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Measurement of ^{90}Sr in Chernobyl arthropods

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Master of Science, Radioecology

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Ivar Ormaasen

Abstract

Following the Chernobyl accident, the areas around the Chernobyl Nuclear Power Plant (ChNPP) were contaminated with many radionuclides, including the fission product ^{90}Sr. Because of its long half-life and chemical similarity to Ca, ^{90}Sr is considered to be of particular radiological concern. When taken up in plants and animals, ^{90}Sr can accumulate in and irradiate Ca rich tissues such as bone, leading to high internal exposure and potential radiation effects.

There is a knowledge gap on the ecosystem transfer of ^{90}Sr to arthropods, largely because of the lack of a reliable and efficient method to determine ^{90}Sr. This makes it difficult to assess factors influencing ^{90}Sr transfer in arthropod communities. This thesis aimed to develop a mass spectrometric method for the determination of ^{90}Sr in individual arthropods and test the method on Chernobyl arthropods. In addition to this, the levels of ^{90}Sr were compared to stable Sr and Ca, and estimations of radiation dose rates absorbed by Chernobyl arthropods were made using the ERICA Assessment Tool.

A chromatographic 'microcolumn' was developed for extraction chromatography (EXC) with the application of Sr-resin in order to separate Sr in low volume, microwave acid digested arthropod samples. The Sr-resin separates Sr from the matrix and interfering ^{90}Zr. For the determination of ^{90}Sr, separated samples were directly analysed in a triple quadrupole inductively coupled plasma mass spectrometer (ICP-QQQ), where remaining ^{90}Zr interference was removed by using a mixture of O_2 and H_2 in the collision-reaction cell (CRC).

Separations with microcolumn led to very good recovery for Sr (93 ± 7%) and highly efficient removal of interfering Ca and Zr (98 ± 2%) in individual arthropods. In addition, ICP-QQQ analysis resulted in a low detection limit (1.1 fg; 5.5 mBq) and precise determination of ^{90}Sr. Determined ^{90}Sr activity concentrations in Chernobyl arthropods ranged from 4.6 to 190 Bq/g and the highest levels were observed in mixed feeders and predators. Uptake of ^{90}Sr by the measured Chernobyl arthropods, resulted in high internal dose, estimated as 1.0 to 21 μGy h^{-1}. Furthermore, a correlation was observed between the levels of ^{90}Sr and stable Sr and Ca, indicating that Ca may influence uptake of stable and radioactive Sr in arthropods.

Sammendrag

Etter Tsjernobyl-ulykken ble områdene rundt kjernekraftverket (ChNPP) kontaminert med mange radionuklider, inkludert fisjonsproduktet ^{90}Sr. På grunn av sin lange halveringstid og kjemiske likhet med Ca, anses ^{90}Sr å være av spesiell radiologisk bekymring. Når den tas opp i planter og dyr, kan ^{90}Sr akkumuleres i og bestråle Ca-rike vev som bein, noe som fører til høy intern eksponering og mulige strålingseffekter.

Det er et kunnskapshull i økosystemoverføringen av ^{90}Sr til leddyr, mest på grunn av mangel på en pålitelig og effektiv metode for bestemmelse av ^{90}Sr. Dette gjør det vanskelig å vurdere faktorer som påvirker ^{90}Sr-overføring i leddyrsamfunn. Målene med denne oppgaven var å utvikle en massespektrometrisk metode for bestemmelsen av ^{90}Sr i individuelle leddyr og å teste metoden på Tsjernobyl-leddyr. I tillegg ble nivåene av ^{90}Sr sammenlignet med stabil Sr og Ca, og estimering av doserater absorbert av Tsjernobyl-leddyr ble utført ved bruk av ERICA Assessment Tool.

En kromatografisk "mikrokolonne" ble utviklet for å utføre separasjon av Sr i små volumprøver av mikrobølge-dekomponerte leddyr ved bruk av ekstraksjonskromatografi (EXC) med Sr-resin. Sr-resinet separerer Sr fra matriks og interfererende ^{90}Zr. For bestemmelsen av ^{90}Sr ble separerte prøver analysert direkte i en trippel kvadrupol induktivt koblet plasma massespektrometer (ICP-QQQ), hvor gjenværende ^{90}Zr interferens ble fjernet ved å bruke en blanding av O_2 og H_2 i kollisjons-reaksjonscellen (CRC).

Separasjon med mikrokolonne førte til veldig god gjenfinning av Sr (93 ± 7%) og svært effektiv fjerning av interfererende Ca og Zr (98 ± 2%) i individuelle leddyr. I tillegg resulterte ICP-QQQ analyse i lav deteksjonsgrense (1.1 fg; 5.5 mBq) og presis bestemmelse av ^{90}Sr. Bestemte aktivitetskonsentrasjoner av ^{90}Sr i Tsjernobyl-leddyr varierte fra 4.6 til 190 Bq/g og de høyeste nivåene ble observert i altetere og rovdyr. Opptak av ^{90}Sr av de målte Tsjernobyl-leddyrene, resulterte i høy intern dose, estimert som 1.0 til 21 µGy h^{-1}. Videre ble det observert en korrelasjon mellom nivåene av ^{90}Sr og stabil Sr og Ca, noe som indikerer at Ca kan påvirke opptaket av radioaktiv og stabil Sr i leddyr.

Abbreviations

BEC	Background equivalent concentration
ChEZ	Chernobyl exclusion zone
ChNPP	Chernobyl Nuclear Power Plant
CPS	Counts per second
CRM	Certified reference material
CRC	Collision-reaction cell
EXAFS	Extended X-ray absorption fine-structure
EXC	Extraction chromatography
DA	Digital autoradiography
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-QQQ	Triple quadrupole ICP-MS
IE	Ionization energy
IS	Internal standard
LOD	Limit of detection
LOQ	Limit of quantification
LSC	Liquid scintillation counting
MS	Mass spectrometry
m/z	Mass-to-charge ratio
PES	Polyethersulfone
PIS	Product ion scan
Q1	First quadrupole mass unit
Q2	Second quadrupole mass unit
TFM	3-trifluoromethyl-4-nitrophenol

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1 INTRODUCTION

1.1 The Chernobyl accident

In the northern Ukraine, shortly after midnight on 26th April 1986, an experiment at reactor unit 4 of the Chernobyl Nuclear Power Plant (ChNPP) went wrong. As a consequence, a steam explosion destroyed the reactor, and a fire broke out in the core. The Chernobyl accident resulted in the largest accidental release of radionuclides into the environment in the history of nuclear power production (Beresford et al., 2016). Plumes of volatile radioactive elements (e.g., radionuclides of iodine, caesium), noble gases and irradiated uranium fuel particles were released into the environment. The long-lived radionuclides of ^{134,137}Cs were transported as condensed particles, causing a widespread contamination in Europe. Additionally, depositions of radioactivity were especially high in areas with heavy rainfall. The local contamination of irradiated fuel particles around the ChNPP, were considered to be a characteristic attribute of the accident. Fuel particle matrices are known to contain long-lived and radiologically significant radionuclides due to low volatility of these elements, and ^{90}Sr and $^{238\text{-}241}$Pu were mainly released in the form of fuel particles (IAEA, 2006; Kuriny et al., 1993). Shortly after the accident, a 30 km radius around the ChNPP, known as the Chernobyl Exclusion Zone (ChEZ), was established and the population evacuated or relocated (Beresford et al., 2016).

After deposition, ^{90}Sr and Pu isotopes, which are biologically important radionuclides, were observed to have low mobility in soil and be unavailable for plant uptake when incorporated within the fuel particles. (Kuriny et al., 1993). Radiocaesium was, on the other hand, more mobile and bioavailable in the form of condensed particles at greater distances from ChNPP. Over time, however, weathering of fuel particles resulted in the leaching of the contained radionuclides, causing mobility of ^{90}Sr in the soils of ChEZ to increase (Kashparov et al., 1999). The dissolution rate was, among other factors, found to be dependent on soil acidity and the oxidation state of the fuel particles. Therefore, oxidized uranium fuel tends to dissolve faster than non-oxidized particles. Hence, as the amount of exchangeable ^{90}Sr in soil increases with particle weathering, activity concentrations of ^{90}Sr in plants increase, including further transfer through the food chain (Kashparov et al., 2000; Kashparov et al., 2004).

1.2 Strontium

Elemental strontium (atomic number 38) is a divalent alkaline earth metal that occurs naturally in the environment as four stable isotopes with the following relative abundances: ^{84}Sr (0.56%), ^{86}Sr (9.86%), ^{87}Sr (7.00%) and ^{88}Sr (82.58%) (Burger & Lichtscheidl, 2019; Dorsey et al., 2004). The oxidation states of strontium are 0 (metal) and +2. It is, however, the divalent form of strontium that is most stable and present in the environment due to the high reactivity of pure strontium with water and oxygen. In nature, strontium is found naturally in soil with a typical concentration of 0.2 mg/kg soil, usually in the form of minerals. Because of the likelihood of forming comparatively soluble compounds, strontium tends to be relatively mobile in ecosystems (Whicker & Schultz, 1982).

Several radioactive isotopes of strontium exist as fission by-products. The most significant radioactive isotopes are ^{89}Sr and ^{90}Sr, with half-lives of 50.56 days and 28.79 years, respectively. They are formed by the nuclear fission of ^{235}U, ^{238}U or ^{239}Pu during nuclear reactor operations and nuclear explosions (Dorsey et al., 2004). Of the two radioactive isotopes of strontium isotopes, ^{90}Sr is the most important one due to its physical long half-life. When ^{90}Sr decays, it emits a beta particle, with a maximum energy of 0.546 MeV, and forms its radioactive daughter isotope, ^{90}Y. The daughter isotope, ^{90}Y has a shorter half-life of 64 hours and forms the stable isotope of ^{90}Zr after beta decay (maximum beta energy of 2.28 MeV) (Vajda & Kim, 2010).

Strontium is a chemical analogue to the alkaline earth element, calcium, due to similar chemical properties. Consequently, strontium deposited in soil and water can be taken up by plants and animals via the same mechanisms as calcium and accumulate in Ca-rich tissues, e.g., bone, teeth, and shells (Whicker & Schultz, 1982). The beta particles emitted during decay are quickly stopped in most media (e.g., air, water, soil). If ingested or inhaled, however, ^{90}Sr can substitute Ca and reside in human bone structure for a long time, due to its long physical and biological half-life (Vajda & Kim, 2010). This can cause high internal exposure and potential radiation effects as a result of the combined disintegrations of ^{90}Sr and its daughter, ^{90}Y (Larson & Ebner, 1958). Nonetheless, the soil-to-plant transfer of ^{90}Sr is dependent on the amounts of exchangeable forms of Ca in the soil. The presence of ^{90}Sr in soil is much less compared to the amounts of Ca, thus, the soil-to-plant transfer factor of ^{90}Sr tends to decrease when exchangeable forms of Ca in soil increases (Yoschenko et al., 2019).

