 2012), there was 1.6-fold variation in shoot K concentrations among 72 field-grown cultivars (White et al., 2010). However, we have identified just one published study reporting (stable) Cs and Sr uptake by *B. oleracea* cultivars, in which a single Brussels sprout cultivar was compared to a single cabbage cultivar (Bibak et al., 1999). The Cs concentration of the edible portion differed between these two genotypes by 2.4-fold and Sr concentration varied by 4.2-fold. Whilst soil-to-human transfer of Cs and Sr via vegetable crops is unlikely to be the major pathway of these elements into the food chain based on typical 'Western' dietary habits with high consumption of animal products and cereals, the contribution of leafy vegetables to dietary intakes of Group I and II essential elements is significant (Broadley and White, 2010). For example, vegetables (excluding potato) are likely to contribute between 5 and 10% of dietary Ca, Mg and K intake in the UK. Therefore, if concentrations of these elements in vegetable crops is manipulated via plant breeding or agronomy, it can significantly affect dietary intakes (Broadley and White, 2010).

## 2. Materials and methods

### 2.1. Overview of experiments

This study comprised four experiments. Experiment 1 aimed to characterise the time course of $^{134}Cs$ accumulation by *B. oleracea* under controlled conditions, to inform subsequent experiments of the number of days after sowing (DAS) for the harvest of a larger number of *B. oleracea* cultivars. Experiment 2 aimed to determine the $^{134}Cs$ concentration ratios (concentration ratio is defined as the quotient of the activity concentration in the shoot (kBq $kg^{-1}$ dry weight (d. wt)) and in the soil (kBq $kg^{-1}$ d. wt); see Equation (1)) in a larger number of *B. oleracea* morphotypes and cultivars. Experiments 1 and 2 were conducted in a growth chamber and used a peat-based potting medium contaminated artificially with $^{134}Cs$. In Experiment 1, shoot $^{134}Cs$ concentration ratio was determined in eight commercial cultivars of *B. oleracea*, between 21 and 118 DAS. As shoot $^{134}Cs$ concentration ratio remained relatively constant during growth, 40 DAS was chosen as a pragmatic time to assay the growth and $^{134}Cs$ concentration ratio of established *B. oleracea* plants in subsequent experiments. In Experiment 2, shoot $^{134}Cs$ concentration ratio was measured for a wider range of commercial cultivars of *B. oleracea* ($n = 27$).

Experiments 3 and 4 aimed to determine variation in $^{137}Cs$ and $^{90}Sr$ concentration ratios under field conditions. In these experiments, shoot $^{137}Cs$ and $^{90}Sr$ concentration ratios were measured in up to 71 cultivars of *B. oleracea* at two different field sites in the Chernobyl Exclusion Zone, Ukraine, in 2003 (Experiment 3) and 2004 (Experiment 4). Field sites were selected for logistical reasons of access and ease of maintenance in each year and had contrasting soil $^{137}Cs$ and $^{90}Sr$ activity concentrations due to the heterogeneous nature of radionuclide deposition following the accident at the Chernobyl Power Plant in 1986. The raw results from Experiments 1–4 can be found in Tables S1–S4.

### 2.2. Cultivars

Commercial *B. oleracea* cultivars were used in the experiments. The rationale for selecting panels of commercial *B. oleracea* cultivars is described in detail by Broadley et al. (2008). Briefly, seeds were selected to provide coverage of the major edible morphotypes of *B. oleracea* and included cultivars from the following varietas: *acephala* (kale), *alboglabra* (Chinese kale), *botrytis* (cauliflower), *capitata* (cabbage), *gemmifera* (Brussels sprout), *gongylodes* (kohlrabi), *italica* (broccoli), *sabauda* (savoy cabbage), and *sabellica* (borecole). Experiment 1 comprised one cultivar from eight varietas, whereas Experiments 2, 3 and 4 included between one and fifteen cultivars from eight or nine varietas (Table 1). All seed were obtained from commercial sources except for three accessions from Horticulture Research International Genetic Resources Unit (now Warwick Genetic Resources Unit) used in Experiment 3 (cultivars Ko 57, Ko 58 and Ko 62: Table S5). Commercial seed had typically been pre-treated with thiram (tetramethylthiuram disulfide) and/or iprodione (3-(3,5-dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide) to reduce fungal contamination.

### 2.3. Experiment 1: time course of $^{134}Cs$ uptake

Seeds of one cultivar of each of eight varietas (see Table S6) were sown at a density of three per pot at 0.5 cm depth in a well-mixed potting medium comprising 25% sand and 75% (v/v) compost (Shamrock medium grade sphagnum peat, Scotts UK, Bromford, Suffolk). Nutrients were incorporated in sufficient amounts per litre of compost to prevent deficiencies: 0.4 g $NH_4NO_3$ $L^{-1}$, 0.75 g $KNO_3$ $L^{-1}$, 0.098 g P $L^{-1}$ as single super phosphate, 2.25 g $CaCO_3$ $L^{-1}$ as ground limestone, 2.25 g ground magnesian limestone $L^{-1}$ and a trace element mixture containing B, Cu, Fe, Mn, Mo and Zn (WM255, Fargro Ltd, Toddington, Littlehampton, West Sussex, UK) at 0.4 g $L^{-1}$. The potting medium was characterised by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) using the methods described by Broadley et al. (2008). Water-soluble nutrients were added at the following concentrations per litre of water:

**Table 1**
Number of each of the nine morphotypes of *Brassica oleracea* grown in each of the four experiments.

| | Morphotype | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Borecole | Broccoli | Brussels sprout | Cabbage | Cauliflower | Chinese kale | Kale | Kohlrabi | Savoy cabbage |
| | *B. oleracea* var. *sabellica* | *B. oleracea* var. *italica* | *B. oleracea* var. *gemmifera* | *B. oleracea* var. *capitata* | *B. oleracea* var. *botrytis* | *B. oleracea* var. *alboglabra* | *B. oleracea* var. *acephala* | *B. oleracea* var. *gongylodes* | *B. oleracea* var. *sabauda* |
| Experiment 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| Experiment 2 | 3 | 5 | 5 | 7 | 3 | 1 | 0 | 1 | 2 |
| Experiment 3 | 6 | 10 | 7 | 14 | 12 | 1 | 6 | 9 | 9 |
| Experiment 4 | 4 | 10 | 7 | 15 | 11 | 1 | 6 | 6 | 10 |

195 mg nitrate-N $L^{-1}$, 30 mg ammonium-N $L^{-1}$, 216 mg K $L^{-1}$, 63 mg Ca $L^{-1}$, 53 mg Mg $L^{-1}$, 30 mg Na $L^{-1}$, 5.5 mg P $L^{-1}$, 0.3 mg Fe $L^{-1}$, 0.06 mg Mg $L^{-1}$, 0.04 mg B $L^{-1}$ and 0.03 mg Zn $L^{-1}$.

