

**CULTIVAR SUBSTITUTION AS A
REMEDICATION STRATEGY IN
RADIOCAESIUM AND
RADIOSTRONTIUM
CONTAMINATED AREAS**

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Abstract

Radioisotopes of caesium (Cs) and strontium (Sr) have been distributed in the environment due to weapons testing, nuclear power production and accidents at nuclear facilities. Radiocaesium and radiostrontium are of major concern in the medium to long term following accidental releases as they have high energies, long half lives ($^{137}\text{Cs}\approx 30$ years; $^{90}\text{Sr}\approx 29$ years) and their easy assimilation into biological systems due to their similarity to the biologically important elements potassium (K) and calcium (Ca), respectively. Radio-caesium and -strontium are transferred to humans primarily via plant root uptake, and therefore minimising this uptake has been the focus of a number of remediation strategies, such as ploughing and fertiliser application. Species or cultivar substitution, where a species/cultivar that has higher uptake is replaced by a species/cultivar that has lower uptake, has been proposed as an effective and socially acceptable remediation strategy for contaminated agricultural land, but not enough is known about its efficacy for it to be recommended internationally.

The aim of this thesis is to evaluate the potential of species or cultivar substitution as a remediation strategy for contaminated agricultural areas. Chapter 2 consists of meta-analysis of the available data (115 experiments) on the inter-cultivar variation in Cs and Sr accumulation by 27 plant species. Chapter 3 includes experiments conducted in the laboratory (UK) and two experiments in the field (Ukraine) investigating inter-cultivar variation in radiocaesium and radiostrontium accumulation in *Brassica oleracea*, and whether consistently lower-accumulating cultivars could be identified. Chapter 4 details analysis of samples from grass breeding experiments in Aberystwyth and Edinburgh (UK) from four forage grass species; hybrid ryegrass (*Lolium perenne* L. x *Lolium multiflorum* Lam.), *L. perenne*, *L. multiflorum* and *Festuca arundinacea* Shreb., and investigates inter-species and inter-cultivar variation in uptake of stable Cs and Sr. Hybrid ryegrass cultivars that were lower-accumulating in Cs and/or Sr were also identified. Chapter 5 compares the stable Cs and Sr uptake in six *L. perenne* and two *F. arundinacea* cultivars grown in Aberystwyth and Narodychi (Ukraine). Chapter 6 compares the performance in terms of yield and forage quality (elemental concentrations, digestibility and water soluble carbohydrate content) of six hybrid ryegrass cultivars and ten *F. arundinacea* cultivars identified as consistently lower-accumulating in Cs and/or Sr against the performance of two commercial hybrid ryegrass cultivars.

The mean inter-cultivar variation in Cs and Sr was 1.8-fold and 2.0-fold, respectively when 27 plant species were studied. Thirty-five-fold variation in radiocaesium and 23-fold variation in radiostrontium was found between c. 70 *Brassica oleracea* cultivars. In two field experiments in Ukraine, five cultivars had consistently lower radiocaesium concentration ratios and two cultivars consistently lower radiostrontium concentration ratios. One cultivar had lower radiocaesium and radiostrontium concentration ratios. *Festuca*

arundinacea cultivars had lower Cs and Sr concentration ratios than cultivars of hybrid ryegrass, *L. perenne* and *L. multiflorum*. Three out of 17 hybrid ryegrass cultivars had consistently lower Cs concentration ratios, two cultivars consistently lower Sr and one consistently lower Cs and Sr. Despite differences in soil properties and environmental conditions, *F. arundinacea* cultivars grown in Aberystwyth and Narodychi accumulated less stable and radioactive Cs and Sr than *L. perenne* cultivars. One *L. perenne* cultivar also accumulated less Cs and Sr at both sites. *Festuca arundinacea* cultivars accumulated less Cs and Sr than commercial hybrid ryegrass cultivars, but also had up to 59% lower yield and a reduction of up to 19% in K accumulation, up to 46% in Ca accumulation, up to 7% in dry matter digestibility and up to 17% in water soluble carbohydrate content. Selecting lower-accumulating cultivars was found to reduce Cs and Sr accumulation less, but with a smaller yield penalty and a smaller reduction in digestibility and water soluble carbohydrate content.

It is concluded that species and cultivar substitution could be an effective remediation strategy in contaminated agricultural land provided implications for yield and quality are considered.

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Abbreviations and acronyms

B	Boron
Bq	Becquerel
Ca	Calcium
Cl	Chlorine
cm	Centimetre
CR	Concentration ratio
Cs	Caesium
Cu	Copper
DMD	Dry matter digestibility
d. wt	Dry weight
DAS	Days after sowing
DW	Dry weight
f. wt	Fresh weight
Fe	Iron
g	Gram
h	Hour
ha	Hectare
ICP-MS	Inductively-coupled plasma mass spectrometry
kBq	Kilobecquerel
kg	Kilogram
L	Litre
m	Metre
Mg	Magnesium
mg	Milligram
mL	Millilitre
mM	Millimolar
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
O	Oxygen
P	Phosphorus
PBq	Petabequerel
S	Sulphur
s	Second
Sr	Strontium
WSC	Water soluble carbohydrate content
Zn	Zinc
μmol	Micromole

Chapter 1. General introduction

1.1 Caesium and strontium

Caesium (Cs) is a soft, gold-coloured alkali metal present in group I of the periodic table. There are 40 known isotopes of Cs with mass numbers from 112-151 (Emsley, 2011). Only one of these 40 isotopes is a stable isotope (^{133}Cs), which originates predominantly from the mineral pollucite ($\text{Cs}_4\text{H}_4\text{Al}_4\text{Si}_9\text{O}_{27}$; Emsley, 2011), the remaining 39 are radioisotopes.

Strontium (Sr) is a soft silvery-white alkaline-earth metal from group II of the periodic table (Stwertka, 2002). There are 33 known isotopes of Sr with mass numbers ranging from 73-105. Four of these are stable isotopes (^{84}Sr , ^{86}Sr , ^{87}Sr and ^{88}Sr), which are primarily found in the minerals celestite (SrSO_4) and strontianite (SrCO_3 ; Emsley, 2011), whilst the other 29 are radioisotopes.

1.2 Caesium and strontium in the environment

Stable Cs and Sr are distributed in the environment via the erosion of Cs and Sr containing minerals, and are present in soils at a concentration of $<1\text{-}30\text{ mg kg}^{-1}$ (Cs) and $5\text{-}3100\text{ mg kg}^{-1}$ (Sr) and in plants at $<0.01\text{-}3\text{ mg kg}^{-1}$ (Cs) and $1.5\text{-}74\text{ mg kg}^{-1}$ (Sr; Kabata-Pendias and Szteke, 2015).

^{134}Cs , ^{137}Cs and ^{90}Sr are the most common radioisotopes produced as by-products when other radioactive materials such as uranium (U) and plutonium (Pu) undergo nuclear fission (Ashraf et al., 2014). The fission of U and Pu creates a huge amount of energy, and thus has been used to make nuclear weapons and for production of nuclear power.