1.3 Background for standard analysis of ^{90}Sr measurements

1.3.1 Radiometric methods

The greater extent of data and published articles from studies on ^{137}Cs behaviour in the environment, is due to its easy measurement by gamma spectrometry that do not necessarily require radiochemical separation. On the other hand, determination of ^{90}Sr in environmental and biological samples for radiometric measurements is a challenging task because it is a pure beta emitter and needs to be separated from other beta emitting radionuclides in the sample of interest, particularly from its beta-emitting daughter-isotope, ^{90}Y. The common radiometric methods to determine ^{90}Sr are gas ionization detectors or liquid scintillation counting (LSC), for which both methods involve counting the decay rate of ^{90}Sr or its daughter, ^{90}Y, directly in order to quantitate ^{90}Sr (Karacan, 2011; Manjón et al., 1997). Because environmental samples contain radionuclides that may interfere with ^{90}Sr, accurate beta counting requires separation of interfering radionuclides followed by ingrowth of ^{90}Y until secular equilibrium between ^{90}Sr and ^{90}Y is reached (~2 weeks) (Taylor et al., 2006). ^{90}Sr emits beta particles with energies up to 0.546 MeV. Compared to its parent, the daughter nuclide ^{90}Y emits more energetic electrons (E_{max}=2.28 MeV). Therefore, ^{90}Y is more suited for radiochemical detection techniques to determine ^{90}Sr activity concentrations after it reaches secular equilibrium with ^{90}Sr (Feuerstein et al., 2008). To achieve acceptable precision, sufficiently long counting times are essential, resulting in long analysis time. If the concentration of ^{90}Sr is low in the sample, preconcentration from a large sample is required. In conclusion, the sample preparation needed for radiometric determination are very often time-consuming, where sample separation and ingrowth time may take weeks. However, limit of detection (LOD) around 10 to 100 mBq/sample can be achieved in environmental samples with these methods (Hou & Roos, 2008; Taylor et al., 2006; Tovedal et al., 2008; Vajda & Kim, 2010).

1.3.2 Mass spectrometric techniques

Instead of determining ^{90}Sr in environmental and biological samples by its characteristic beta radiation, one can also use mass spectrometric techniques, e.g., resonance ionization mass spectrometry (RIMS), thermal ionization mass spectrometry (TIMS), accelerator mass spectrometry (AMS) and inductively coupled plasma mass spectrometry (ICP-MS) (Bu et al., 2016). Mass spectrometry (MS) methods are not affected by the presence of other beta-emitting radionuclides from the sample, because ^{90}Sr is determined by measuring the mass-to-charge ratio (*m/z*) of ^{90}Sr ions (Skoog et al., 2018). Thus, samples can be measured directly, or

following separation of Sr from interfering elements. Mass spectrometry is characterized by a high sensitivity and low detection limit, including relatively shorter sample preparation and analysis time compared to radiometric methods (Bu et al., 2016; Hou & Roos, 2008). The limit of detection achievable by either mass spectrometry and radiometric methods depends on the specific activity of the radionuclide and there are often low detection limits for long lived radionuclides. In radiometric methods the detection limit is determined by the total Bq in the sample, while in mass spectrometry it is the concentration (g/L). Hence, improvement of detection limits by concentrating the samples applies to both methods. When it comes to the determination of ^{90}Sr, ICP-MS is a more desirable instrument to use compared to other MS techniques because of its high sensitivity, easy operation, relatively low cost, and its availability (Bu et al., 2016; Feuerstein et al., 2008).

1.4 Chemical separation of Sr

Chemical separation of Sr to obtain a homogenous sample solution and separate Sr from matrix and interfering elements are required during radiometric methods. However, separation techniques are often applied prior to MS analysis as well. Additionally, many MS methods, such as ICP-MS, have the ability to remove interferences during analysis by, e.g., using a collision-reaction cell (CRC). For separation of strontium, different separation procedures can be applied, such as ion exchange chromatography and extraction chromatography (Vajda & Kim, 2010). However, due to high volume samples, large separation columns (>2 mL) and relatively high volumes to rinse and elute the columns with are often required to ensure efficient separation (Gaca et al., 2006; Taylor et al., 2006; Tovedal et al., 2008). A method to determine ^{90}Sr in ashed fish bone has been under development at NMBU, by performing extraction chromatography with Sr-resin and instrument-based separation in a triple quadrupole ICP-MS (ICP-QQQ), using a mixture of O_2 and H_2 inside the CRC. This removes isobaric ^{90}Zr interference in the samples and achieving a quantification limit of 3 pg/L (~1 Bq/g) for ^{90}Sr (Reinoso-Maset et al., 2021).

1.4.1 Extraction chromatography with Sr-Resin

Extraction chromatography (EXC) is a technique performed on a packed resin column for sample clean-up and extraction of a variety of metal ions from a broad-spectrum of sample types. Using EXC with Sr-resin, provides a simple and effective method for separation and preconcentration of strontium from nitric acid media. Additionally, the resin allows strontium

to be isolated from large amounts of calcium and other interfering elements, such as zirconium (Horwitz et al., 1991). The three major components of an EXC system consist of an inert support, a stationary phase, and a mobile phase. The two Sr-resins used in this project (Eichrom and Triskem) both contain 4'4(5')-di-t-butylcyclohexano-18-crown-6 (crown ether) dissolved in 1-octanol as the stationary phase, which is sorbed on an inert substrate. For Sr-resin, nitric acid solution is used as the mobile phase.

Strontium is extracted from nitric acid and sorbed onto the crown ether when the acid concentration is equal or greater than 1M. By washing the crown ether with either water or 0.05M nitric acid, the extracted strontium will easily desorb from the crown ether (Horwitz et al., 1992). The extraction equilibrium for this behaviour is assumed to be:

Eq. 1 $$Sr^{2+} + 2NO_3^- + DtBuCH18C6_{resin} \leftrightarrow Sr(NO_3)_2(DtBuCH18C6)_{resin}$$

Dietz and Jensen (Dietz & Jensen, 2004) have used extended X-ray absorption fine-structure (EXAFS) to investigate the coordination environment of the structure $Sr(NO_3)_2$(DtBuCH18C6) complex. The complex structure, shown in Figure 1.1, illustrates that the strontium cation resides in the centre of the crown ether ring and the nitrate anions are bonded to the strontium as bidentate ligands.

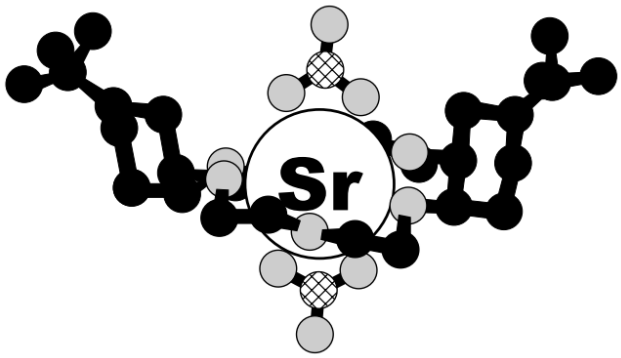

Figure 1.1: Schematic structure of the complex $Sr(NO_3)_2$(DtBuCH18C6) sorbed onto the resin. Carbon atoms are shown in black, oxygen atoms in white and nitrogen atoms in white with crosshatching. Figure is obtained from (Dietz & Jensen, 2004).

As shown in Figure 1.2, the Sr-resin's affinity for strontium increases with increasing nitric acid concentration (Eichrom Technologies LLC., s.a.). Strontium reaches a maximum retention factor, k'_{Sr}, of 90 between 3 and 8M HNO_3. At 0.05M HNO_3 and lower, the affinity for strontium decreases and the k'-value falls to less than 1. The retention factor, k', is defined as the ratio of the dissolved component in both the stationary phase and the mobile phase, and the factor will increase when more of the component is sorbed on the stationary phase.

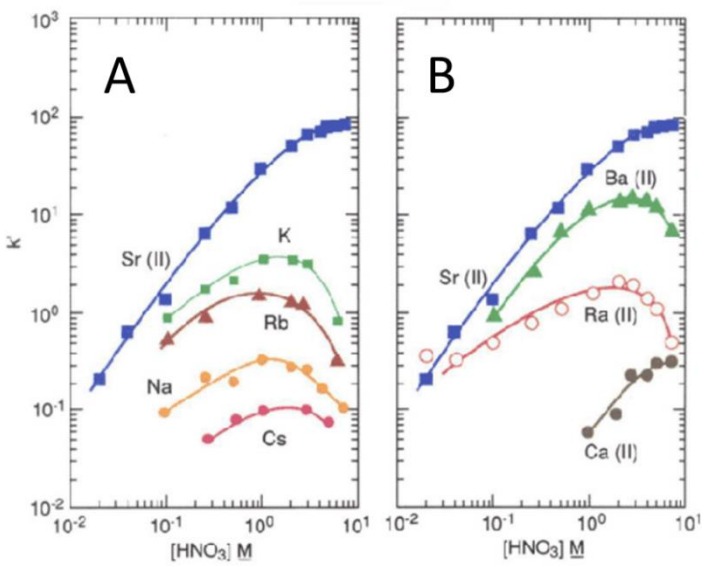

Figure 1.2: Graphs showing k' values of strontium, (A) alkali metals and (B) alkaline earth metals sorbed onto Sr-resin with increasing nitric acid concentration. Figure is obtained from (Eichrom Technologies LLC., s.a.).

Compared to strontium, the uptake of alkali and alkaline earth metals by Sr-resin is much lower across the concentration range of nitric acid (Eichrom Technologies LLC., s.a.). Calcium has the lowest uptake on the resin among the alkaline earth metals, which makes it relatively easy to separate strontium from calcium. Barium uptake peaks at 3M HNO_3, showing a greater affinity for the resin than Ca and Ra. However, by loading Sr on the resin from 8M HNO_3, barium uptake will be sufficiently low. With an adequate rinse afterwards, barium will be washed from the column.

Other elements such as Pb and actinides show significant retention on the Sr-resin. The radionuclide ^{210}Pb and its daughter products ^{210}Bi and ^{210}Po emit beta and alpha particles, respectively. When these radionuclides are present, an additional clean-up step is necessary to prevent these nuclides obscuring the ^{90}Sr signal when performing LSC (Gaca et al., 2006). By adding competitive complexing agents, these elements will lose affinity towards the resin and prevent retention (Eichrom Technologies LLC., s.a.). However, when using mass spectrometry, decontamination of these elements is unnecessary due to the measuring of *m/z* and the removal of remaining ions in the samples during ICP-MS analysis of ^{90}Sr.