A solution containing 1 μM $^{133}$Cs (a concentration typical of soil solutions; White and Broadley, 2000) as $^{133}$CsCl and $^{134}$CsCl at 17.76 kBq $^{134}$Cs $\mu mol^{-1}$ $^{133}$Cs was prepared. Preliminary experiments had been conducted to determine an appropriate method to homogenise the distribution of Cs in the potting medium (Payne, 2005). The optimal method for incorporating the CsCl solution into the potting medium was to mix 1200 g compost; enough to fill four pots; and 800 mL of solution for 2 min in a large domestic food mixer (Kenwood KM310, Hampshire, UK). Pots were of dimensions 9 × 9 cm (top), 6 × 6 cm (bottom) × 10 cm (depth), and were filled with compost to 9 cm depth (compost volume = 4812 $cm^3$ $pot^{-1}$, about 300 g compost). Pots were placed on a gravel tray in a growth chamber with illumination of 16 h $d^{-1}$. Temperature was maintained at 26 °C (day) and 16 °C (night) throughout the experiment.

The experimental design comprised an alpha-type randomised design (Patterson and Williams, 1976) of 256 pots, comprising 8 cultivars with 8 sequential harvests and 4 replicates per harvest. Due to uneven germination noted at 14 DAS, only two seeds per pot were retained in replicates one and two, and one seed per pot was retained in replicates three and four. To minimise the potential for soil splash and external contamination of plant surfaces, and also leaching and contamination of the growth chamber, pots were watered from below using deionised water, on alternate days.

Whole shoots were harvested destructively at eight times (21, 28, 33, 42, 49, 56, 75, 118 DAS), four replicate pots per time. Shoot fresh weight (f. wt) was determined in all samples. After harvesting, the compost from each pot was emptied out, mixed and a sample of approximately 5 g was taken from each pot. Shoot material and compost samples were oven dried for 72 h at 40 °C. Dried shoot samples were weighed, and then ground using a domestic coffee grinder. Dried compost samples were placed into 20 mL scintillation vials for determination of $^{134}$Cs activity concentration. Plant shoot and compost $^{134}$Cs activity concentrations were determined for each sample by counting $^{134}$Cs γ-emissions until the error decreased to 1.65 sigma or for a maximum of 60 min on an automatic well-type γ-counter (MiniaxiAuto-Gamma 5000 Series; Packard Instrument Company, Downers Grove, Illinois, USA). The detector of the γ-counter was normalised for $^{134}$Cs according to the manufacturer's instructions and calibrated against results from calibrated hyper-pure germanium detectors. The background count rate was 64 counts per minute (±SE 2.6) when a blank tube or a tube filled with deionised water was present in the detection well and this activity concentration was subtracted from counts for each sample.

### 2.4. Experiment 2: pot experiment

A total of 27 commercial cultivars of *B. oleracea* were grown (see Table S7), including the eight cultivars grown in Experiment 1 plus a further 19 cultivars which together comprised three borecole, five broccoli, five Brussels sprout, seven cabbage, three cauliflower, one kohlrabi, one oriental kale and two Savoy cabbage cultivars (Table 1). All growth conditions were identical to Experiment 1, except a radiolabel of 30 kBq $^{134}$Cs $\mu mol^{-1}$ $^{133}$Cs was used. Two seeds were planted per pot and thinned to leave one seedling per pot at 14 DAS. Shoot f. wt, d. wt and $^{134}$Cs activity concentration were determined for all plant and compost samples 40 DAS using the same methods as described previously.

### 2.5. Experiments 3 and 4: field experiments in the Chernobyl Exclusion Zone

Two field experiments were conducted over two years. Experiment 3 (2003) and Experiment 4 (2004) included the 27 cultivars from Experiment 2, and an additional 43 (Experiment 4) or 44 (Experiment 3) cultivars from nine *B. oleracea* types (Table S5). Four of the cultivars used in Experiment 3 could not be used in Experiment 4 due to seed availability. These were replaced by three additional cultivars (Table S5).

### 2.6. Site description, experimental design and sowing

Both experimental sites were located in the Chernobyl Exclusion Zone in Ukraine (Experiment 3: 51.266422°N, Fig. 1; 30.225606°E; Experiment 4: 51.290662°N/E, 30.194946°E), where the soil was classified as having a $^{137}$Cs deposition density of <185 kBq $^{137}$Cs $m^{-2}$ soil as a consequence of the Chernobyl accident in 1986 (Shestopalov, 1996).

Soil $^{137}$Cs and $^{90}$Sr activity concentrations were measured in 2003 and 2004 for Experiments 3 and 4, respectively. However, other soil characteristics such as pH and clay content were not measured at this time. Therefore, soil was sampled and analysed further in May 2013 from the sites used for experiments in 2003 and 2004. To our knowledge, no cultivation activities had been undertaken on these sites during the intervening period. Soil pH, cation exchange capacity (CEC), concentrations of exchangeable P, K, Ca and Mg, organic matter and clay content were measured (Table 2; for details of the methods used, see Table S8).

The soil was cultivated using a hand-held rotavator to a depth of 15–20 cm and then hand-weeded. Soil beds were prepared prior to sowing and fencing was placed around the beds to minimise the risk of animal herbivory and for site security. Trickle irrigation pipes were laid on each bed, and connected to a water supply. Black and

**Fig. 1.** Experiment 3 field site in the Chernobyl Exclusion Zone, Ukraine. Clockwise, from top-left: (i) and (ii) prepared soil beds (May 2003), (iii) Experiment 3 cabbages growing in late June, 2003, (iv) field site after harvest of Experiment 3 (August 2003); only 'guard' cabbages remain in the ground.