Radioisotopes of Cs and Sr have been widely deposited in the environment due to weapons testing, nuclear power production and accidents at nuclear facilities (Shaw, 2007). The two largest nuclear accidents occurred at the Chernobyl nuclear power plant, Ukraine, in 1986 and at the Fukushima Daiichi nuclear power plant, Japan, in 2011 (Steinhauser, 2014). The primary radionuclide of concern for human radiation dose immediately following both of these accidents was another fission by-product, radioiodine (principally ^{131}I ; Alexakhin et al., 2006; Matsuzaki et al., 2012), which has a short half-life of c. 8 days and concentrates in the thyroid (Baverstock et al., 1992; Cardis et al., 2005). The radiation produced due to this accumulated radioiodine can cause thyroid cancer, especially in children and adolescents (Reiners et al., 2013). In the long term, radioisotopes of Cs and Sr with longer half-lives ($^{137}\text{Cs}\approx 30$ years) and ($^{90}\text{Sr}\approx 29$ years) are of principal concern to human health. This is due not only to their relatively long half-lives, but also their high energy emissions and assimilation into biological systems due to their chemical similarity to the biologically important elements potassium (K; Cs) and calcium (Ca; Sr).

1.3 Radiocaesium and radiostrontium from the Chernobyl and Fukushima accidents

The Chernobyl accident caused many petabequerels (PBq) of ^{137}Cs and ^{90}Sr to be released into the environment (Table 1), and approximately 125,000 km² of land in Belarus, Ukraine and Russia were contaminated with radiocaesium deposition greater than 37 kBq m² following the accident. Around 52,000 km² of this land was under agricultural use at the time of the accident (NEA, 2002). Although the releases of ^{137}Cs and ^{90}Sr from the Fukushima accident were one to two orders of magnitude less than those of Chernobyl, 4221 ha of rice paddy and 1332 ha of 'dry field' (fields containing crops other than rice) were contaminated with >10 kBq kg⁻¹ soil (Atomic Energy Society of Japan, 2014).

Table 1 Activity concentrations (PBq) of ^{137}Cs and ^{90}Sr released during the Chernobyl (NEA, 2002) and Fukushima accidents (UNSCEAR, 2014; Casacuberta et al., 2013)

	Total release during the accident	
	^{137}Cs (PBq)	^{90}Sr (PBq)
Chernobyl	85	10
Fukushima	8.8	0.08-0.09

1.4 Caesium and strontium transfer and exposure pathways

There are a number of transfer and exposure pathways for ^{137}Cs and ^{90}Sr to humans (Fig. 1). From terrestrial and freshwater environments, external exposure from soil and atmospheric dispersion and internal exposure via inhalation, the drinking of contaminated surface water and ingestion of freshwater fish are important exposure pathways. However, the most important transfer pathways are primarily from the consumption of contaminated plant and animal products from agricultural land, and these therefore are the focus of this thesis

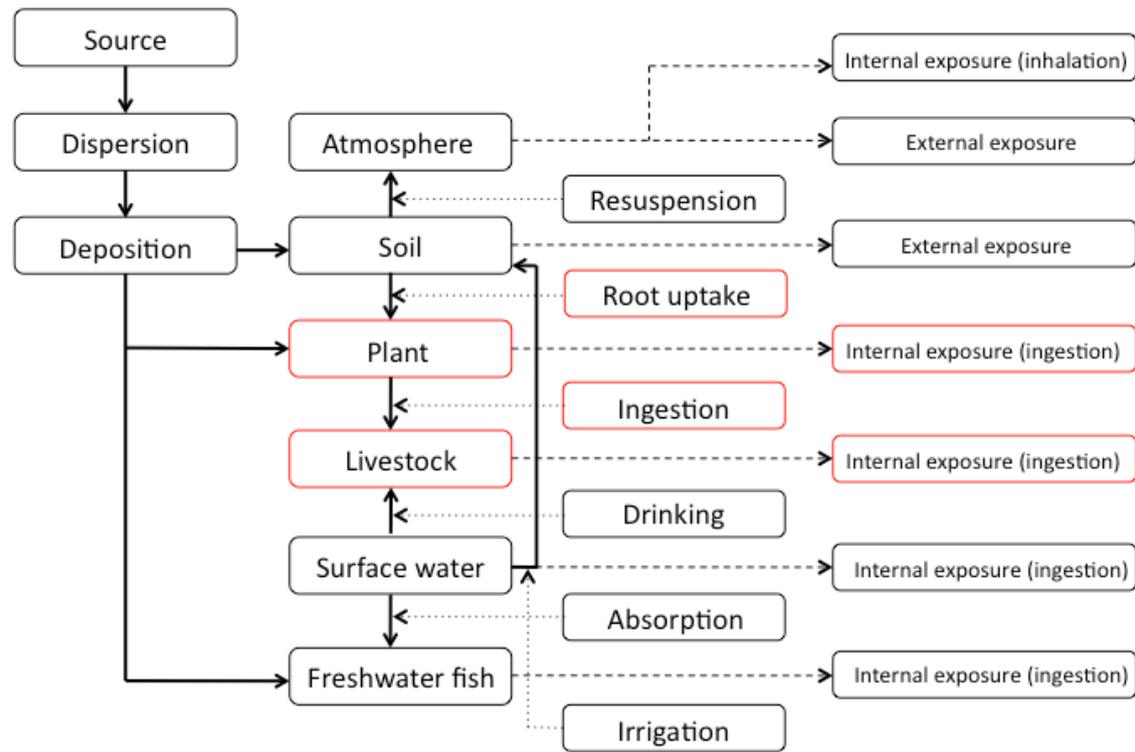


Figure 1 Some major transfer and exposure pathways of aerial radionuclide releases following a nuclear accident (redrawn from Takahashi, 2014). The transfer and exposure pathways shown in red are the main pathways discussed in this thesis

Agricultural food products that provided the largest contribution to daily ^{137}Cs intake in rural populations in areas of the former Soviet Union affected by the Chernobyl accident were found to be bread (6.8-11 %), potatoes (9.5-19 %) and milk (13-50 %; Beresford et al., 2001). Following the Fukushima accident, consumption of food from contaminated areas was rigorously restricted, and therefore there was little transfer of radionuclides to humans via food products.

1.5 Plant uptake of Cs and Sr

Plants have no known biological requirement for either Cs or Sr, but accumulate these elements because of their chemical similarities to the plant macronutrients K and Ca. Caesium is predominantly taken up by plants via root cell membrane K^+ transporters and K^+ channels (White and Broadley, 2000; Zhu and Smolders, 2000) and is transported easily and quickly around the plant (Middleton et al., 1960; Buysse et al., 1995). Strontium is thought to be taken up and transported in the plant in the same way as its chemical analogue Ca (Willey and Fawcett, 2006), via Ca^+ channels and apoplastic pathways (White and Broadley, 2003).