Strontium may be recovered from the resin using deionized water. However, it is recommended using 0.05M HNO_3 for a consistent high recovery of Sr when eluting Sr from Sr-resin (Eichrom Technologies LLC., s.a.).

1.5 Radionuclide transfer and impact on wildlife

In the early period after the Chernobyl accident, the local area around the ChNPP were essentially exposed to acute radiation, causing numerous adverse effects in biota located within a few kilometres from the reactor source. By dry deposition, large quantities of radioactive isotopes from the volatile radioactive plume deposited on to plant and ground surfaces, which resulted in accumulation of large doses that affected biota (IAEA, 2006). The deposited contamination on the forest canopy caused increased mortality of coniferous forest stands, resulting in the so-called "red forest", which did not restore its viability until five years later (Arkhipov et al., 1994). Deciduous trees, however, were more resistant to the ionizing radiation, though early loss of leaves and damage to their branches was observed (Beresford et al., 2016).

The acute radiation caused also severe effects to animals, including invertebrates. Within two months, a decrease of small pine-litter fauna populations was observed, causing mortality of eggs and early life stages, in addition to reproductive failure in adults (Krivolutzkii & Pokarzhevskii, 1992). This was a consequence of the short-lived and highly radioactive isotopes which were transferred from forest canopy to soil and accumulated in litter, exposing invertebrates with high energy beta radiation (IAEA, 2006). The population numbers slowly recovered after the first year after the accident and increased primarily due to migration of invertebrates from the surrounding areas into the contaminated area (Krivolutzkii & Pokarzhevskii, 1992).

The impact acute radiation had on biota and the severe effects it caused during the early period after the Chernobyl accident are apparent compared to the chronic low doses of radiation the non-human biota are exposed to today. Now, more than 30 years after the accident, there are studies showing contradictory results and there is no consensus on the effects of long term chronic low dose radiation and its significance on wildlife (Beresford et al., 2020b). In a study by Møller & Mousseau (2009), a decrease in the abundance of different arthropod populations with increasing radiation, at dose rates below 1 $\mu Gy\ h^{-1}$, was observed. Although these dose rates are considered to be in the range of natural background exposures (Beresford et al., 2008). Additionally, there are studies with contrasting results about radiation effects on ecosystem processes such as decomposition. A study reported a decrease in decomposition rate of litter with increasing radiation (Mousseau et al., 2014), while a second study reported increased litter mass loss with increasing radiation (Bonzom et al., 2016).

In a forest ecosystem, ^{137}Cs and ^{90}Sr can be taken up by plants by the same mechanisms as their chemical analogues and plant nutrients K and Ca, respectively (Yoschenko et al., 2019). From there, the radionuclides can be transferred to organisms, such as arthropods, by the plant-based food chain and the detritus-based food chain. In the plant-based food chain, herbivore arthropods accumulate radionuclides by grazing on plants, which in turn are eaten by carnivores. In the detritus-based food chain, radionuclides accumulated in the leaf litter are eaten by detritivore arthropods and subsequently eaten by carnivores (Ishii et al., 2017). It has been observed over the years since the Chernobyl accident, that ^{137}Cs and ^{90}Sr uptake in vegetation presently exceeds the downward migration in soils, which indicates that ^{137}Cs and ^{90}Sr are still involved in the biological cycle in forest ecosystems within the ChEZ. ^{137}Cs is reportedly taken up actively in fungi, while ^{90}Sr is mainly accumulated in the arboreal vegetation (Shcheglov et al., 2014). Similarly, observations in forests of South Germany indicated high ^{90}Sr soil-to-plant transfer, leading to a constant supply of ^{90}Sr accumulated litter due to leaf turnover. Whereas, ^{137}Cs had lower soil-to-plant uptake compared to ^{90}Sr, but higher accumulation in fungi (Bruchertseifer et al., 2002). Overall, the studies indicate a high potential of radionuclide transfer through the food chain.

1.6 Arthropods

Arthropods are invertebrate animals, which includes classes such as insects and spiders. They consist of species with high diversity when it comes to e.g., their appearance, preferred diet, and way of living. Furthermore, arthropods perform essential ecosystem processes, such as decomposition (Galante & Marcos-Garcia, 2008), plant seed dispersal (Warren & Giladi, 2014) and pollination (Garibaldi et al., 2013). Lastly, they are an important food source for many organisms and, thus, constitutes a major route of radionuclide transfer to organisms in higher trophic levels, such as fish, small mammals, and birds and further up in the food chain (Ishii et al., 2017).

1.6.1 Previous studies on the determination of ^{137}Cs and ^{90}Sr in arthropods

Few studies have previously investigated the determination of radionuclides in arthropods, however, more research on ^{137}Cs has been conducted compared to ^{90}Sr. Common for these studies are the determination of radionuclides in samples consisting of numerous arthropod individuals, and not the determination on an individual level. The only papers measuring ^{90}Sr in arthropods used either LSC (Dragović et al., 2010; Mietelski et al., 2010) or beta

spectrometry (Beresford et al., 2020a), where sample preparation and analysis time during the LSC methods took weeks and hours, respectively.

Several articles reports high accumulation of ^{137}Cs and ^{90}Sr in invertebrates that feeds on litter and fungi (Copplestone et al., 1999; Ishii et al., 2017), especially in millipedes (Mietelski et al., 2010; Renkas, 2019), indicating that contaminated litter and other forest components are important sources for transfer of radionuclides into detritivore species. In general, the determined activity concentrations of ^{137}Cs and ^{90}Sr are relatively similar in studies from the Chernobyl area that measure both isotopes, however, ^{137}Cs tends to be higher (Beresford et al., 2020a; Dragović et al., 2010).

1.6.2 **Challenges in measuring ^{90}Sr in Chernobyl arthropods**

The challenge in measuring arthropods is a small mass combined with varying ^{90}Sr concentrations. Arthropods come in a variety of sizes, and some are extremely small, with a mass ranging from a few micrograms to hundred milligrams. This will lead to small volume samples.

Since detection limits will be improved if the sample can be concentrated into a small volume for ICP-MS measurement, a solution is to develop a chromatographic “microcolumn” designed for extraction chromatography (EXC) with the application of a Sr-selective resin. With this separation method, all ions except strontium are removed and both stable and radioactive Sr isotopes extracted from small sample volumes.

1.7 Aims of thesis

^{90}Sr is shown to be a biological important radionuclide because of its long half-life of 29 years and by being a chemical analogue of Ca. There is a knowledge gap on ecosystem transfer of ^{90}Sr and effects on arthropods. Information on ^{90}Sr levels in arthropods can contribute to knowledge on the impact of ecosystem function (herbivore, detritivore, omnivore, carnivore) on radionuclide transfer and cycling. The lack of a reliable and efficient method for determination of ^{90}Sr in arthropods is a challenge. Few studies have previously measured ^{90}Sr in arthropods, but only by radiometric methods and not on an individual level. Therefore, the aims of the thesis were:

- Development of a mass spectrometric method that could perform precise and rapid determination of ^{90}Sr accumulated in individual arthropods.
- Testing the method by measurement of ^{90}Sr in arthropods collected at the Chernobyl Exclusion Zone (ChEZ).
- Investigating the correlation between ^{90}Sr and stable Sr and Ca levels in arthropods from the ChEZ.
- Estimations of radiation dose rates absorbed by Chernobyl arthropods based on determined ^{90}Sr activity concentrations.

2 MATERIALS AND METHODS

2.1 Microcolumn resin separation

2.1.1 **Microcolumns**

When developing a microcolumn for the purpose of separating Sr in small volume samples, there are several things to keep in mind. Because ^{90}Sr needs to be measured in many individual arthropod samples, the microcolumns should be convenient to pack with Sr-resin and separation should be practical and time efficient.

In columns used for EXC, resin (the stationary phase) is contained between two filters to prevent resin leakage. With that in mind, a microcolumn was developed by connecting two syringe filters. The syringe filters used were Captiva Premium Syringe Filters by Agilent Technologies, Inc., that had a polypropylene housing and a 4 mm diameter polyethersulfone (PES) membrane filter with a 0.45 µm pore size (Figure 2.1A). When these two syringe filters are filled with resin and connected together, the resin is contained and will not leak. A 10 µL filter pipette tip, shown in Figure 2.1B, was connected to the end of the column to reduce the droplet size. Instead of using gravitational force to push the liquid through the column, the column was loaded from a 1 mL syringe, which fits perfectly to the syringe filter. The syringe was used to push the liquid through the column at a steady rate, by a constant low pressure on the syringe plunger. This resulted in a steady flow of droplets out of the column, at a rate of 0.2 mL/min.

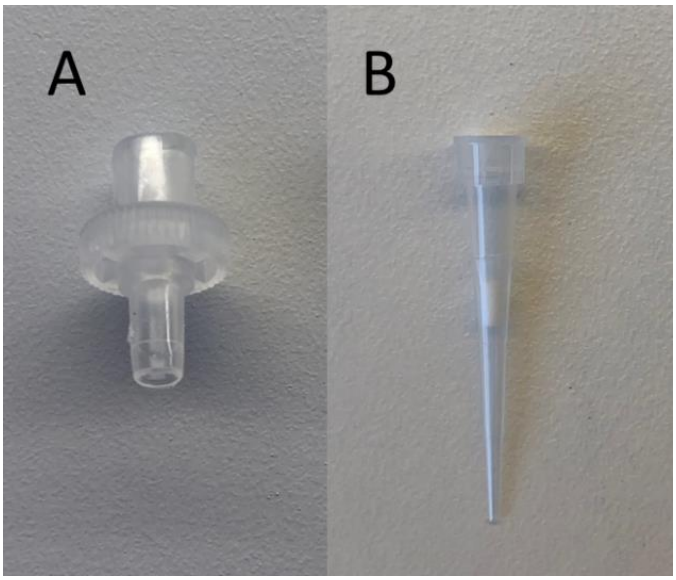

Figure 2.1: Microcolumn components, (A) Captiva Premium Syringe Filter (Agilent Technologies, Inc.) and (B) 10 µL filter pipette tip.

The microcolumn had a volume of ~150 µL, containing around 55 mg of commercial Sr-resin® from Eichrom Technologies, LLC., with a particle size of 50-100 µm. This first microcolumn was called model A (Figure 2.2A).