**Table 2**
Soil characteristics for the experimental sites used in Experiment 3 and 4.

| Experiment | pH ($H_2O$) | Cation exchange capacity (mg-eq (100) $g^{-1}$) | Exchangeable P (mg $kg^{-1}$) | Exchangeable K (mg $kg^{-1}$) | Exchangeable Ca (mg-eq (100) $g^{-1}$) | Exchangeable Mg (mg-eq (100) $g^{-1}$) | Organic matter (%) | Clay content (%) |
|---|---|---|---|---|---|---|---|---|
| Experiment 3 | 6.2 | 11.2 | 107.8 | 51.8 | 2.66 | 0.47 | 1.5 | 7.6 |
| Experiment 4 | 6.4 | 10 | 101.4 | 66.8 | 3.44 | 0.41 | 1.6 | 10.0 |

white polythene sheeting was used to cover the beds, to minimise both the re-suspension of soil particles and the re-establishment of weeds. Each experiment was laid out in a randomised block design of three replicates. These were allocated across five cultivation beds. Each bed comprised four rows of planting stations at 20 cm inter-row spacing. The outer two rows comprised guard rows of a cabbage cultivar. The inner two rows had 45 planting stations per bed. A small hole was cut into the polythene sheet at each planting station. Two seeds of the *B. oleracea* cultivars were hand-sown into each planting station. These seedlings were thinned to one plant per planting station at the 1–2 leaf stage. The plants were harvested 10 weeks after sowing in Experiment 3, and 13 weeks after sowing in Experiment 4.

### 2.7. Sample preparation and analysis

Shoots were cut above the polythene sheets, bagged and fresh weight recorded. Topsoil samples (10 cm deep) were also taken from each plot using a soil auger. Shoots and soils were dried for 72 h at 40 °C and dry weight was recorded. Soil and shoots were homogenised using a domestic coffee grinder. $^{137}Cs$ were determined using a high-purity germanium γ-spectrometer detector (Model GC3019, Canberra-Packard, Connecticut, USA). A standard source (OISN-16; Applied Ecology Laboratory of Environmental Safety Centre, Odessa, Ukraine) was used for calibration. The standard source contained epoxy granules (<3.0 mm) with the density of 1 g $cm^{-3}$ with a $^{152}Eu$ specific activity of about 158 kBq $kg^{-1}$ (as of 8 October 2001). The effectiveness of recording the 662 keV gamma line was 0.0206 pulse $Bq^{-1}$ $s^{-1}$. The minimal detectable activity was 0.18 Bq per sample. Following homogenization, $^{90}Sr$ activity concentrations for the soil and plant samples were measured using a β-spectrometer (SEB-01-150, Atom Komplex Prylad, Kiev, Ukraine) with a thin-filmed (0.1 mm) plastic scintillator detector. This approach does not need any radiochemical pretreatment; the sample (approximately 2 g dry mass) was analysed as a solid matrix. The obtained spectra were processed by a correlation with measured spectra from the standard sources e.g. $^{90}Sr + ^{90}Y$, $^{137}Cs$ and $^{90}Sr + ^{90}Y$, and $^{137}Cs$ combinations as well as from background measurements; the equipment was calibrated daily. A more detailed description of method can be found in Bondarkov et al. (2002, 2011) and Gaschak et al. (2011).

### 2.8. Data analysis

All data analyses were conducted using R (R Core Team, 2013). Concentration ratios were calculated using Equation (1) and inter-cultivar variation was calculated using Equation (2):

$$Concentration\ ratio = \frac{Activity\ concentration\ in\ the\ plant}{Activity\ concentration\ in\ the\ soil} \qquad (1)$$

$$Inter-cultivar\ variation = \frac{Mean\ \ concentration\ ratio\ in\ the\ highest\ accumulating\ cultivar}{Mean\ \ concentration\ ratio\ in\ the\ lowest\ accumulating\ cultivar} \tag{2}$$

Activity concentration was measured in kBq kg$^{-1}$ (d.wt) in all experiments.

Concentration ratios (as calculated using the above equation) are used throughout this paper. Using concentration ratios instead of concentrations minimises the effect of different concentrations of radiocaesium and radiostrontium in the soil of the different experiments.

Where $x$ is used, this represents the arithmetic mean. The arithmetic mean was used throughout this paper, as all data were found not to be log-normally distributed.

## 3. Results

### 3.1. Soil characteristics

Characteristics of the soils from the sites for Experiment 3 and Experiment 4 are similar. The soils were slightly acidic (pH 6.2–6.4) and similar to most of the Chernobyl area (Davydchuk et al., 1994). The organic matter, clay content, exchangeable K, Ca and Mg were relatively low in comparison to agricultural soils in Western Europe.

### 3.2. Experiment 1

Caesium-134 concentration ratios were relatively constant during growth (Fig. 2), decreasing by around 50% between Harvest 2 (28 DAS; $x = 0.24$) and Harvest 8 (118 DAS; $x = 0.12$). A harvest of 40 DAS was chosen for convenience for Experiment 2.

### 3.3. Experiment 2

The 27 cultivars varied 2.7-fold in shoot $^{134}$Cs concentration ratio (Fig. 3). The highest concentration ratio was found in Bro 11 ($x = 0.65$), and the lowest concentration ratio in BrSp 17 ($x = 0.24$).

### 3.4. Experiment 3

Caesium-137 concentration ratios varied 35-fold in the 71 cultivars sampled in Experiment 3 (Fig. 4a). Cab 26 had the lowest mean $^{137}$Cs concentration ratio ($x = 0.03$) and the highest concentration ratio ($x = 1.05$) was found in SaCa 68.

Ka 53 had the highest $^{90}$Sr concentration ratio (x = 46) whereas the lowest $^{90}$Sr concentration ratio was found in cultivar SaCa 72 ($x = 2$). The $^{90}$Sr concentration ratio varied by 23-fold between the 71 cultivars.

### 3.5. Experiment 4

Caesium-137 concentration ratios varied 5-fold between the cultivar with the highest CR (Cab 32, $x = 0.16$) and cultivar with the lowest CR (Cau 46, $x = 0.03$) studied in Experiment 4 (Fig. 5a).