1.6 Soil characteristics affecting plant uptake of Cs and Sr

The extent to which Cs and Sr are taken up by plants is strongly affected by soil characteristics such as K, Ca and NH_4 concentrations, pH and soil type. Cs uptake is significantly reduced by increasing soil K^+ concentrations (Shaw and Bell, 1991; Zhu and Shaw, 2000; Kubo et al., 2015), and increases in soil K^+ concentrations has also been shown to reduce plant uptake of Sr (Frere et al., 1967; Lembrechts, 1993). Increasing soil Ca concentration has been shown to decrease uptake of both Cs (White and Broadley, 2000; Zhu and Smolders, 2000) and Sr (Frere et al., 1967; Lembrechts, 1993). Conversely, an excess of NH_4^+ in soil appears to increase Cs uptake (Livens and Loveland, 1988), though NH_4^+ is not present in high concentrations in the soil solution in aerobic soils, and therefore under normal conditions its effect on Cs plant uptake is thought to be minimal (Zhu and Smolders, 2000). Soil type and pH strongly affect the mobility of nutrients in the soil solution, and therefore has a strong, principally indirect effect, on plant uptake of Cs and Sr (Prister et al., 1992)

1.7 Current remediation strategies to reduce transfer of Cs and Sr from agricultural land

Current remediation strategies for radiologically contaminated land fall into three main categories: mechanical soil amendments, chemical soil amendments and treatment of livestock.

Ploughing to 20-30 cm using a common single-furrow plough can reduce uptake of Cs and Sr by up to 4-fold by burying the radionuclides from top-soil to a depth deeper than the crop rooting zone, thus reducing their availability for plant uptake (IAEA, 2012). Deep ploughing, which can be utilised in soils with a depth exceeding 50 cm, is used to invert the top 20-45 cm of soil. This buries the radionuclides to an even greater depth and can reduce the transfer of Cs and Sr by 2-4 fold, with a maximum recorded reduction of 10-fold (Maubert et al., 1993; Vovk et al., 1993; Bogdevitch, 2002; Fesenko et al., 2007). Following the accident in Fukushima, the preferred mechanical soil amendment for agricultural areas contaminated at an activity concentration $>5 \text{ kBq m}^{-2}$ (IAEA, 2014) has been removal of the surface soil layer (c. 5 cm; Nakano and Yong, 2013).

Chemical soil amendments aimed at reducing plant uptake of ^{137}Cs and ^{90}Sr have also been widely applied following contamination incidents. Mainly due to their ability to increase the concentration of plant-available K and Ca, the application of organic and mineral fertilisers is known to reduce the uptake of ^{137}Cs and ^{90}Sr by 1.3-3-fold (organic fertilisers) and 2-5-fold (mineral fertilisers; IAEA, 2012). The addition of lime (calcium and/or magnesium rich minerals) is known to reduce Cs and Sr uptake by 1.5-4-fold (IAEA, 2012).

Animal-based remediation strategies can also be used after a contamination incident to reduce the transfer of radionuclides to humans via animal

products. Natural (e.g. clay minerals) or artificial (e.g. 'Prussian blue') binding agents can be added to the livestock diet to reduce Cs uptake from the gut, and thus transfer to products such as meat and milk by up to 5-fold (clay minerals) and 8-fold (Prussian blue type compounds; IAEA, 2012). Sr transfer can be reduced by supplementing the diet with Ca (Beresford et al., 1998). Clean feeding, where animals are fed with uncontaminated fodder was utilised widely after the Fukushima accident (Manabe et al., 2013) and can be highly effective in reducing the transfer of radionuclides to animal products, but can be expensive as a long-term strategy and relies on the supply of uncontaminated feedstuffs (IAEA, 2012).

1.8 Plant-based remediation strategies

It has been known since the 1950s that plant species vary in the degree to which they take up Cs and Sr (e.g. Fuller and Flocker, 1955; Middleton et al., 1960). It has been proposed that plant species with high uptake of Cs and Sr could be used to remove Cs and Sr from contaminated land, a strategy known as phytoremediation (Entry et al., 1996). However, due to biological constraints on the amount of Cs and Sr a plant can accumulate, this has been shown to produce a large amount of low-level radioactive waste that needs to be disposed of (Vandenhove, 2013).

Therefore, another approach where species with lower Sr and/or Cs uptake are selected as 'safer' crops that can limit transfer of radionuclides from the soil to humans has been proposed (White et al., 2003; IAEA, 2012). Variation in Cs and Sr accumulation between different plant species can exceed 100-fold (Fesenko et al., 2000; Sanzharova, 2009). However, the knowledge and skills required to produce the 'safer' selected crop must be sufficient (Beresford et al., 2006) and there must be an available economically viable market for the selected crop (IAEA, 2012).

There is not only variation in uptake between species, there is also variation in uptake within species (between cultivars). Selection of lower-accumulating 'safer' cultivars has been shown to reduce transfer by up to 4.5-fold (Alexakhin, 1993), but the available information regarding inter-cultivar variation has not been sufficient for it to be internationally recommended as remediation measure (Beresford et al., 2006).

1.9 Aims and thesis structure

The aims of this thesis are to:

- To assess current knowledge of the variation in Cs and Sr accumulation by plant cultivars
- To integrate existing datasets regarding inter-cultivar variation in Cs and Sr accumulation into a usable database

- To quantify Cs and Sr accumulation among a large number of cultivars grown under the same conditions in long-term pasture-grass breeding trials
- To identify whether lower Cs and/or Sr accumulating cultivars consistently display lower-accumulation at multiple sites and harvests
- To identify whether there is variation in Cs and Sr accumulation between forage grass species
- To quantify the potential reduction in soil-cow Cs and Sr transfer by exploiting the variation in Cs and Sr accumulation between forage grass cultivars
- To elucidate the potential effects of selecting 'safer', lower-accumulating forage grasses on yield and forage quality

Thesis structure:

Chapter one introduces caesium and strontium, the distribution of radioisotopes of these elements in the environment, the problems arising from their transfer to humans and remediation strategies to minimise this transfer.

Chapter two is a meta-analysis of inter-cultivar variation in Cs (69 experiments) and Sr (58 experiments) accumulation, comprising a total of 27 plant species.

Chapter three presents the findings of four experiments investigating variation in Cs and Sr accumulation between cultivars of *Brassica oleracea*, two laboratory experiments conducted in the UK, and two field experiments conducted in the Chernobyl Exclusion Zone, Ukraine. Cultivars identified as lower accumulating in Cs and Sr are tested to evaluate whether they are consistently lower-accumulating in between the laboratory and field experiments, and between the two field experiments.

Chapter four concerns the results of analyses of Cs and Sr concentrations in 397 cultivars of four forage grass species; hybrid ryegrass (101), *Lolium perenne* (269), *Lolium multiflorum* (17) and *Festuca arundinacea* (10) grown in Aberystwyth and Edinburgh. The variation in Cs and Sr accumulation between these species and between cultivars of these species was calculated. Seventeen hybrid ryegrass cultivars grown in Aberystwyth and Edinburgh are tested to see if any cultivars could be identified as consistently lower accumulating in Cs and/or Sr at both sites and in spring and summer harvests

in two years. The number of cultivars required to encompass the maximum inter-cultivar variation between forage grass cultivars was also investigated.