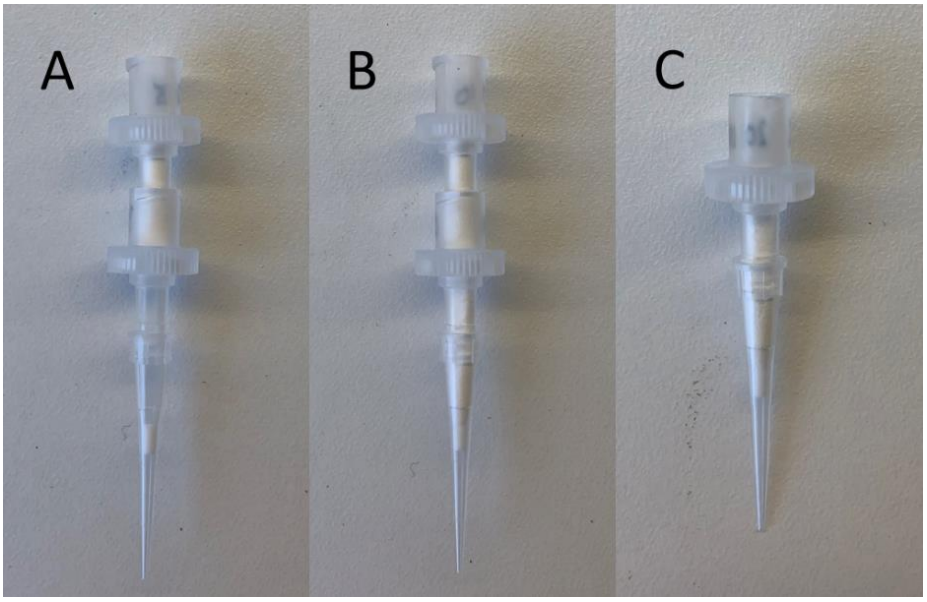

Figure 2.2: Different models of developed microcolumns, (A) model A, (B) model B and (C) model C.

2.1.2 **Microcolumn test standard**

As a substitute for arthropods, a standard was prepared using the stable Sr isotope, ^{84}Sr, for the method development. The standard consisted of 30 µg/L ^{84}Sr (78.3% ^{84}Sr) in 8M HNO_3 to which different elements that can potentially interfere with strontium during analysis were added. The ^{84}Sr solution is traceable to a certified isotope-enriched product (Neonest AB, 2019). The interfering elements and their final concentrations in the standard were 5 mg/L Na, 5 mg/L Mg, 20 mg/L K, 20 mg/L Ca, and 20 µg/L Zr. The enriched stable ^{84}Sr isotope was used as analyte because of its low relative natural abundance of (0.56%), to reduce the risk of Sr contamination during the method testing. The standard was prepared in 8M HNO_3 since this is the recommended sample load conditions (table 2.2).

2.1.3 **Microcolumn tests**

During the development, many tests were conducted to reach the final method. The tests which had the main contribution to positive progress have been included and presented below. Table 2.1 shows an overview of the column parameters for these tests.

Table 2.1: Overview of the different column parameters for each microcolumn test.

Test no.	Column model	Volume (µL)	Resin d.w. (mg)	Sr-resin	Resin particle size (µm)	P/L/R*
#1	A	150	52 - 55	Eichrom (E)	50-100	3/8/3
#2	A	150	57	Eichrom (E)	50-100	8/8/8
#3	A	150	150	Triskem (T)	100-150	8/8/8
	B	280	100			
Final	C	146	42-50	Triskem (T)	100-150	8/8/8

*****Precondition/Load/Rinse, 3M/8M/3M or 8M/8M/8M.

2.1.3.1 Initial tests of microcolumns – Eichrom resin

In test #1, three resin column replicates were preconditioned with 3M HNO_3 to make the resin available and ready for uptake of ^{84}Sr. All steps (table 2.2), except preconditioning, were collected in 2 mL Eppendorf tubes in case the elution of strontium occurred before the last step. The ^{84}Sr standard was loaded onto the columns and each column was rinsed with 3M HNO_3. The rinse was collected in four tubes to examine adequate rinse volume. The volume was roughly the same in each. Strontium was eluted from the resin with 2 x 0.6 mL 0.05M HNO_3 and collected in two Eppendorf tubes, to see how much volume was needed to elute strontium from the resin. See details on each step of the column separation in Table 2.2.

Table 2.2: Column separation details for test #1.

Column separation details		
Steps	**HNO_3 concentration**	**Volume (mL)**
Preconditioning	3M	1.5
Sample load	8M	0.5
Rinse	3M	1.5
Elution	0.05M	1.2
Samples were prepared in 8M HNO_3 to prevent barium from binding to resin if it was present in the sample.		

In order to have the same acid concentration and volume in the samples, the samples were evaporated to dryness in an oven at 90 °C. Subsequently, the samples were diluted and redissolved in 2 mL, consisting of 2 µg/L In and 5% (V/V) HNO_3. Indium was used as an internal standard (IS) for strontium (see section 2.4.4 for further details). Concentrations of ^{84}Sr and the interfering elements in the samples were determined on an Agilent 8800 ICP-QQQ (Agilent Technologies, Inc.).

The results acquired during analysis show the total recovery of ^{84}Sr was close to 100%, which means all of the strontium sent through the resin columns was recovered. However, only

1.8-5.6% of the total ^{84}Sr was eluted from the resin in the elution step, while the majority of the Sr (76-93%) was washed out during the rinse stage. Strontium was supposed to elute in the elution step and not before. Although, compared to interfering elements, strontium was retained in the resin during the load phase, both strontium and interfering elements were easily washed out during the rinse.

To check if the difference in molarity between the sample load concentration (8M HNO_3) and the rinse concentration (3M HNO_3) caused strontium to elute earlier from the resin, rinse concentration was replaced with 8M HNO_3 during test #2. The resin in prepacked columns from Triskem International, is packed with deionized water. To see if water had any effect on the dry Sr-resin from Eichrom, column 1 to 3 were loaded with deionized water to make the resin bed wet before separation. The fourth column was not loaded with water to see if it had the same effect without. Acid concentration for preconditioning was changed to 8M, because the columns should be conditioned with the same concentration as the sample load. The rinse was also changed to 8M to make sure that interfering elements were removed from the resin. An overview of concentrations and volumes used in each step regarding the column separation for test #2 can be seen in Table 2.3.

Table 2.3: Column separation details for test #2.

Column separation details		
Steps	**HNO_3 concentration**	**Volume (mL)**
Preconditioning	8M	0.8
Sample load	8M	0.5
Rinse	8M	0.8
Elution	0.05M	1
Resin bed in column 1 to 3 was solved with deionized water.		

As shown in Figure 2.3, the resin columns loaded with deionized water were able to recover 20-29% of the strontium in the sample during elution, while the fourth column without deionized water eluted 22%. Even though the result from each column was very similar, too much of strontium was eluted in the rinse rather in the elution step.

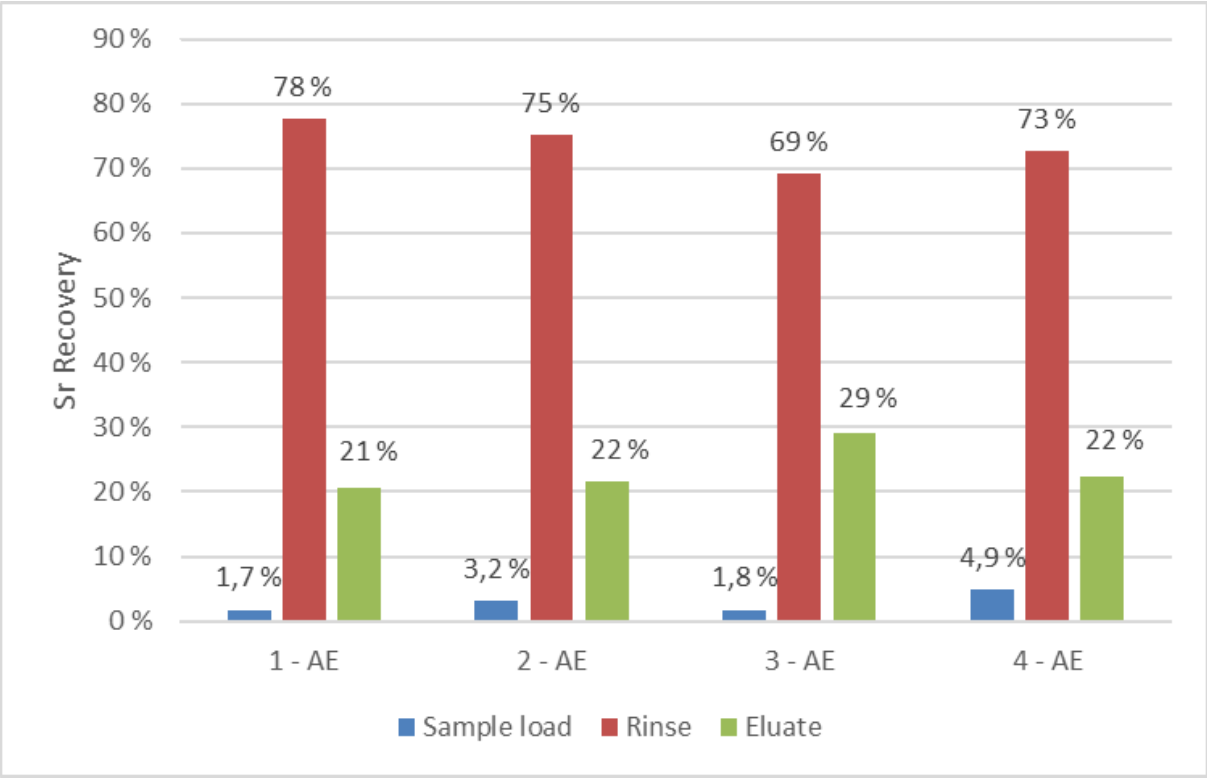

Figure 2.3: Strontium recovery from each separation step after EXC with four replicates of model A columns, packed with Eichrom resin.

2.1.3.2 Further development of columns – Triskem resin

The resin's weak ability to retain strontium, led to a suspicion that the resin was not working properly or that it had expired. Therefore, Sr-resin from prepacked 2 mL columns from Triskem International (SR-C50-A, Triskem International), was used instead of the Eichrom resin. This resin had a bigger particle size of 100-150 µm. The prepacked columns were packed with deionized water to keep the resin from drying out. Resin and the deionized water were mixed into a slurry and removed from the prepacked column and into a plastic tube. Using a syringe and a short plastic tube made it easy to pack the resin slurry into the microcolumn. A new model of the column was developed, model B, which was packed with resin between two syringe filters and between the lower syringe filter and the 10 µL filter pipette tip. This column had an inner volume of ~280 µL and could contain around 100 mg resin (Figure 2.2B).

For test #3, one column model A and two model B columns were packed with resin from Triskem International. The concentrations and volumes used during the separations can be found in Table 2.4.

Table 2.4: Column separation details for test #3.