The $^{90}$Sr concentration ratios varied 12-fold between cultivars studied in Experiment 4 (Fig. 5b). SaCa 70 had the highest $^{90}$Sr concentration ratio ($x = 129$) and Cau 43 had the lowest $^{90}$Sr concentration ratio ($x = 10$).

## 4. Discussion

### 4.1. Comparisons with previous studies

The inter-cultivar variation in $^{137}$Cs concentration ratios found in Experiments 2 (2.7-fold; Fig. 3) and 4 (6.3-fold; Fig. 5a) were higher, but in the same order of magnitude as those found in the paper of Bibak et al. (1999) reporting variation in concentrations of stable Cs in *B. oleracea*, and as the mean inter-cultivar variation in Cs (both stable and radioactive) uptake in 23 plant species as found in Penrose et al. (2015) (2.0-fold). The inter-cultivar variation in $^{137}$Cs concentration ratios in Experiment 3 was substantially greater (35-fold; Fig. 4a) than in Penrose et al. (2015) and Bibak et al. (1999). The variation in $^{90}$Sr concentration ratios in Experiments 3 (23-fold; Fig. 4b) and (12-fold; Fig. 5b) were both an order

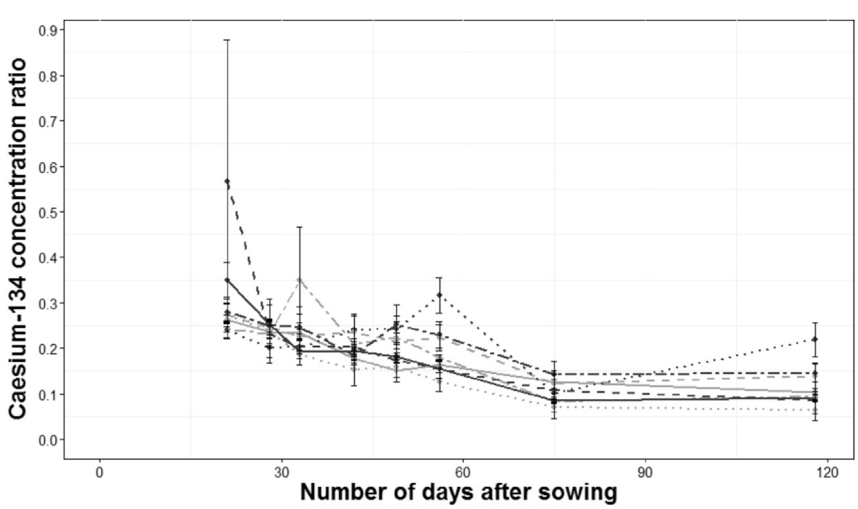


**Fig. 2.** Mean shoot $^{134}$Cs concentration ratio (in eight Brassica oleracea cultivars at harvest 21–118 days after sowing. - - - = Ch Ka 50; - - - = Bro 12; - — - = Sa Ca 69; - — - = Cab 31; ▪ ▪ ▪ = Ko 60; ▪ ▪ ▪ = Br Sp 21; — = Cau 41; — = Bor 4. Data are means (n = 4) +/− standard error of the mean.

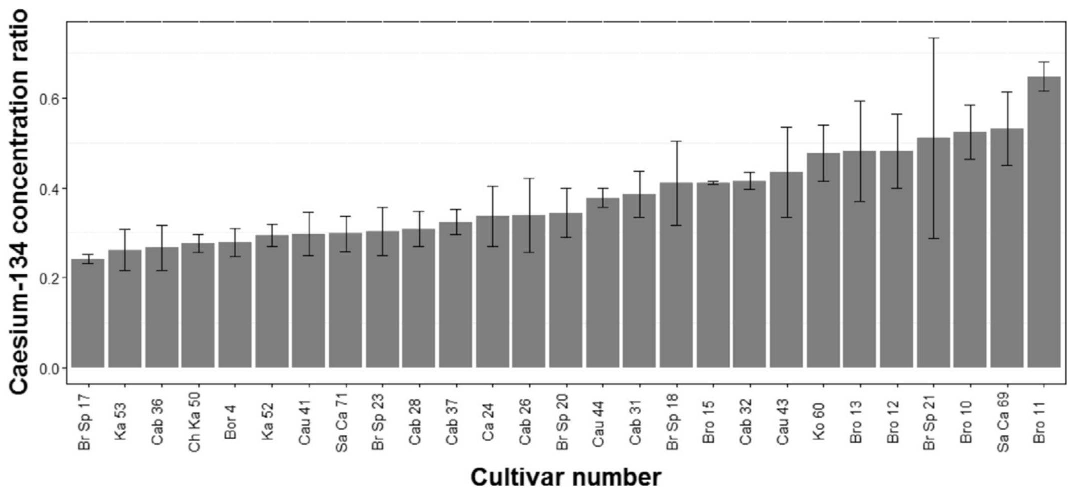


**Fig. 3.** Mean $^{134}$Cs concentration ratio for 27 B. oleracea cultivars from Experiment 2. Data are means (n = 3) +/− standard error of the mean.