Chapter five reports the findings of experiments comprising of *L. perenne* and *F. arundinacea* cultivars grown in Aberystwyth and Narodychi. Forage grass species and cultivars consistently lower-accumulating in Cs and Sr at both experimental sites are identified. The relationship between Cs and K and Sr and Ca are investigated.

Chapter six compares the dry and fresh weight yield, percentage dry weight, Cs, Sr, K and Ca concentrations, water soluble carbohydrate content and dry matter digestibility in hybrid ryegrass cultivars considered consistently lower Cs and/or Sr accumulating in chapter four and cultivars of *F. arundinacea* found to be a lower-accumulating species in chapters four and five with two commercially grown hybrid ryegrass cultivars. The potential effect on yield and forage quality is also evaluated.

Chapter seven includes a general discussion of the thesis contents and provides recommendations for future work.

1.10 References

Alexakhin, R.M., 1993. Countermeasures in agricultural production as an effective means of mitigating the radiological consequences of the Chernobyl accident. *Sci. Total Environ.* 137, 9-20. [http://dx.doi.org/10.1016/0048-9697\(93\)90374-f](http://dx.doi.org/10.1016/0048-9697(93)90374-f).

Alexakhin, R., Anspaugh, L., Balonov, M., Batandjieva, B., Besnus, F., Biesold, H., Bogdevich, I., Byron, D., Carr, Z., Deville-Cavelin, G., Ferris, I., Fesenko, S., Gentner, N., Golikov, V., Gora, A., Hendry, J., Hinton, T., Howard, B., Kashparov, V., Kirchner, G., LaGuardia, T., Linsley, G., Louvat, D., Moberg, L., Napier, B., Prister, B., Proskura, M., Reisenweaver, D., Schmieman, E., Shaw, G., Shestopalov, V., Smith, J., Strand, P., Tsaturov, Y., Vojtsekhovich, O., Woodhead, D., 2006. Environmental consequences of the Chernobyl accident and their remediation: Twenty years of experience. Report of the Chernobyl Forum Expert group "Environment". International Atomic Energy Agency, Vienna, Austria.

Ashraf, M.A., Khan, A.M., Ahmad, M., Akib, S., Balkair, K.S., Bakar, N.K., 2014. Release, deposition and elimination of radiocesium (^{137}Cs) in the terrestrial environment. *Environ. Geochem. Health* 36, 1165-1190. <http://dx.doi.org/10.1007/s10653-014-9620-9>.

Atomic Energy Society of Japan, 2014. The Fukushima Daiichi Nuclear Accident- Final report of the AESJ Investigation Committee by Atomic Energy Society of Japan. Maruzen Publishing Co., Ltd., Tokyo, Japan.

Baverstock, K., Egloff, B., Pinchera, A., Ruchti, C., Williams, D., 1992. Nat. 359, 21-22. <http://dx.doi.org/10.1038/359021b0>.

Beresford, N.A., Mayes, R.W., Hansen, H.S., Crout, N.M.J., Hove, K., Howard, B.J., 1998. Generic relationship between calcium intake and radiostrontium transfer to the milk of dairy ruminants. *Radiat. Environ. Biophys.* 37, 129-131. <http://dx.doi.org/10.1007/s004110050105>

Beresford, N.A., Voigt, G., Wright, S.M., Howard, B.J., Barnett, C.L., Prister, B., Balonov, M., Ratnikov, A., Travnikova, I., Gillett, A.G., Mehli, H., Skuterud, L., Lepicard, S., Semiochkina, N., Perepeliantnikova, L., Goncharova, N., Arkhipov, A. 2001. Self-help countermeasure strategies for populations living within contaminated areas of Belarus, Russia and Ukraine. *J. Environ. Radioactiv.* 56, 215-239. [http://dx.doi.org/10.1016/S0265-931X\(01\)00055-8](http://dx.doi.org/10.1016/S0265-931X(01)00055-8).

Beresford, N.A., Barnett, C.L., Howard, B.J., Rantavaara, A., Rissanen, K., Reales, N., Gallay, F., Papachristodoulou, C., Ioannides, K., Nisbet, A., Hesketh, N., Oughton, D., Bay, I., 2006. EURANOS Compendium of Countermeasures for the Management of Food Production Systems. Version 1.3. Available from: <http://www.euranos.fzk.de>.

Bibak, A., Sturup S, Knudsen, Beresford, N.A., Barnett, C.L., Howard, B.J., Rantavaara, A., Rissanen, K., Reales, N., Gallay, F., Papachristodoulou, C., Ioannides, K., Nisbet, A., Hesketh, N., Oughton, D., Bay, I., 2006. EURANOS Compendium of Countermeasures for the Management of Food Production Systems. Version 1.3. Available from: <http://www.euranos.fzk.de>.

Bogdevitch, I.M. (Ed.), 2002. Recommendations on agricultural production in conditions of radioactive contamination of lands in Belarus, approved by the Ministry of Agriculture and Food of Belarus Republic, BRISSA. [in Russian]

Buysse, J., Van de Brande, K., Merckx, R., 1995. The distribution of radiocaesium and potassium in spinach plants grown at different shoot temperatures. *J. Plant Physiol.* 146, 263-267. [http://dx.doi.org/10.1016/S0176-1617\(11\)82051-1](http://dx.doi.org/10.1016/S0176-1617(11)82051-1).

Cardis, E., Kesminiene, A., Ivanov, V., Malakhova, I., Shibata, Y., Khrouch, V., Drozdovitch, V., Maceika, E., Zvonova, I., Vlassov, O., Bouville, A., Goulko, G., Hoshi, M., Abrosimov, A., Anoshko, J., Astakhova, L., Chekin, S., Demidchik, E., Galanti, R., Ito, M., Korobova, E., Lushnikov, E., Maksioutov, M., Masyakin, V., Nerovnia, A., Parshin, V., Parshkov, E., Piliptsevich, N., Pinchera, A., Polyakov, S., Shabeka, N., Suonio, E., Tenet, V., Tsyb, A., Yamashita, S., Williams, D., 2005. Risk of thyroid cancer after exposure to ¹³¹I in childhood. *J. Natl. Cancer Inst.* 97, 724-732. <http://dx.doi.org/10.1093/jnci/dji129>.

Casacuberta, N., Masque, P., Garcia-Orellana, J., Garcia-Tenorio, R., Buessler, K.O., 2013. ^{90}Sr and ^{89}Sr in seawater off Japan as a consequence of the Fukushima Dai-ichi nuclear accident. *Biogeosciences*. 10, 3649-3659. <http://dx.doi.org/10.5194/bg-10-3649-2013>.

Emsley, J., 2011. *Nature's building blocks: an A-Z guide to the elements*. Oxford University Press, Oxford, UK.

Entry, J.A., Vance, N.C., Hamilton, M.A., Zabowski, D., Watrud, L.S., Adriano, D.C., 1996. Phytoremediation of soil contaminated with low concentrations of radionuclides. *Water Soil Poll.* 88, 167-176. <http://dx.doi.org/10.1007/BF00157420>T.