Column separation details		
Steps	**HNO_3 concentration**	**Volume (mL)**
Preconditioning	8M	0.8
Sample load	8M	0.5
Rinse	8M	0.8
Elution	0.05M	1

The resin from Triskem retained strontium quite strongly compared to the one from Eichrom. In fact, as shown in Figure 2.4, almost no strontium was eluted during the sample load and rinse. Strontium was eluted where it was supposed to, however, not all the strontium was eluted during elution, only 59-81%. Because of the incomplete elution, the known standard concentration of ^{84}Sr was set as 100%. The difference in recovery between the two column models were not substantial. During a rerun of the test, a considerable amount of strontium was still retained in the column when a larger elution volume was applied to the columns. The length of the column, and perhaps the difference in diameter between the top and the bottom of the syringe filters, may have caused a stronger retention of strontium. Poor recovery of strontium led to the column being modified.

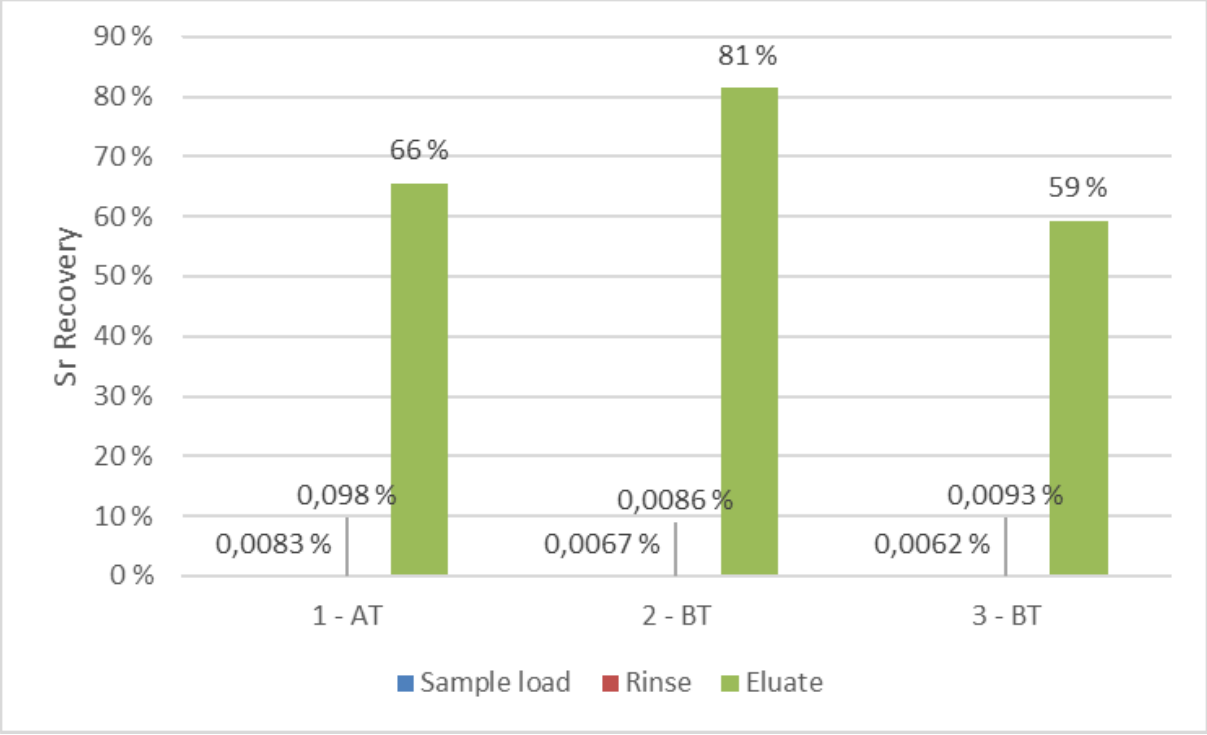

Figure 2.4: Strontium recovery from each separation step after EXC. Performed with one replicate of model A and two replicates of model B, packed with Triskem resin.

2.1.3.3 Final modification and test

The final modification of the microcolumn, model C, consisted of one syringe filter connected to a 10 µL filter pipette tip (Figure 2.2C). This column had an inner volume of ~146 µL and could contain around 50 mg of dry Sr-resin. Three replicates of column model C were used in the final test and Table 2.5 presents the column separation details used during this separation.

Table 2.5: Column separation details for the final test.

Column separation details		
Steps	**HNO_3 concentration**	**Volume (mL)**
Preconditioning	8M	1
Sample load	8M	0.5
Rinse	8M	1.5
Elution	0.05M	1.5

These columns were able to elute 98.7 ± 0.7% of strontium in the elution step, that was originally loaded onto the columns (Figure 2.5). The interfering elements were easily eluted from the resin during rinse.

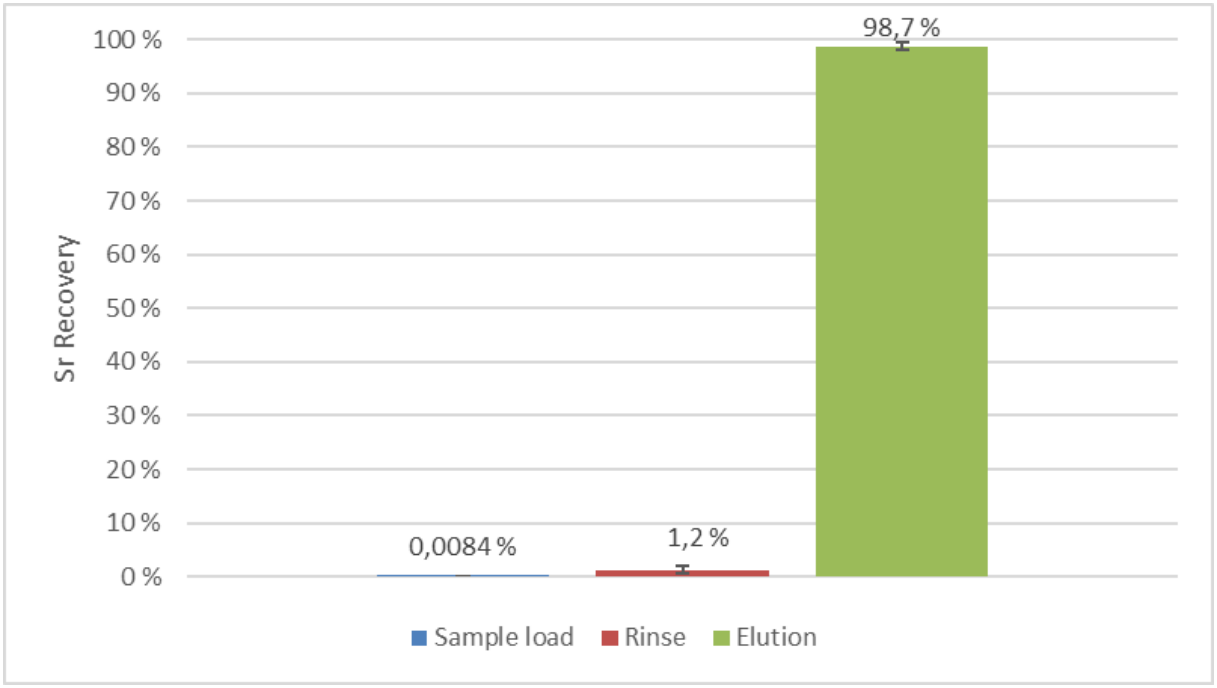

Figure 2.5: Average and standard deviation of strontium recovery from each separation step after EXC with three replicates of model C columns, packed with Triskem resin.

Column model C was used further to see when the resin begins to lose affinity for strontium and how much volume is required to elute all of the strontium with 0.05M HNO_3. In the elution step, 97% of strontium from the sample load was recovered. As shown in Figure 2.6, strontium begins to elute after 0.3 mL and most of it has been eluted after 1 mL 0.05M HNO_3. Therefore, 1 mL elution volume should be enough to elute strontium from the resin in the final method.

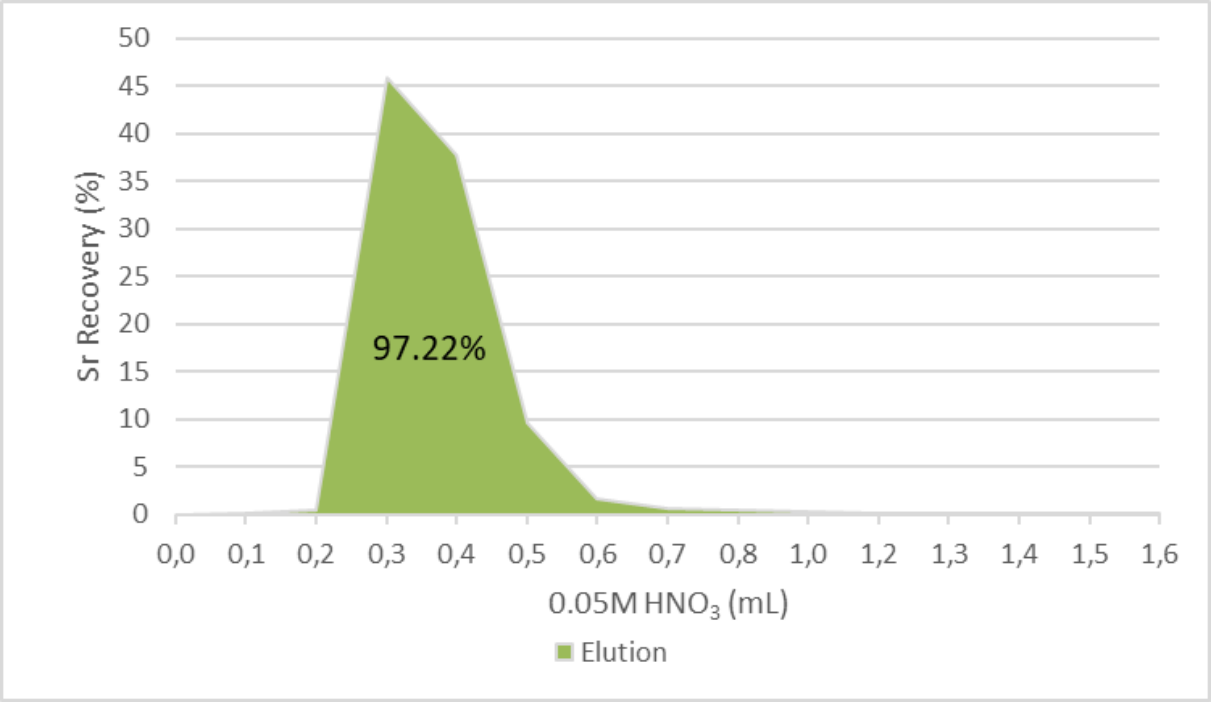

Figure 2.6: Elution of strontium with increasing volume of 0.05M HNO_3.