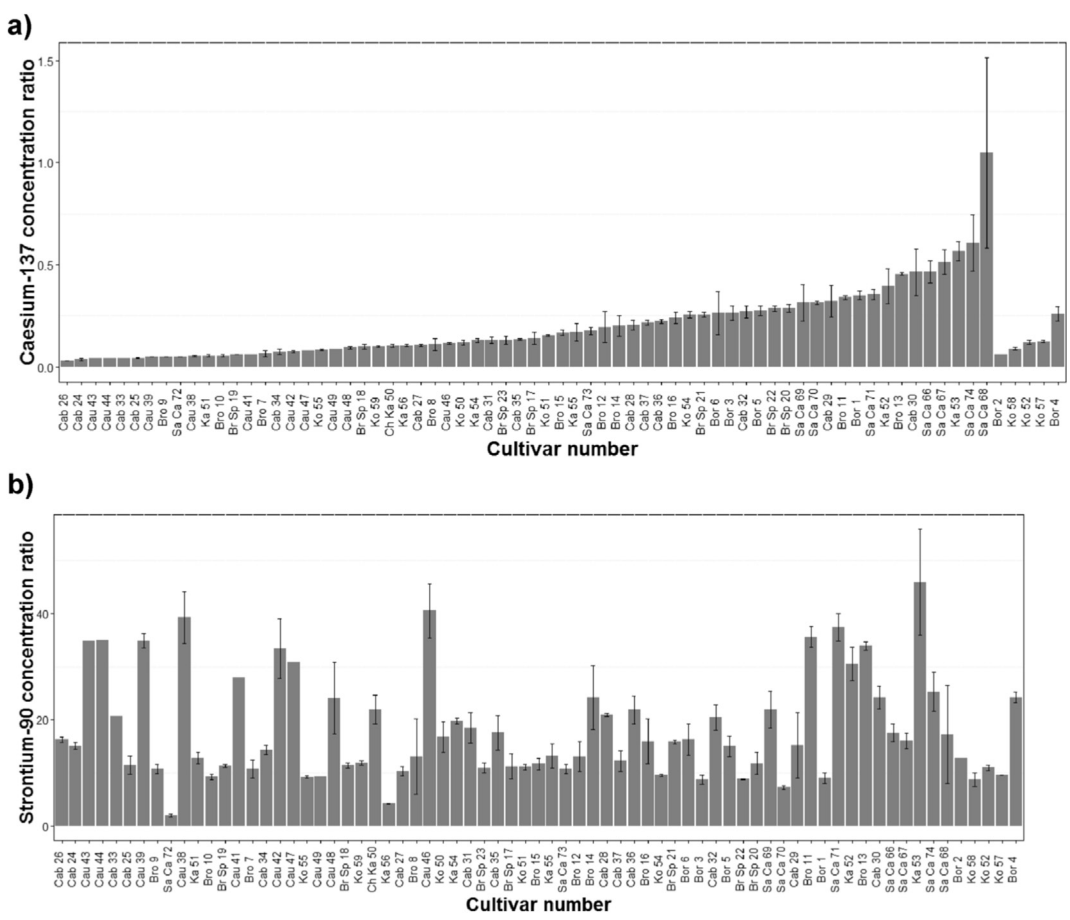


**Fig. 4.** (a) Caesium-137 concentration ratios and (b) strontium-90 concentration ratios for 71 B. oleracea cultivars grown in Experiment 3. Cultivars studied in both Experiments 3 and 4 are ordered by their $^{137}$Cs concentration ratio. Cultivars found only in Experiment 3 are added to the right of the cultivars studied in both experiments. Data are means (n = 1–3) +/− standard error of the mean.

of magnitude greater than the 4.2-fold variation observed by Bibak et al. (1999), and the average inter-cultivar variation in Sr uptake by 20 plant species (2.0-fold) reported in Penrose et al. (2015). However, once again fewer cultivars were studied by Bibak et al. (1999, $n = 2$) and in the studies reported by Penrose et al. (2015, $n \approx 7$) than in Experiments 3 or 4 ($n = 71$ and 70, respectively). The larger range in concentration ratios found in our experiments may be due to a larger number of cultivars used in Experiments 2 ($n = 27$), 3 ($n = 71$) and 4 ($n = 70$) in comparison to the number used in Bibak et al. (1999; $n = 2$) and the average number of cultivars used in the experiments reviewed by Penrose et al. (2015; $n \approx 7$); variation will be likely to increase with sample size. As there is extensive