Fesenko, S.V., Alexakhin, R.M., Balonov, M.I., Bogdevitch, I.M., Howard, B.J., Kashparov, V.A., Sanzharova, N.I., Panov, A.V., Voigt, G., Zhuchenka, Y.M., 2007. An extended review of twenty years of countermeasures used in agriculture after the Chernobyl accident. *Sci. Total. Environ.* 383, 1-24. <http://dx.doi.org/10.1016/j.scitotenv.2007.05.011>.

Fesenko, S.V., Alexakhin, R.M., Sanzharova, N.I., 2000. Site characterisation techniques used in restoration of agricultural areas on the territory of the Russian Federation contaminated after the accident at the Chernobyl NPP, in: IAEA, *Site characterization techniques used in environmental restoration activities*, IAEA-TECDOC-1148. IAEA, Vienna, Austria.

Frere, M.H., Menzel, R.G., Roberts Jr, H., Myhre, D.L., Amemiya, M., Beale, O.W., Timmons, D.R., Wood, E.H., 1967. Reduction in the plant uptake of Sr-90 by soil management treatments. In: *Technical Bulletin No. 1378*. Agricultural Research Service, United States Department of Agriculture, Washington D.C., USA.

Fuller, W.H., and Flocker, W.J., 1955. The uptake of radiostrontium by certain type crops from calcareous soils. *Univ. Ariz. Agri. Stn. Tech. Bull.* 130.

IAEA, 2012. *Guidelines for Remediation Strategies to Reduce the Radiological Consequences of Environmental Contamination*. Technical Report Series No. 475. International Atomic Energy Agency, Vienna, Austria.

IAEA, 2014. *The Fukushima Daiichi accident technical volume 5 post-accident recovery*. International Atomic Energy Agency, Vienna, Austria.

Kabata-Pendias, A., Szteke, B., 2015. *Trace Elements in Abiotic and Biotic Environments*. CRC Press, Florida

Kubo, K., Nemoto, K., Kobayashi, H., Kuriyama, Y., Harada, H., Matsunami, H., Eguchi, T., Kihou, N., Ota, T., Keitoku, S., Kimura, T., Shinano, T., 2015. Analyses and countermeasures for decreasing radioactive cesium in buckwheat in areas affected by the nuclear accident in 2011. *Field Crop. Res.* 170 40-46. <http://dx.doi.org/10.1016/j.fcr.2014.10.001>.

Lembrechts, J.F., 1993. A review of literature on the effectiveness of chemical amendments in reducing the soil-to-plant transfer of radiostrontium and radiocaesium. *Sci. Total Environ.* 137, 81-98. [http://dx.doi.org/10.1016/0048-9697\(93\)90379-K](http://dx.doi.org/10.1016/0048-9697(93)90379-K).

Livens, F.R., Loveland, P.J., 1988. The influence of soil properties on the environmental mobility of caesium in Cumbria. *Soil Use Manag.* 4, 69-75. <http://dx.doi.org/10.1111/j.1475-2743.1988.tb00739.x>

Manabe, N., Takahashi, T., Li, J.-Y., Tanoi, K., 2013. Changes in the transfer of fallout radiocaesium from pasture harvested in Ibaraki Prefecture, Japan, to cow milk two months after the Fukushima Daiichi Nuclear Power Plant accident, in: Nakanishi, T.M., Tanoi, K. (Eds.), *Agricultural Implications of the Fukushima Nuclear Accident*. Springer, Tokyo, Japan. <http://dx.doi.org/10.1007/978-4-431-54328-2>.

Matsuzaki, H., Fujiwara, T., Saito, T., Yamagata, T., Honda, M., Muramatsu, Y., 2012. Isotopic ratio of radioactive iodine ($^{129}\text{I}/^{131}\text{I}$) released from Fukushima Daiichi NPP accident. *Geochem. J.* 46, 327-333. <http://dx.doi.org/10.2343/geochemj.2.0210>.

Maubert, H., Vovk, I., Roed, J., Arapis, G., Jouve, A., 1993. Reduction of soil-plant transfer factors: mechanical aspects. *Sci. Total Environ.* 137, 163-167. [http://dx.doi.org/10.1016/0048-9697\(93\)90384-I](http://dx.doi.org/10.1016/0048-9697(93)90384-I).

Middleton, L.J., Handley, R., Overstreet, R., 1960. Relative uptake and translocation of potassium and cesium in barley. *J. Plant Physiol.* 35, 913-918.

Nakano, M., Yong, R.N., 2013. Overview of rehabilitation schemes for farmlands contaminated with radioactive cesium released from Fukushima power plant. *Engineering Geology.* 155, 87-93. <http://dx.doi.org/10.1016/j.enggeo.2012.12.010>.

Nuclear Energy Agency (NEA), 2002. *Chernobyl: Assessment of Radiological and Health Impacts*. <http://www.nea.fr/html/rp/chernobyl/> (accessed 25.09.15).

Prister, B., Loshchilov, N., Perepelyatnikova, L., Perepelyatnikov, G., Bondar, P., 1992. Efficiency of measures aimed at decreasing the contamination of agricultural products in areas contaminated by the Chernobyl NPP accident. 112, 79-87. [http://dx.doi.org/10.1016/0048-9697\(92\)90240-S](http://dx.doi.org/10.1016/0048-9697(92)90240-S).

Reiners, C., Biko, J., Haensheid, H., Hebestreit, H., Kirinjuk, S., Baranowski, O., Marlowe, R.J., Demidchik, E., Drozd, V., Demidchik, Y., 2013. Twenty-Five Years After Chernobyl: Outcome of Radioiodine Treatment in Children and Adolescents With Very High-Risk Radiation-Induced Differentiated Thyroid Carcinoma. *J. Clin. Endocrinol. Metab.* 98, 3039-3048.
<http://dx.doi.org/10.1210/jc.2013-1059>.

Sanzharova, N., Shubina, O., Vandenhove, H., Olyslaegers, G., Fesenko, S., Zang, Z.R., Reed, E., Velasco, H., 2009. Root uptake: temperate environment, in: Quantification of radionuclide transfer in terrestrial and freshwater environments for radiological assessments, IAEA-TECDOC-1616. IAEA, Vienna, Austria.

Shaw, G., Bell, J.N.B., 1991. Competitive effects of potassium and ammonium on caesium uptake kinetics in wheat. *J. Environ. Radioact.*, 13,283–296.
[http://dx.doi.org/10.1016/10.1016/0265-931X\(91\)90002-W](http://dx.doi.org/10.1016/10.1016/0265-931X(91)90002-W)

Shaw, G., (Ed.), 2007. Radioactivity in the terrestrial environment. Elsevier, Oxford, UK.

Steinhauser, G., Bradl, A., Johnson, T.E., 2014. Comparison of the Chernobyl and Fukushima nuclear accidents: A review of the environmental impacts. *Sci. Total Environ.* 470-471, 800-817.
<http://dx.doi.org/10.1016/j.scitotenv.2013.10.029>.

Stwertka, A., 2002. A guide to the elements 2nd ed. Oxford University Press, Oxford, UK.