2.1.3.4 Test of Sr separation for fly samples

The method was tested on microwave acid digested flies, collected in Norway, to see how well strontium separation worked on high-matrix samples. To give an indication of natural levels of strontium, calcium, and zirconium, an aliquot (1/6) of the digested samples did not undergo separation and went straight to ICP-MS. This also enabled the use of natural strontium as yield monitor: by measuring strontium before and after separation, the recovery of strontium could be calculated. Natural strontium can be used as a yield monitor due to the same properties as ^{90}Sr. The recovery of Sr is used to correct the low ^{90}Sr levels in ^{90}Sr contaminated arthropod samples, and in addition, stable strontium levels in the samples can be obtained.

Nine flies were dried and weighed, and each put in a 3-trifluoromethyl-4-nitrophenol (TFM) vial together with 0.6 mL 8M HNO_3 with added Rh. Rhodium was used as an IS for calcium

and strontium. A Milestone UltraWAVE ECR (Sorisole (BG), Italy) was used to digest samples at 260 °C for 20 min (see section 2.3 for further details). After digestion, the samples were transferred from the TFM vials and into Eppendorf tubes with deionized water, and then evaporated to dryness. Subsequently, the samples were redissolved in 0.6 mL 8M HNO_3 before they were split into two aliquots: 0.1 mL of the sample to be directly measured on ICP-MS for determination of natural levels of Sr, Ca and Zr, and 0.5 mL of the sample to undergo Sr extraction.

A separation microcolumn was prepared for each arthropod. Rinse volume was set to a total of 1 mL to see when the matrix and interferences was being washed out of the resin. Elution volume was set to a total of 1.5 mL to make sure most of the strontium was eluted from the resin. More information about preconditioning and sample load is presented in Table 2.6.

Table 2.6: Column separation details for test with flies.

Column separation details		
Steps	**Acid concentration**	**Volume (mL)**
Preconditioning	8M HNO_3	1
Sample load	8M HNO_3	0.5
Rinse	8M HNO_3	1
Elution	0.05M HNO_3	1.5

After separation, the samples were evaporated and redissolved in 0.5 mL, consisting of In and 5% (V/V) HNO_3. The unseparated samples were added In as IS and diluted from 0.1 mL to 0.5 mL to avoid potentially high concentrations of Ca. Both unseparated and separated samples from each fly was analysed on the ICP-MS. The samples were analysed for the natural abundant isotopes of strontium, ^{84}Sr and ^{86}Sr, to check if the levels for these two isotopes were similar. The natural strontium isotope, ^{88}Sr, was not analysed to avoid potentially high counts per second (CPS) levels in the detector.

Most of the Ca and Zr in the samples were washed out after 0.5 mL rinse. Little strontium was washed out after the first rinse of 0.5 mL but began to detach from the resin when another portion of 0.5 mL 8M HNO_3 was added. Therefore, 0.5 mL should be enough to rinse the column for interferences and yet avoid release of strontium. Elution volume of 1 mL 0.05M HNO_3 should be enough to elute strontium in high-matrix samples instead of 1.5 mL. The difference in recovery between 1 mL and 1.5 mL elution volume was not significant. Final concentrations of unseparated samples had acceptable levels of Ca when diluted to 0.5 mL.

The levels of ^{84}Sr and ^{86}Sr in the arthropods were similar. Considering that ^{84}Sr has a lower relative natural abundance (0.56%) than ^{86}Sr (9.86%) and ^{88}Sr (82.58%), it was decided to use ^{84}Sr as yield monitor to measure natural strontium and to correct the low ^{90}Sr levels in the final method. The use of ^{84}Sr will avoid high CPS-values in the detector because of its low abundance. In the final method, the levels of stable Sr of the analysed samples were about 20 mg/kg.

2.2 In-house arthropod standard and control arthropods

It is important when choosing control samples and a reference material, that they contain the same analyte and matrix as the samples to be analysed. Because there are no certified reference materials (CRM) for arthropods available, an in-house standard was made, consisting of different types of arthropods. An in-house standard is used to evaluate the method's precision and accuracy and control the calibration on the instrument. Various arthropods collected in Norway were used as control arthropods and to produce an in-house standard for the Chernobyl arthropods.

In the final method, each individual arthropod was to be dissolved in 0.6 mL 8M HNO_3 before separation and direct measurements of strontium, zirconium, and calcium levels. The equivalent volume for the in-house standard was 15 mL, representing 25 arthropods. Because arthropods show a wide variation in mass, a theoretical mass of 0.010 g per arthropod was set, 0.25 g in total. 16 small flies, 2 medium sized flies, 2 big flies, 3 wasps, 14 green lacewings and 3 deer flies were combined to give a total mass of 0.2519 g. The arthropods were dried at 35-40 °C for 72 h. Then they were added into a TFM vial together with 7.5 mL concentrated HNO_3 and, subsequently, microwave acid digested. After digestion, the standard was spiked with ^{90}Sr and diluted to 15 mL. This gave a final concentration of spiked ^{90}Sr and an acid concentration of 144.2 pg/L in 8M HNO_3. The ^{90}Sr spike is traceable to a certified source solution (Eckert & Ziegler, 2017).

Individual arthropods from Norway were used as control samples, to calculate limit of detection and quantification (LOD & LOQ) for ^{90}Sr. The control samples have the same interferences as the Chernobyl arthropods, but with non-detectable amounts of ^{90}Sr. The arthropods were dried at 35-40 °C in 72 h prior to digestion.

2.3 Microwave acid digestion

Milestone UltraWAVE ECR (Sorisole (BG), Italy) is a closed vessel microwave digestion system and was utilized for dissolution of all arthropod samples. The microwave digestion technique involves the use of acid digestion and is often used to digest solid samples before determining trace elements with a variety of spectrometric techniques, including ICP-MS (Lohne & Jensen, 2019). If the solid sample contains organic material, the carbon will be oxidized into CO_2-gas during the digestion. An oxidation reaction such as this needs a strong oxidizing acid. Nitric acid (HNO_3) is a good choice of acid, because it is a strong oxidizing agent that oxidizes well in high temperatures and causes few interferences. When carbon is oxidized by nitric acid, the acid will be reduced to nitrogen oxides (NO_x):

Eq. 2 $$[CH_2]_{n\,(s)} + NO_{3\,(aq)}^{-} \rightarrow CO_{2\,(g)} + NO_{x\,(g)}$$

This technique provides good precision, because the samples receives the same temperature and pressure when lowered into a load in the reaction chamber. Good accuracy is achieved, because there is little or no organic residue left in the samples after digestion. The vials are made of 3-trifluoromethyl-4-nitrophenol (TFM) and can endure temperatures up to 300°C. Samples are transferred to TFM vials and added nitric acid, and the vials are placed in a rack that are lowered into a load in the reaction chamber. The load contains water and nitric acid. The chamber is pressurized with an inert gas, e.g., N_2, that prevents boiling of the solutions and cross contamination. Microwaves are introduced into the chamber and a magnetic field is formed. Ions from the dissociated acid and water molecules will move along the magnetic field inside the chamber and sample vials. Dipole rotation and ionic migration from the water molecules and the ions, respectively, will cause friction and a rise in temperature (Lohne & Jensen, 2019).

The nitrogen oxide gas formed when organic material oxidizes will take up more space than the ions, which will cause the pressure to increase. To prevent difference in pressure between the TFM vials and the chamber, the vial caps have small holes to equalize the pressure, allowing the nitrogen oxide to move freely between vials and chamber.

2.4 Inductively Coupled Plasma Mass Spectrometry – ICP-MS

Inductively coupled plasma mass spectrometry is a quick and sensitive multi-element technique to determine concentrations of elements from g/L and down to pg/L. Because of its low detection limits, high degree of selectivity and good precision and accuracy, ICP-MS has become one of the most important techniques for elemental analysis (Skoog et al., 2018).

Using ICP-MS, ions are produced in an inductively coupled plasma and a mass spectrometer (usually a quadrupole mass analyser unit) separates the ions according to their mass-to-charge ratios (m/z) (Skoog et al., 2018). In commercial ICP-MS systems, three main types of mass spectrometers are used: quadrupole, time-of-flight, and magnetic sector (PerkinElmer Inc, 2011). An inductively coupled plasma quadrupole MS (ICP-QMS) is equipped with a collision-reaction cell (CRC) and a single quadrupole mass unit. Another instrument is the double-focusing sector field ICP-MS (ICP-SFMS) which uses a magnetic sector as a mass analyser unit instead of a quadrupole. The use of a magnetic sector achieves a higher mass resolution compared to the quadrupole. For this project, all samples were analysed using an Agilent 8900 ICP-QQQ (Agilent Technologies, Inc.), a triple quadrupole ICP-MS (ICP-QQQ) with two quadrupole mass units and a CRC. With the use of two quadrupole mass units for mass discrimination and by introducing a reaction gas into the CRC, will suppress interfering signals, improve transport efficiency, and lower the detection limit (Bu et al., 2016).

2.4.1 **Sample introduction – Micro autosampler and Apex Q**

Sample introduction was changed to permit uptake of small volume samples. A micro autosampler was made from a probe, a peek tubing, and a micro nebulizer. The peek tubing had an i.d. of 0.18 mm and an inner volume of 73 µL. A PFA concentric nebulizer (Savillex, LLC) with a free aspiration rate of 50 µL/min was used and connected to an Apex Q (Elemental Scientific, Inc.). Additionally, a peristaltic pump tubing with flared ends and two stops, coloured orange/blue was used (0.25 mm i.d.). The sample introduction had a sample flow of 107 µL/min, resulting in a total acquisition volume of 186 µL for each sample. If necessary, this volume could be reduced with ‘pre-emptive rinse’ enabled. ‘Pre-emptive rinse’ will move the probe to the rinse port at a pre-set time before sample acquisition has finished. The remaining sample in the uptake tubing will be used for the remaining data acquisition (Wilbur et al., 2006). With ‘pre-emptive rinse’ enabled, a sample volume of 100 µL would be sufficient.

Apex Q is a fully integrated quartz introduction system for ICP that is directly connected to the ICP torch injector, enhancing the sensitivity up to 10 times and efficiently transporting more than 90% of the sample analyte to the plasma (Elemental Scientific Inc., s.a.-a). Including an o-ring free quartz sample flow path designed for a rapid rinse-out, it features a heated cyclonic spray chamber and a Peltier-cooled desolvation system. The samples are taken up by the micro autosampler through the nebulizer and nebulized into the heated cyclonic spray chamber (140 °C) and vaporized completely. Subsequently, the Peltier-cooled desolvation system (2 °C) condenses and removes the solvent vapor, which reduces formation of oxides and increases the plasma robustness. The Apex has the possibility to add nitrogen as a second gas flow to be mixed with the sample aerosol stream together with argon. Nitrogen is introduced in the last loop of the Peltier-cooled condenser, and the nitrogen gas helps to increase signal intensity, improve stability, and reduce oxide formations (Elemental Scientific Inc., s.a.-b).