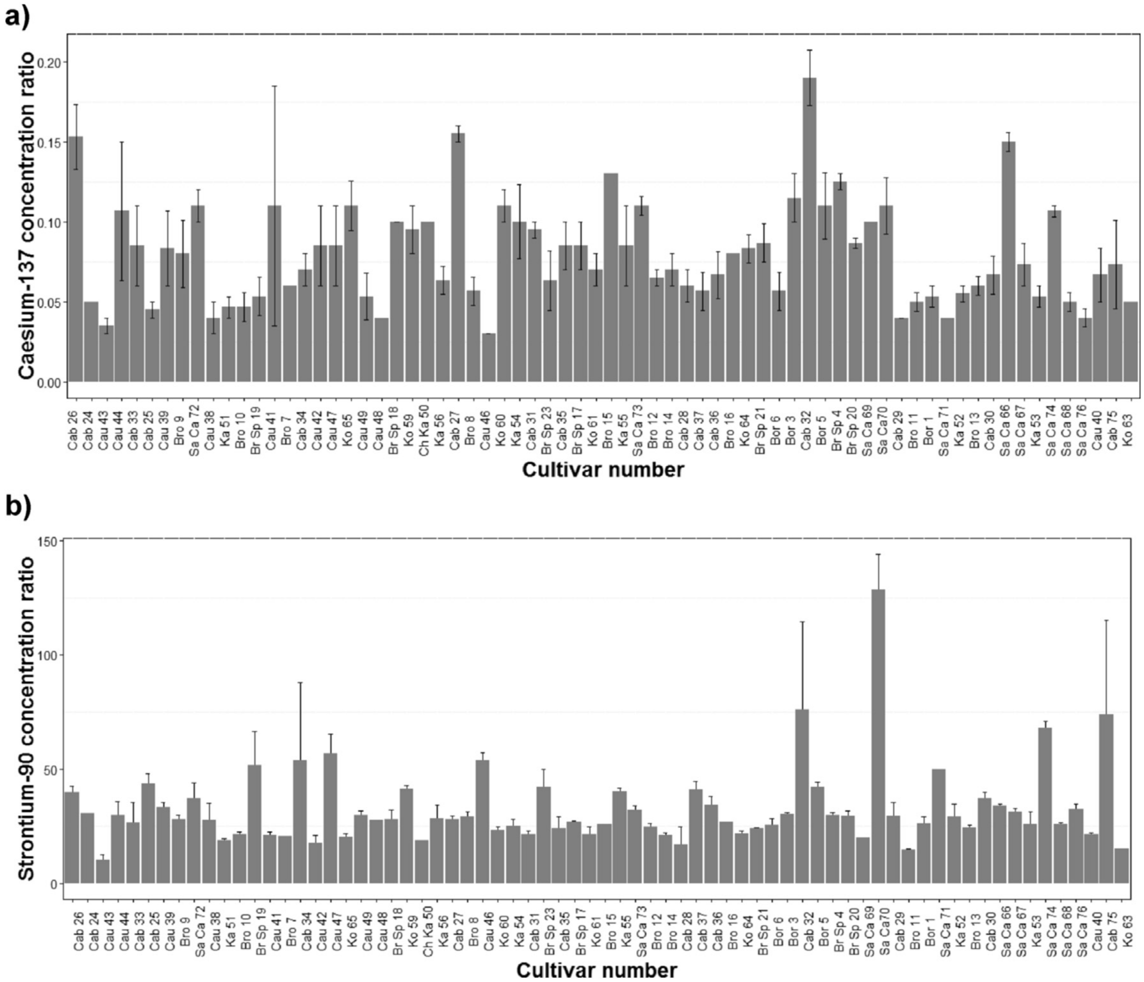


**Fig. 5.** (a) Caesium-137 concentration ratios and (b) strontium-90 concentration ratios for 70 *B. oleracea* cultivars grown in Experiment 4. Cultivars studied in both Experiments 3 and 4 are ordered by their $^{137}$Cs concentration ratio in Experiment 3. Cultivars studied only in Experiment 4 are added to the far right of the cultivars studied in both experiments. Data are means ($n$ = 1–6) +/− standard error of the mean.

morphological variation in *B. oleracea*, it is possible that Cs and Sr concentration ratios vary more in this species than others. However, multi-variety field experiments would need to be conducted for many species and in multiple locations to determine whether *B. oleracea* has inherently more or less variation in Cs and Sr concentration ratios than other species.

### 4.2. Soil sampling

As the soil samples were only taken from the top 10 cm instead of the top 20 cm as recommended by the IAEA (IAEA, 2010), care should be taken when using these values for human food chain assessment. However, as the soil had been rotavated prior to sowing, the authors believe that the soil was well mixed and therefore soil activity concentrations were representative of the top soil as a whole.

### 4.3. Consistency between experiments

To test statistically whether cultivars consistently accumulated more or less than the other cultivars over multiple experiments, cultivars grown in Experiments 2, 3 and 4 were ranked for their mean radiocaesium concentration ratio in each of the three experiments (Table 3). These ranks were then added together to create a total rank for each cultivar.

To identify cultivars with consistently lower or higher concentration ratios, three random numbers between 1 and 27 (the minimum and maximum rank) were added together to create a simulated 'total rank'. This process was iterated 1000 times, and the 5th and 95th percentile calculated. Any cultivar with a total rank below the 5th percentile (i.e. below 20 for this simulation) was considered consistently low-ranking, and any cultivar with a total rank above the 95th percentile (i.e. 66 for this simulation) was deemed consistently high-ranking. Cab 24 (Total rank = 18.5) had a consistently lower radiocaesium concentration ratio, and SaCa 69 (Total rank = 67) was identified as a cultivar with a consistently higher radiocaesium concentration ratio.

The 66 cultivars used in both Experiments 3 and 4 were ranked in terms of their $^{137}$Cs and $^{90}$Sr concentration ratios, to test consistency using the same method as for the comparison of Experiments 2, 3 and 4 (Table 4). This time, two random numbers between one and 66 were added together to create a simulated 'total rank'.

Five cultivars, Cau 43 (Total $^{\mathbf{137}}$Cs rank = 6), Cab 24 (Total $^{\mathbf{137}}$Cs rank = 13), Cab 25 (Total $^{137}$Cs rank = 13), Cau 38 (Total $^{\mathbf{137}}$Cs rank = 14.5) and Ka 51 (Total $^{\mathbf{137}}$Cs rank = 20) had consistently lower $^{137}$Cs concentration ratios. Two cultivars, Cab 32 (Total $^{\mathbf{137}}$Cs rank = 116) and SaCa 66 (Total $^{\mathbf{137}}$Cs rank = 125) had consistently higher $^{137}$Cs concentration ratios.

Two cultivars, Ko 65 (Total $^{90}$Sr rank = 14) and Bro 7 (Total $^{90}$Sr

**Table 3**
Cultivars grown in Experiments 2, 3 and 4 and their rankings for radiocaesium concentration ratio. The lowest ranking (1) refers to the cultivar with the lowest concentration ratio, the highest ranking (27) refers to the cultivar with the highest concentration ratio.

| Cultivar number | Morphotype | Experiment 2 $^{134}$Cs concentration ratio rank | Experiment 3 $^{137}$Cs concentration ratio rank | Experiment 4 $^{137}$Cs concentration ratio rank | Total rank |
|---|---|---|---|---|---|
| Bor 4 | Borecole | 5 | 19 | 24 | 48 |
| Bro 10 | Broccoli | 25 | 5 | 3 | 33 |
| Bro 11 | Broccoli | 27 | 23 | 4.5 | 54.5 |
| Bro 12 | Broccoli | 23 | 14 | 12 | 49 |
| Bro 13 | Broccoli | 22 | 26 | 9.5 | 57.5 |
| Bro 15 | Broccoli | 18 | 13 | 25 | 56 |
| BrSp 17 | Brussels sprout | 1 | 12 | 14 | 27 |
| BrSp 18 | Brussels sprout | 17 | 7 | 19 | 43 |
| BrSp 20 | Brussels sprout | 14 | 21 | 15.5 | 50.5 |
| BrSp 21 | Brussels sprout | 24 | 18 | 15.5 | 57.5 |
| BrSp 23 | Brussels sprout | 9 | 10.5 | 11 | 30.5 |
| Cab 24 | Cabbage | 12 | 2 | 4.5 | 18.5[a] |
| Cab 26 | Cabbage | 13 | 1 | 26 | 40 |
| Cab 28 | Cabbage | 10 | 15 | 9.5 | 34.5 |
| Cab 31 | Cabbage | 16 | 10.5 | 17 | 43.5 |
| Cab 32 | Cabbage | 19 | 20 | 27 | 66 |
| Cab 36 | Cabbage | 3 | 17 | 13 | 33 |
| Cab 37 | Cabbage | 11 | 16 | 8 | 35 |
| Cau 41 | Cauliflower | 7 | 6 | 22.5 | 35.5 |
| Cau 43 | Cauliflower | 20 | 3.5 | 1 | 24.5 |
| Cau 44 | Cauliflower | 15 | 3.5 | 21 | 39.5 |
| ChKa 50 | Chinese kale | 4 | 8 | 19 | 31 |
| Ka 52 | Kale | 6 | 25 | 7 | 38 |
| Ka 53 | Kale | 2 | 27 | 6 | 35 |
| Ko 60 | Kohlrabi | 21 | 9 | 22.5 | 52.5 |
| SaCa 69 | Savoy cabbage | 26 | 22 | 19 | 67[b] |
| SaCa 71 | Savoy cabbage | 8 | 24 | 2 | 34 |

[a] Denotes cultivars that consistently accumulated less.
[b] Denotes cultivars that consistently accumulated more.

rank = 21) had consistently lower $^{90}$Sr concentration ratios. Three cultivars, SaCa 71, Cau 46 and Cau 47 had consistently higher $^{90}$Sr concentration ratios (Total $^{90}$Sr rank = 118, 126 and 119, respectively).