Takahashi, S., ed., 2014. Radiation Monitoring and Dose Estimation of the Fukushima Nuclear Accident. Springer, Tokyo, Japan.

United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), 2014. Sources, effects and risks of ionizing radiation. Report Volume I, Report to the general assembly scientific Annex A: Levels and effects of radiation exposure due to the nuclear accident after the 2011 great east-Japan earthquake and tsunami. Available from:
http://www.unscear.org/docs/reports/2013/14-06336_Report_2013_Annex_A_Ebook_website.pdf.

Vandenhove, H., 2013. Phytoremediation options for radioactively contaminated sites evaluated. *Ann. Nucl. Energy* 62, 596-606.
<http://dx.doi.org/10.1016/j.anucene.2013.02.005>.

Vovk, I.F., Blagoyev, V.V., Lyashenko, A.N., Koyalev, I.S., 1993. Technical approaches to decontamination of terrestrial environments in the CIS. *Sci. Total Env.* 137, 49-63. [http://dx.doi.org/10.1016/0048-9697\(93\)90377-I](http://dx.doi.org/10.1016/0048-9697(93)90377-I).

White, P.J., Broadley, M.R., 2000. Mechanisms of caesium uptake by plants. *New Phytol.* 147, 241-256. [http://dx.doi.org/ 10.1046/j.1469-8137.2000.00704.x](http://dx.doi.org/10.1046/j.1469-8137.2000.00704.x)

White, P.J., Broadley, M.R., 2003. Calcium in plants. *Ann. Bot.* 487-511.

White, P.J., Swarup, K., Escobar-Gutiérrez, A.J., Bowen, H.C., Willey, N.J., Broadley, M.R., 2003. Selecting plants to minimise radiocaesium in the food chain. *Plant Soil* 249, 177-186.

Willey, N., Fawcett, K., 2006. A phylogenetic effect on strontium concentrations in angiosperms. *Environ. Exp. Bot.* 57, 258-269. <http://dx.doi.org/10.1016/j.envexpbot.2005.06.005>.

Zhu, Y.G., Shaw, G., 2000. Soil contamination with radionuclides and potential remediation. *Chemosphere* 41,121-128. [http://dx.doi.org/10.1016/S0045-6535\(99\)00398-7](http://dx.doi.org/10.1016/S0045-6535(99)00398-7)

Zhu, Y.G., Smolders, E., 2000. Plant uptake of radiocaesium: a review of mechanisms, regulation and application. 51, 1635-45. <http://dx.doi.org/10.1093/jexbot/51.351.1635>

Chapter 7. General discussion

7.1 Genetic variation in caesium and strontium in plants

This study has shown that there is genetic variation in the uptake of caesium (Cs) and strontium (Sr) within a number of different plant species. This is in accordance with previous studies that have shown wide variation in the concentration of plant macronutrients such as calcium (Ca), potassium (K) and magnesium (Mg; e.g. Broadley et al., 2004; Vreugdenhil et al., 2004; White and Broadley, 2005; Harada and Leigh, 2006; Waters and Grusak, 2008; El-Nashaar et al., 2009; Garcia-Oliviera et al., 2009) and with studies that have shown variation in plant uptake of Cs and Sr between plant families (e.g. Broadley and Willey, 1997; Broadley et al., 1999; Willey and Fawcett, 2006; Watanabe et al., 2007; Willey, 2010), species (e.g. Andersen, 1967; Zhu and Smolders, 2000) and cultivars (e.g. Rasmusson et al., 1963; Payne et al., 2004; Ohmori et al., 2014). It has been suggested that this variation can be used to reduce the transfer of radioisotopes of Cs and Sr to crop plants following a contamination incident.

For the first time, within species (inter-cultivar) variation from all available studies was investigated, finding an average of 1.8-fold variation in Cs and 2.0-fold variation in Sr in 27 plant species from a total of 115 experiments (Chapter 2). However, most of these experiments were conducted on fewer than seven cultivars, and focussed on main food crop species, especially wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*).

The experimental work in Chapters 3, 4 and 5 regarding *Brassica oleraceae* (Chapter 3), hybrid ryegrass (Chapter 4) *Lolium perenne* (Chapters 4 and 5) and *Lolium multiflorum* (Chapter 4) showed higher variation than the average found in Chapter 2 in Cs (up to: 35-fold; 14-fold; 13-fold; 2-fold) and Sr (up to 23-fold; 4.4-fold; 2.5-fold and 2.9-fold) concentration ratios. This may be due to the larger numbers of cultivars in these experiments (number of cultivar were up to: 71, 100, 189 and 29, respectively). The study detailed in Chapter 4 including 397 cultivars of forage grass (hybrid ryegrass=101; *L. perenne*=269; *L. multiflorum*=17; *F. arundinacea*=10), is likely to be the largest study regarding inter-cultivar variation in Cs and Sr accumulation to date. Recent studies including larger numbers of cultivars such as Ohmori et al., (2014) who studied ¹³⁷Cs activity concentrations in 85 rice (*Oryza sativa*) cultivars have also found larger inter-cultivar variation (10-fold) than the average found in Chapter 2. The relationship between the number of cultivars and inter-cultivar variation in Cs and Sr accumulation has not been investigated before; Chapter 4 includes an investigation into this relationship, and these results suggest that there is a positive relationship between the number of cultivars and the magnitude of the inter-cultivar variation. However, this relationship appears to start to plateau, suggesting there is a maximum number of cultivars needed to be able to reach the maximum inter-cultivar variation.

This relationship was also shown to be different for each plant species studied and the maximum number of cultivars was different in each location. It is possible that this relationship was affected by climate, small differences in soil properties, differences in the soil concentrations of the analogous elements K and Ca or differences in soil concentrations of Cs and Sr. Further work needs to be carried out in order to understand the factors influencing this relationship.

Although the relationships between the number of cultivars and inter-cultivar variation generally appeared to be starting to plateau, they suggest that the maximum inter-cultivar variation is larger than the inter-cultivar variations found in our experiments. This suggests that the potential reduction in transfer of Cs and Sr using species or cultivar substitution could be even higher than reported in this thesis. It is recommended that future research on inter-cultivar variation includes as many cultivars as possible in order to encompass the maximum variation.

7.2 Consistently lower-accumulating cultivars

Though inter-cultivar variation appears to vary with location, we have been able to identify cultivars of *Brassica oleracea* (Chapter 3), hybrid ryegrass (Chapter 4), *L. perenne* (Chapter 5) and *F. arundinacea* (Chapter 5) that were significantly consistently lower accumulating in multiple locations. Previously, consistently lower accumulating cultivars have been found in the experiments of Csupka et al., (1969; wheat), Øhlenschläger and Gissel-Nielsen, (1989; barley), Øhlenschläger et al., (1993 barley), Sarfraz et al., (2007; rice). Other experiments where the same cultivars were grown in multiple sites found little consistency in lower-accumulation of Cs and Sr (Csupka et al., 1969, wheat; Gertsman and Schimmack, 2006, wheat; Øhlenschläger and Gissel-Nielsen, 1991, barley), though cultivars in these experiments were defined as consistently lower accumulating if the lowest accumulating cultivar was the same in experiments in multiple locations. Using this method of defining lower accumulation, one is less likely to find lower-accumulating cultivars, as the cultivars always have to be the lowest, not just lower. The likelihood of a cultivar being the lowest decreases significantly with the number of cultivars, the number of locations and the number of sampling events. The likelihood of a cultivar being in the lowest 5th percentile-which is how lower accumulation is defined in this thesis- also decreases with number of cultivars, locations and sampling events, though to a lesser degree. Furthermore, it is not as important for cultivars to be the lowest accumulating as it is for them to be *amongst* the lowest accumulating cultivars. It is therefore recommended that when comparing large numbers of cultivars and/or several locations or sampling events, it is recommended that instead of trying to find a cultivar that is consistently lowest accumulating the statistical methods used in Chapters 3-5 are applied.