2.4.2 **Collision-reaction cell and gasses**

When working at ppq levels, it is important to know and remove interferences. Especially when the absolute amount of ^{90}Sr in arthropods is very low for ICP-MS analysis, which in this project ranged from 4 to 200 fg in the analysed Chernobyl arthropods. Mass spectrometry of ^{90}Sr has many interferences, but most importantly, an isobar from ^{90}Zr (Bu et al., 2016). The ^{90}Zr interference can cause overlap with the ^{90}Sr signal during ICP-MS analysis and samples with high matrix concentrations can increase the uncertainty of the ^{90}Sr determination due to ionization effects in the instrument. The levels of ^{90}Zr are usually quite low in biological samples, but nevertheless, much higher than ^{90}Sr.

The ICP-MS instrument, Agilent 8900 ICP-QQQ (Agilent Technologies, Inc.) is a triple quadrupole ICP-MS, comprised of two quadrupole unit mass filters on either side of an octopole CRC which makes it more efficient of overcoming isobaric interferences by applying a reaction gas. Since all the zirconium is not removed after EXC, the use of a reaction gas during ICP-QQQ analysis is necessary to remove the remaining zirconium in the samples. By adding a mixture of O_2 and H_2 as a reaction gas into the CRC, these two masses can be separated by the second quadrupole mass unit (Q2) (Reinoso-Maset et al., 2021).

In the CRC, oxygen is used to separate ^{90}Zr from ^{90}Sr. It has previously been shown that Zr^+ can be suppressed by oxidation with O_2, while Sr^+ will not to the same extent oxidize with O_2 because of low O-atom affinity (Bandura et al., 2001):

Eq. 3 $$Zr^+ + O_2 \leftrightarrow ZrO^+ + O \qquad \Delta H_r = -88.7\ kcal\ mol^{-1}$$

Eq. 4 $$Sr^+ + O_2 \leftrightarrow SrO^+ + O \qquad \Delta H_r = 47.8\ kcal\ mol^{-1}$$

The reaction enthalpies for the oxidations (Eq. 3 and 4) shows that the reaction between Zr and O is exothermic (negative) and preferred over the reaction between Sr and O, which is endothermic (not energetically favourable) (Bandura et al., 2001; Wang et al., 2021). This enables the separation between ^{90}Sr and ^{90}Zr in the CRC. By adding hydrogen to the CRC, will promote the reaction between Zr^+ and O_2 (Agilient Technologies, 2020).

To check all possible reactions when the O_2 and H_2 mix was present in the CRC, the ICP-QQQ was used to run a Product ion scan (PIS) for strontium and zirconium. The PIS showed that reaction with strontium and the reaction gas is endothermic, resulting in 96% of strontium being quantified as its original mass. The reaction with Zirconium is exothermic and reacted to $^{90}ZrH_xO_y^+$ with an efficiency close to 100%. Most of the zirconium reacted and was quantified with the following masses: 159 amu (16%), 174 amu (2%), 177 amu (67%) and 192 amu (9%). See Appendix A for results from the PIS for strontium and zirconium.

The ^{90}Sr and ^{90}Zr pathway and reaction inside the ICP-QQQ instrument is illustrated in Figure 2.10. The first quadrupole mass unit (Q1) removed all masses different from 90 amu, ^{90}Sr and ^{90}Zr passed through the mass unit and was sent further to the octopole CRC. The reaction gas reacts with ^{90}Zr and to produce clusters where the main product has a mass of 177 amu. The only possible combination of relevant elements with m/z = 177 is $^{90}Zr(OH)_3(H_2O)_2^+$. The second quadrupole (Q2), set on 90 m/z, filtered ^{90}Sr from the Zr clusters before being passed to the detector.

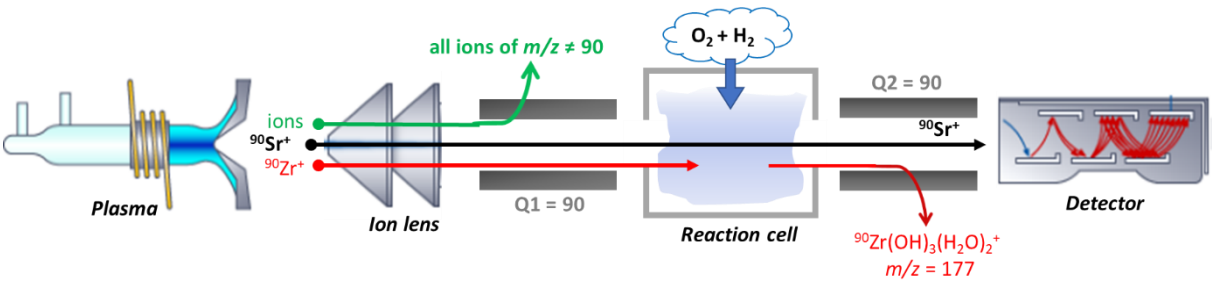

Figure 2.10: Schematic illustration of ^{90}Sr and ^{90}Zr ICP-QQQ pathway and reaction with O_2 and H_2 reaction gases inside the collision-reaction cell (Jensen, 2020).

The natural ^{84}Sr isotope was used both as a yield monitor for ^{90}Sr and determination of natural Sr levels in the samples (see section 2.1.3.4 for more details about the choice of the yield monitor). This Sr isotope has an isobaric interference from the noble gas, ^{84}Kr, which has a relative abundance of 56.99%. Because Kr (14.00 eV) has a higher ionization energy (IE) than O_2 (12.07 eV), but lower than H_2 (15.43 eV), potential krypton interference was easily removed by oxygen as a charge transfer reaction in the CRC during analysis.

2.4.3 **Instrumental settings for ICP-QQQ**

Instrumental settings for the ICP-QQQ analysis of ^{90}Sr in Chernobyl arthropods are given in Table 2.7. The monitored isotopes during the analysis were ^{44}Cs, ^{84}Sr, ^{90}Sr and ^{90}Zr. The Ca isotope was selected due to its relative natural abundance of 2.086% and to avoid potentially high CPS-values. This isotope reacted to $^{44}Ca^{16}O^{1}H^{+}$ in the CRC and monitored at Q2 with mass 61 amu. Both ^{84}Sr and ^{90}Sr was measured "on-mass". Because of its low relative natural abundance (0.56%), the ^{84}Sr isotope was selected to quantify natural Sr and to avoid potentially high CPS-values. Zirconium reacted to $^{90}Zr(OH)_3(H_2O)_2^{+}$ and was monitored at Q2 with mass 177 amu to observe its decontamination. During the analysis, seven replicates were measured for each sample and analysis time was ~3 min/sample.

The nebulizer gas flow was set to 1.05 L min^{-1} because this led to the best signal intensity. In the CRC, oxygen and hydrogen was used as reaction gas to separate ^{90}Zr interferences from ^{90}Sr. The combination of high flow of oxygen and hydrogen promote the reaction between Zr and the reaction gas, producing $^{90}Zr(OH)_3(H_2O)_2^{+}$. This equilibrium will be further promoted with Octopole bias. Negative voltage will contribute energy to the CRC, i.e., to the reaction, increasing the efficiency of the reaction. Reaction with Sr is endothermic and will not react to the same extent.

Axial acceleration is a focused voltage that extracts ions from the CRC. This is important when the gas density in the cell is high. Otherwise, the ions will be scattered after collisions with other gas molecules (scattering effects). Energy discrimination is a field between the CRC and Q2. When this field has a negative voltage, the ions will be extracted from the CRC into Q2.

Table 2.7: Instrument settings for ICP-QQQ analysis of ^{90}Sr in Chernobyl arthropods. Q denotes quadrupole mass filter.

Parameter	**Setting**	
Scan type	MS/MS	
Monitored mass pairs (Q1 → Q2):	44→61	($^{44}Ca^{+} \rightarrow {}^{44}Ca^{16}O^{1}H^{+}$)
	84 → 84	($^{84}Sr^{+} \rightarrow {}^{84}Sr^{+}$)
	90 → 90	($^{90}Sr^{+} \rightarrow {}^{90}Sr^{+}$)
	90 → 177	($^{90}Zr^{+} \rightarrow {}^{90}Zr(OH)_3(H_2O)_2^{+}$)
Integration time	0.5 s for Ca; 0.5 s for ^{84}Sr; 5 s for ^{90}Sr; 0.5 s for Zr	
Replicates	7	
RF Power	1600 W	
Sample depth plasma	8.0 mm	
Nebulizer gas flow	1.05 L min^{-1}	
Spray chamber temperature	140 °C	
Condenser temperature	2 °C	
Makeup gas flow	0.1 L min^{-1}	
Collision-reaction cell:		
O_2 flow rate	0.75 mL min^{-1}	
H_2 flow rate	10 mL min^{-1}	
Octopole bias	-5.5 V	
Axial acceleration	2 V	
Energy discrimination	-13.0 V	
Deflect lens	0.8 (optimized before every analysis)	

2.4.4 **Internal standards**

An internal standard (IS) is a chemical substance that is used to correct loss of analyte and matrix effects during sample preparation and analysis, respectively. Moreover, drift in the analytical instrument can be limited using an IS. The IS is added in known amounts to samples, blanks, and calibration standards. An internal standard should have similar but not identical properties as the analyte, e.g., atomic weight and ionization energy (IE). It is important that the internal standard is not present in significant concentrations in the samples. The corrections are calculated by the ratio of analyte response and internal standard response as a function of analyte concentration from the calibration standards. The use of IS requires a multi-element technique, e.g., ICP-MS.

In this thesis, indium was used as IS for strontium while rhodium was used as IS for both calcium and strontium. Their atomic weight and first IE, together with calcium and strontium, can be found in Table 2.8. The IE of In and Sr is quite similar, which makes it a suitable IS for Sr. Rhodium was selected as IS prior to microwave acid digestion instead of In due to the

uncertainty of whether In would follow Sr though the separation. Therefore, In was added after the chemical separation, and Rh was used to control for loss of Sr during digestion.

Table 2.8: Atomic weight and first ionization energy of the following elements: calcium, strontium, rhodium, and indium.

Element	Atomic Weight (amu)	First Ionization Energy (eV)
Calcium	40.08	6.11
Strontium	87.62	5.69
Rhodium	102.9	7.46
Indium	114.8	5.79

2.5 Final method

A schematic overview of the final method is given in Figure 2.7.