Bro 10 had consistently lower $^{137}$Cs and $^{90}$Sr concentration ratios (Total $^{137}$Cs rank = 20; Total $^{90}$Sr rank = 21). Cultivar SaCa 74 had consistently higher $^{137}$Cs and $^{90}$Sr concentration ratios (Total $^{137}$Cs rank = 116.5; Total $^{90}$Sr rank = 117).

In a recent meta-analysis comprising 27 plant species, cultivars that had consistently the lowest Cs or Sr concentration ratios over multiple sites were found in five out of eight sets of experiments (Penrose et al., 2015). In three out of the eight sets of experiments, no cultivar was found to have consistently the lowest concentration ratio. However, there were far fewer (range = 2–28) cultivars included in the experiments reported by Penrose et al. (2015), and thus consistency in concentration ratios in these studies was measured by testing whether the cultivar with the lowest concentration ratio at one site had the lowest concentration ratio at all sites. As the study reported here includes more cultivars (27–66), this method of testing for consistency in concentration ratios was not appropriate, thus the 5th and 95th percentile were used. Our research supports the hypothesis that it is possible to identify cultivars that have consistently lower radiocaesium or radiostrontium concentration ratios at multiple sites.

### 4.4. *Brassica oleracea* as a contributor to human radioactive dose

This study has identified *B. oleracea* cultivars that have consistently lower radiocaesium and/or radiostrontium concentration ratios, which suggests that cultivar selection or substitution might be an effective remediation strategy in radiologically contaminated areas. The evidence for genetic variation in Cs accumulation in *B. oleracea* shown in this study could have an immediate beneficial effect for populations who inhabit and consume crops that have been grown on radiocaesium contaminated land. Utilising these cultivars with lower concentration ratios could be a cost effective way to reduce both individual and collective doses of radiocaesium and radiostrontium. Additionally, these findings could provide an example to breeders of other crop species showing that substantial variation in uptake of radiocaesium and radiostrontium can occur between plant cultivars. However, it may be that consumers have a preference for one varietas in favour of another, and therefore we recommend that further studies include more cultivars from each varietas to investigate further the variation within varietas. Additionally, due to relatively low consumption of leafy vegetables in the typical diet, *B. oleracea* is unlikely to be a main contributor to human radioactive dose. For example, a study investigating the contribution of foodstuffs to daily $^{137}$Cs intake in two settlements in Ukraine found that vegetables only contributed to 3.6–4.5% of $^{137}$Cs intake from foodstuffs, whereas bread, potatoes and milk contributed 6.8–11%, 9.5–19% and 13–50%, respectively (Beresford et al., 2001). It is therefore recommended that future research regarding cultivar substitution should focus on crop species that are likely to be major contributors to human radioactive dose, such as grains, potato and animal fodders.

## 5. Conclusion

Up to 35-fold variation in radiocaesium concentration ratios and 23-fold variation in radiostrontium concentration ratios was found among 71 cultivars of *B. oleracea* in individual experiments (Figs. 3–5). When results of three experiments (one performed in a controlled environment and two field experiments performed in the Chernobyl Exclusion Zone) were compared, only one (a cabbage) out of the 27 cultivars studied was found to have lower radiocaesium concentration ratios in all three experiments. When results of the two field experiments were compared, five (two