7.3 *Cultivar substitution as a remediation strategy for contaminated land*

Substituting higher-accumulating species or cultivars for lower accumulating ones has been proposed as a remediation strategy for radiologically contaminated land since the 1950s (e.g. Fuller and Flocker, 1955; Middleton et al., 1960; Rasmusson et al., 1963), but the lack of information meant it was not possible previously to evaluate its efficacy (Beresford et al., 2006). From the work of the studies included in this thesis, it is concluded that there is considerable variation in Cs and Sr concentration ratios between plant species and cultivars, which is in agreement with the findings of e.g. Prister et al., (1992), Alexakhin, (1993) and White et al., (2003). Therefore species or cultivar substitution is recommended as an effective remediation strategy in contaminated agricultural areas.

Other remediation strategies to minimise root uptake of radionuclides by plants fall into two main categories; mechanical soil amendments and chemical soil amendments. Mechanical soil amendments such as ploughing can reduce transfer of radionuclides by up to 4-fold using a single-furrow plough and up to 10-fold using deep ploughing techniques (IAEA, 2012). Chemical soil amendments such as the application of organic fertilisers have been found to reduce ^{137}Cs and ^{90}Sr by up to 3-fold, mineral fertilisers by 2-5 fold and lime by 1.5-4 fold (IAEA, 2012). The possible reductions in transfer of these elements using species substitution found in Chapter 4 (up to 19-fold in Cs, if hybrid ryegrass is replaced by *F. arundinacea*; up to 2.6-fold in Sr, if *L. multiflorum* is replaced by *F. arundinacea*) or cultivar substitution found in Chapter 3 (up to 35-fold for Cs, up to 23-fold for Sr; *Brassica oleracea*) and Chapter 4 (up to 14-fold for Cs, up to 4.4-fold for Sr, hybrid ryegrass) are in the same order of magnitude or higher than these established techniques, and therefore is potentially an effective remediation strategy following a contamination incident. Furthermore, crop substitution could be implemented in conjunction with one or more of these existing soil-based remediation strategies to produce an even larger reduction of transfer of Cs and Sr.

7.4 *The effects of cultivar substitution on crop yield and quality*

The effect of lower-accumulation on crop yield and quality has not been extensively studied before. This is possibly because previously there were too few cultivars identified as lower accumulating in Cs and/or Sr. Results from the experiments in Chapter 6 suggest that species substitution could affect crop yield and quality. If *F. arundinacea* was planted instead of commercial hybrid ryegrasses, the fresh weight yield could be reduced by up to 59%, though the quality parameters dry matter digestibility (DMD) and water soluble carbohydrate content (WSC) were less affected (a 7% and 16% reduction, respectively).

Results of the experiment in Chapter 6, however, suggest that cultivar substitution has little negative effect on crop yield and quality. Substituting commercial hybrid ryegrass cultivars for lower accumulating hybrid ryegrass cultivars showed to have little effect on the yield, Ca and K concentrations and DMD and WSC content. However, it is not known how the yield might be affected by cultivar substitution in other species. In addition to this, quality parameters vary between crop species, so it is not known how these might be affected if lower-accumulating cultivars or species were selected. Many of the existing remediation strategies have been evaluated in terms of their acceptability to stakeholders (e.g. Nisbet et al., 2009). Species and cultivar substitution were not included in these evaluations, as too little was known about its effectiveness as a remediation strategy. Therefore how stakeholders such as farmers and consumers would feel about species or cultivar substitution following a contamination incident is not known. It is therefore suggested that the acceptability of species and cultivar substitution is assessed prior to being recommended or implemented.

References

- Alexakhin, R.M., 1993. Countermeasures in agricultural production as an effective means of mitigating the radiological consequences of the Chernobyl accident. *Sci. Total Environ.* 137, 9-20. [http://dx.doi.org/10.1016/0048-9697\(93\)90374-f](http://dx.doi.org/10.1016/0048-9697(93)90374-f).
- Andersen, A.J., 1967. Investigations on the plant uptake of fission products from contaminated soils. I. Influence of plant species and soil types on the uptake of radioactive strontium and caesium. *Risø Report No. 170*. Risø National Laboratory, Copenhagen.
- Beresford, N.A., Barnett, C.L., Howard, B.J., Rantavaara, A., Rissanen, K., Reales, N., Gallay, F., Papachristodoulou, C., Ioannides, K., Nisbet, A., Hesketh, N., Oughton, D., Bay, I., 2006. EURANOS Compendium of Countermeasures for the Management of Food Production Systems. Version 1.3. Available from: <http://www.euranos.fzk.de>.
- Broadley, M.R., Willey, N.J., 1997. Differences in root uptake of radiocaesium by 30 plant taxa. *Environ. Pollut.* 97, 11-15. [http://dx.doi.org/10.1016/S0269-7491\(97\)00090-0](http://dx.doi.org/10.1016/S0269-7491(97)00090-0)
- Broadley, M.R., Willey, N.J., Mead, A., 1999. A method to assess taxonomic variation in shoot caesium concentration among flowering plants. *Environ. Pollut.* 106, 341-349. [http://dx.doi.org/10.1016/S0269-7491\(99\)00105-0](http://dx.doi.org/10.1016/S0269-7491(99)00105-0).