**Table 4**
Cultivars grown in Experiments 3 and 4 and their rankings for $^{137}Cs$ and $^{90}Sr$ concentration ratios.

| Cultivar number | Morphotype | Experiment 3 $^{137}Cs$ concentration ratio rank | Experiment 4 $^{137}Cs$ concentration ratio rank | Experiment 3 $^{90}Sr$ concentration ratio rank | Experiment 4 $^{90}Sr$ concentration ratio rank | Total $^{137}Cs$ concentration ratio rank | Total $^{90}Sr$ concentration ratio rank |
|---|---|---|---|---|---|---|---|
| Bor 6 | Borecole | 48 | 19 | 35 | 22 | 67 | 57 |
| Bor 1 | Borecole | 57 | 14.5 | 5 | 26 | 71.5 | 31 |
| Bor 5 | Borecole | 51 | 56 | 29 | 57 | 107 | 86 |
| Bor 4 | Borecole | 47 | 61 | 51 | 40 | 108 | 91 |
| Bor 3 | Borecole | 49 | 60 | 4 | 43 | 109 | 47 |
| Bro 10 | Broccoli | 11.5 | 8.5 | 7 | 14 | 20[a] | 21[a] |
| Bro 7 | Broccoli | 15 | 22 | 12 | 9 | 37 | 21[a] |
| Bro 9 | Broccoli | 8 | 33.5 | 11 | 34 | 41.5 | 45 |
| Bro 8 | Broccoli | 27 | 19 | 26 | 36 | 46 | 62 |
| Bro 12 | Broccoli | 39 | 26 | 25 | 20 | 65 | 45 |
| Bro 11 | Broccoli | 56 | 11 | 62 | 2 | 67 | 64 |
| Bro 14 | Broccoli | 40 | 30 | 52 | 11 | 70 | 63 |
| Bro 16 | Broccoli | 44 | 33.5 | 33 | 29 | 77.5 | 62 |
| Bro 13 | Broccoli | 60 | 22 | 58 | 19 | 82 | 77 |
| Bro 15 | Broccoli | 36 | 62 | 20 | 23 | 98 | 43 |
| BrSp 19 | Brussels sprout | 13.5 | 14.5 | 17 | 60 | 28 | 77 |
| BrSp 23 | Brussels sprout | 31 | 24.5 | 14 | 56 | 55.5 | 70 |
| BrSp 18 | Brussels sprout | 22.5 | 48.5 | 18 | 33 | 71 | 51 |
| BrSp 17 | Brussels sprout | 34 | 39.5 | 16 | 28 | 73.5 | 44 |
| BrSp 21 | Brussels sprout | 46 | 43.5 | 32 | 18 | 89.5 | 50 |
| BrSp 20 | Brussels sprout | 52 | 43.5 | 21 | 39 | 95.5 | 60 |
| Cab 25 | Cabbage | 6 | 7 | 19 | 58 | 13[a] | 77 |
| Cab 24 | Cabbage | 2 | 11 | 30 | 44 | 13[a] | 74 |
| Cab 33 | Cabbage | 4 | 39.5 | 44 | 27 | 43.5 | 71 |
| Cab 34 | Cabbage | 16 | 30 | 28 | 62 | 46 | 90 |
| Cab 29 | Cabbage | 55 | 4.5 | 31 | 38 | 59.5 | 69 |
| Cab 37 | Cabbage | 42 | 19 | 23 | 54 | 61 | 77 |
| Cab 28 | Cabbage | 41 | 22 | 45 | 3 | 63 | 48 |
| Cab 26 | Cabbage | 1 | 64 | 36 | 52 | 65 | 88 |
| Cab 36 | Cabbage | 43 | 27.5 | 46 | 49 | 70.5 | 95 |
| Cab 35 | Cabbage | 33 | 39.5 | 40 | 17 | 72.5 | 57 |
| Cab 31 | Cabbage | 31 | 45.5 | 41 | 13 | 76.5 | 54 |
| Cab 30 | Cabbage | 61 | 27.5 | 50 | 50 | 88.5 | 100 |
| Cab 27 | Cabbage | 26 | 65 | 10 | 32 | 91 | 42 |
| Cab 32 | Cabbage | 50 | 66 | 43 | 65 | 116[b] | 108 |
| Cau 43 | Cauliflower | 4 | 2 | 59 | 1 | 6[a] | 60 |
| Cau 38 | Cauliflower | 10 | 4.5 | 64 | 30 | 14.5[a] | 94 |
| Cau 48 | Cauliflower | 21 | 4.5 | 49 | 31 | 25.5 | 80 |
| Cau 46 | Cauliflower | 28 | 1 | 65 | 61 | 29 | 126[b] |
| Cau 49 | Cauliflower | 20 | 14.5 | 8 | 41 | 34.5 | 49 |
| Cau 39 | Cauliflower | 8 | 35.5 | 60 | 47 | 43.5 | 107 |
| Cau 44 | Cauliflower | 4 | 51.5 | 61 | 42 | 55.5 | 103 |
| Cau 42 | Cauliflower | 17 | 39.5 | 57 | 4 | 56.5 | 61 |
| Cau 47 | Cauliflower | 18 | 39.5 | 56 | 63 | 57.5 | 119[b] |
| Cau 41 | Cauliflower | 13.5 | 56 | 54 | 10 | 69.5 | 64 |
| ChKa 50 | Chinese kale | 24 | 48.5 | 47 | 5 | 72.5 | 52 |
| Ka 51 | Kale | 11.5 | 8.5 | 24 | 6 | 20[a] | 30 |
| Ka 56 | Kale | 25 | 24.5 | 2 | 35 | 49.5 | 37 |
| Ka 52 | Kale | 59 | 17 | 55 | 37 | 76 | 92 |
| Ka 55 | Kale | 37 | 39.5 | 27 | 53 | 76.5 | 80 |
| Ka 53 | Kale | 64 | 14.5 | 66 | 25 | 78.5 | 91 |
| Ka 54 | Kale | 31 | 48.5 | 42 | 21 | 79.5 | 63 |
| Ko 61 | Kohlrabi | 35 | 30 | 15 | 12 | 65 | 27 |
| Ko 59 | Kohlrabi | 22.5 | 45.5 | 22 | 55 | 68 | 77 |
| Ko 65 | Kohlrabi | 19 | 56 | 6 | 8 | 75 | 14[a] |
| Ko 64 | Kohlrabi | 45 | 35.5 | 9 | 15 | 80.5 | 24 |
| Ko 60 | Kohlrabi | 29 | 56 | 37 | 16 | 85 | 53 |
| SaCa 71 | Savoy cabbage | 58 | 4.5 | 63 | 59 | 62.5 | 122[b] |
| SaCa 72 | Savoy cabbage | 8 | 56 | 1 | 51 | 64 | 52 |
| SaCa 68 | Savoy cabbage | 66 | 11 | 38 | 24 | 77 | 62 |
| SaCa 73 | Savoy cabbage | 38 | 56 | 13 | 46 | 94 | 59 |
| SaCa 67 | Savoy cabbage | 63 | 32 | 34 | 45 | 95 | 79 |
| SaCa 69 | Savoy cabbage | 53.5 | 48.5 | 48 | 7 | 102 | 55 |
| SaCa 70 | Savoy cabbage | 53.5 | 56 | 3 | 66 | 109.5 | 69 |
| SaCa 74 | Savoy cabbage | 65 | 51.5 | 53 | 64 | 116.5[b] | 117[b] |
| SaCa 66 | Savoy cabbage | 62 | 63 | 39 | 48 | 125[b] | 87 |

[a] Denotes cultivars that consistently accumulated less.
[b] Denotes cultivars that consistently accumulated more.

cauliflower, two cabbage, one kale) out of the 66 cultivars common to both experiments were found to be consistently lower $^{137}$Cs accumulators, and two (one kohlrabi, one broccoli) were found to be consistently lower $^{90}$Sr accumulators. One cultivar (a broccoli) was found to consistently accumulate less $^{137}$Cs and $^{90}$Sr than the other cultivars. Cultivars that consistently accumulate less radiocaesium and/or radiostrontium indicate that cultivar selection or substitution might be an effective remediation strategy in radiologically contaminated areas. However, as *B. oleracea* is a relatively minor crop, it is recommended that future research focuses on plant species such as grains, potatoes and animal fodders, that have greater contributors to human radioactive dose either directly or indirectly through their importance in the diet of farm animals.


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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvrad.2016.02.020.

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