- Broadley, M.R., Bowen, H.C., Cotterill, H.D., Hammond, J.P., Meacham, M.C., Mead, A., White, P.J., 2004 Phylogenetic variation in the shoot mineral concentration of angiosperms. *J. Exp. Bot.* 396, 321-336. <http://dx.doi.org/10.1093/jxb/erh002>
- Csupka, S., Carach, J., Petrásová, M., 1969. Strontium-90 level in different varieties of wheat in Slovakia. *Pol'nohospodarstvo* 15, 964-971.
- El-Nashaar, H.M., Banowetz, G.M., Griffith, S.M., Casler, M.D., Vogel, K.P., 2009. *Bioresource Technol.* 100, 1809-1814. <http://dx.doi.org/10.1016/j.biortech.2008.09.058>
- Fuller, W.H., and Flocker, W.J., 1955. The uptake of radiostrontium by certain type crops from calcareous soils. *Univ. Ariz. Agri. Stn. Tech. Bull.* 130.
- Garcia-Oliveira, A.L., Tan, L.B., Fu, Y.C., Sun, C.Q., 2009. Genetic Identification of Quantitative Trait Loci for Contents of Mineral Nutrients in Rice Grain. *J. Integr. Plant Biol.* 51, 84-92. <http://dx.doi.org/10.1111/j.1744-7909.2008.00730.x>
- Gerstmann, U.C., Schimmack, W., 2006. Soil-to-grain transfer of fallout Sr-90 for 28 winter wheat cultivars. *Radiat. Environ. Biophys.* 45, 187-194. <http://dx.doi.org/10.1007/s00411-006-0060-5>
- Harada, H., Leigh, R.A., 2006. Genetic mapping of natural variation in potassium concentrations in shoots of *Arabidopsis thaliana*. *J. Exp. Bot.* 57, 953-960. <http://dx.doi.org/10.1093/jxb/erj081>
- IAEA, 2012. Guidelines for Remediation Strategies to Reduce the Radiological Consequences of Environmental Contamination. Technical Report Series No.475. International Atomic Energy Agency, Vienna.
- Middleton, L.J., Handley, R., Overstreet, R., 1960. Relative uptake and translocation of potassium and cesium in barley. *J. Plant Physiol.* 35, 913-918.
- Nisbet, A.F., Howard, B.J., Jones, A., Jullien, T., Pupin, V., Ollagnon, H., Turcanu, C., Camps, J., Papachristodoulou, C., Ioannides, K., Hänninen, R., Rantavaara, A., Solatie, D., Kostianen, E., Oughton, D., Andersson, K.G., 2009. EURANOS. Generic handbook for assisting in the management of contaminated food production systems in Europe following a radiological emergency. Abstract from Final EURANOS contractors meeting, Madrid, Spain.

Øhlenschläger, M., Gissel-Nielsen, G., 1989. Transfer of Radiocaesium to Barley, Rye, Grass and Pea. Risø-M-2831. Risø National Laboratory, Copenhagen.

Øhlenschläger, M., Gissel-Nielsen, G., 1991. Differences in the ability for barley and rye grass varieties to absorb caesium through the roots. Acta Agr Scand. 41, 321-328.

Øhlenschläger, M., Gissel-Nielsen, G., Nielsen, S.P., 1993. Differences in the sensitivity of barley varieties to direct cesium contamination from the Chernobyl accident. Health Phys. 64, 535-537.
<http://dx.doi.org/10.1097/00004032-199305000-00012>.

Ohmori, Y., Inui, Y., Kajikawa, M., Nakata, A., Sotta, N., Kasai, K., Uruguchi, S., Tanaka, N., Nishida, S., Hasegawa, T., Sakamoto, T., Kawara, Y., Aizawa, K., Fujita, H., Li, K., Sawaki, N., Oda, K., Futagoishi, R., Tsusaka, T., Takahashi, S., Takano, J., Wakuta, S., Yoshinari, A., Uehara, M., Takada, S., Nagano, H., Miwa, K., Aibara, I., Ojima, T., Ebana, K., Ishikawa, S., Sueyoshi, K., Hasegawa, H., Mimura, T., Mimura, M., Kobayashi, N.I., Furukawa, J., Kobayashi, D., Okouchi, T., Tanoi, K., Fujiwara, T., 2014. Difference in cesium accumulation among rice cultivars grown in the paddy field in Fukushima Prefecture in 2011 and 2012. J. Plant Res. 127, 57-66. <http://dx.doi.org/10.1007/s10265-013-0616-9>

Payne, K.A., Bowen, H.C., Hammond, J.P., Hampton, C.R., Lynn, J.R., Mead, A., Swarup, K., Bennett, M.J., White, P.J., Broadley, M.R., 2004. Natural genetic variation in caesium (Cs) accumulation by *Arabidopsis thaliana*. New Phytol. 162, 535-548. <http://dx.doi.org/10.1111/j.1469-8137.2004.01026.x>

Prister, B., Loshchilov, N., Perepelyatnikova, L., Perepelyatnikov, G., Bondar, P., 1992. Efficiency of measures aimed at decreasing the contamination of agricultural products in areas contaminated by the Chernobyl NPP accident. 112, 79-87. [http://dx.doi.org/10.1016/0048-9697\(92\)90240-S](http://dx.doi.org/10.1016/0048-9697(92)90240-S).

Rasmusson, D.C., Smith, L.H., Myers, W.M., 1963. Effect of genotype on accumulation of Strontium-89 in barley and wheat. Crop Sci. 3, 34e37. <http://dx.doi.org/10.1038/1981008a0>

Sarfraz, M., Mehdi, S.M., Hassan, G., Abbas, S.T., 2007. Metal contamination in Nullah Dek water and accumulation in rice. Pedosphere 17, 130-136. [http://dx.doi.org/10.1016/s1002-0160\(07\)60018-6](http://dx.doi.org/10.1016/s1002-0160(07)60018-6).

Vregdenhil, D., Aarts, M.G.M., Koornneef, M., Nelissen, H., Ernst, W.H.O., 2004. Natural variation and QTL analysis for cationic mineral content in seeds of *Arabidopsis thaliana*. Plant Cell Environ. 27, 828-839. <http://dx.doi.org/10.1111/j.1365-3040.2004.01189.x>

Watanabe, T., Broadley, M.R., Jansen, S., White, P.J., Takada, J., Satake, K., Takamatsu, T., Tuah, S.J., Osaki, M., 2007. Evolutionary control of leaf element composition in plants. *New Phytol.* 174, 516e523. <http://dx.doi.org/10.1111/j.1469-8137.2007.02078.x>.

Waters, B.M., Grusak, M.A., 2008. Quantitative trait locus mapping for seed mineral concentrations in two *Arabidopsis thaliana* recombinant inbred populations. *New Phytol.* 179, 1033-1047. <http://dx.doi.org/10.1111/j.1469-8137.2008.02544.x>

White, P.J., Swarup, K., Escobar-Gutiérrez, A.J., Bowen, H.C., Willey, N.J., Broadley, M.R., 2003. Selecting plants to minimise radiocaesium in the food chain. *Plant Soil* 249, 177-186.

White, P.J., Broadley, M.R., 2005. Biofortifying crops with essential mineral elements. *Trends Plant Sci.* 10, 586-593. <http://dx.doi.org/10.1016/j.tplants.2005.10.001>

Willey, N., Fawcett, K., 2006. A phylogenetic effect on strontium concentrations in angiosperms. *Environ. Exp. Bot.* 57, 258-269. <http://dx.doi.org/10.1016/j.envexpbot.2005.06.005>.

Willey, N.J., 2010. Phylogeny can be used to make useful predictions of soil-to-plant transfer factors for radionuclides. *Radiat. Environ. Biophys.* 49, 613-623. <http://dx.doi.org/10.1007/s00411-010-0320-2>

Zhu, Y.G., Smolders, E., 2000. Plant uptake of radiocaesium: a review of mechanisms, regulation and application. 51, 1635-45. <http://dx.doi.org/10.1093/jexbot/51.351.1635>