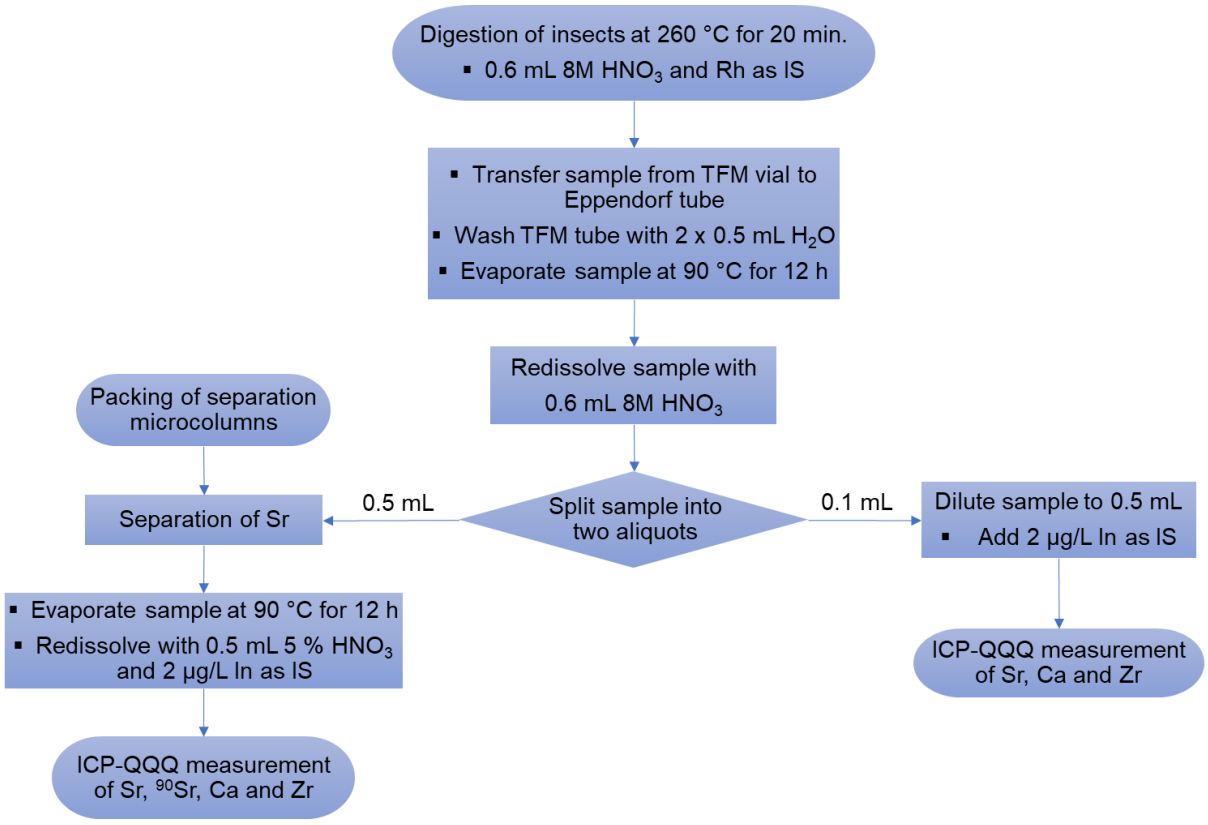

Figure 2.7: Flow chart of the final method.

2.5.1 **Collection of arthropod samples**

The arthropods used in this project and its pilot work (see Appendix D.1 for more details regarding the pilot study), were collected within the ChEZ in 2017 by Greg Lamarre and Emmanuel Lapied. A combination of pan and pitfall traps were applied for the collection of arthropods. Depending on the ambient air dose rate and soil contamination, the arthropods were divided into four levels: control, intermediate (7.3-8.3 µGy h^{-1}) , high (16.4-66.5 µGy h^{-1}), and very high (192 µGy h^{-1}). Arthropods collected at areas with intermediate (I1, I2), high (C1, C2, C3) and very high (C4) contamination has been used in this project. More details on the arthropod sampling can be found in Renkas (2019). Some of the arthropods used for this project have been previously measured for ^{137}Cs by Renkas (2019).

2.5.2 **Digestion of samples**

Dried Chernobyl and control arthropods were weighed and transferred to the TFM vials. The arthropods were individually microwave acid digested in 0.6 mL 8M HNO_3 and Rh (10 µg/L) at 260 °C for 20 min using Milestone UltraWAVE (see 2.3 for further details). Rhodium was used as IS for Ca and Sr to observe potential sample loss between digestion and EXC. After digestion, samples were transferred from TFM vials into 2 mL Eppendorf tubes. The TFM vials were rinsed two times with a total volume of 1 mL deionized water and added to the samples. Undiluted HNO_3 could have been used during microwave acid digestion instead of 8M HNO_3, due to the following evaporation in the next step. Samples were evaporated to dryness at 90 °C for 12 h for control of volume and concentration. The Eppendorf tubes used were robust and could withstand temperatures up to at least 90 °C and high acid concentrations.

Residues of the arthropod samples were dissolved with 0.6 mL 8M HNO_3 and subsequently split into two parts. Five 0.6 mL replicates of the arthropod in-house standard were split in two aliquots: 0.1 mL went directly to ICP-MS measurement and 0.5 mL to Sr separation.

2.5.3 **Unseparated samples: ICP-QQQ**

The 0.1 mL sample aliquot was diluted to 0.5 mL of 10% (V/V) HNO_3 with indium (2 µg/L) added as IS, resulting in a final Rh concentration of 2 µg/L. Additionally to Rh, indium was used as IS to correct loss of Sr and matrix effects. Natural levels of Sr, Ca and Zr in arthropods and in-house standard replicates were analysed directly and determined by ICP-QQQ.

2.5.4 **Sr-resin separation samples**

One microcolumn per sample was packed with Sr-resin and prepared for separation of strontium. The 0.5 mL sample aliquot was used for EXC of strontium. Stable and radioactive Sr were separated from matrix and interfering zirconium (see section 2.5.4 and 2.5.5 for detailed information on packing of columns and EXC with Sr-resin, respectively).

Following separation, samples were evaporated to dryness at 90 °C for 12 h to have control of volume and concentration prior to analysis. To redissolve the samples, 0.5 mL 5 % (V/V) HNO_3 with indium (2 µg/L) was added. This volume quantity could have been lower but was selected to have the option of analysing the samples twice (see section 4.1 for further discussion). Levels of natural Sr, ^{90}Sr, Ca and Zr in separated, preconcentrated arthropod and in-house standard samples were analysed directly by ICP-QQQ. Calcium and zirconium concentrations were determined to observe the decontamination after separation.

2.5.5 **Packing of microcolumns**

Microcolumn model C consisted of a syringe filter and a 10 µL filter pipette tip coupled together. This column was designed to perform EXC of small sample volumes, e.g., samples of individual arthropods (details on the development of microcolumn model C can be found in section 2.1.3.3). If many samples are to be analysed, packing of microcolumns should be easy and efficient. Packing of each column took approximately 5-10 min and the packing process is demonstrated in Figure 2.8. Instead of using Sr-resin from prepacked columns, loose Sr-resin sold in bottles by Triskem International was used in the final method (SR-B25-A, Triskem International). The Sr-resin that comes in bottles is the same as the resin in prepacked columns, but it is not stored in deionized water.

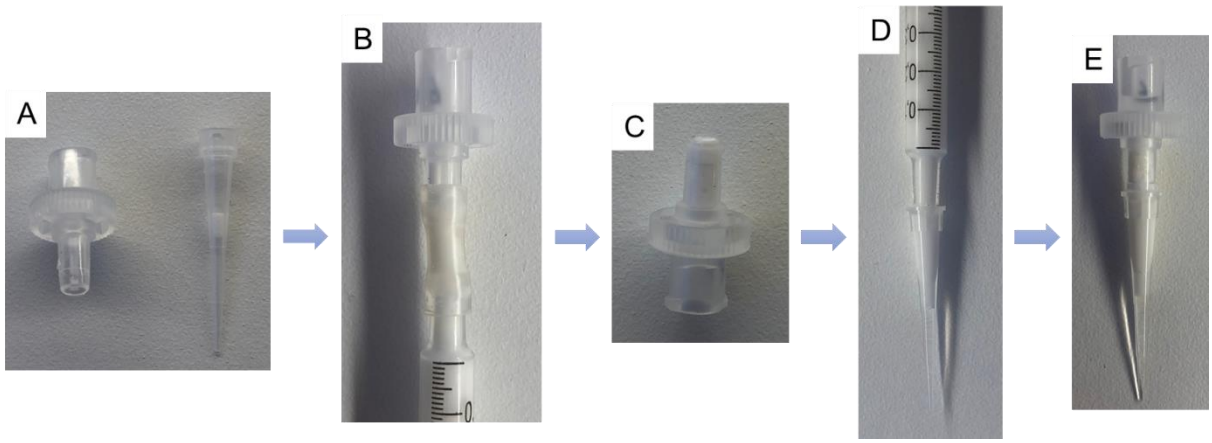

Figure 2.8: Process of packing microcolumns with resin.

Before the column components were packed with resin, the syringe filter and pipette tip were connected to ensure that they fit together (Figure 2.8A). To avoid damage to the syringe filter and reduce risk of leakage, the coupling of the filter pipette tip was expanded with hot water to better fit the end of the syringe filter.

Sr-resin and deionized water were mixed together into a slurry in a plastic tube vial. Slurry was easily transferred to the syringe filter from a 1 mL syringe, via a short plastic tube (Figure 2.8B). Resin was pushed into the syringe filter and was made as compact as possible to prevent formation of air bubbles in the resin bed (Figure 2.8C). Air bubbles in the packed resin bed may limit contact with the resin and the mixture compounds, leading to a decreased separation efficiency.

The filter pipette tip was filled with resin by pushing slurry from a 1 mL syringe into the pipette tip (Figure 2.8D). Syringe filter and pipette tip were connected tight together. The column could contain up to 50 mg of Sr-resin (Figure 2.8E). The resin bed was kept wet until separation to avoid the bed to become dry. A dry resin bed could lead to formation of channels in the bed that would lead to poor separation. Deionized water was added to the column and to avoid evaporation during storage column was wrapped in parafilm.

2.5.6 **Extraction chromatography with Sr-resin**

Microcolumns were easily loaded with nitric acid and sample by connecting a 1 mL syringe to the columns. Liquid was pushed through the column using the syringe, and flow rates of ~0.2 mL/min were typically employed. To ensure adequate Sr separation from matrix, the sample was prepared in 8M HNO_3 from the beginning. A schematic step-by-step of the separation process is given in Figure 2.9.

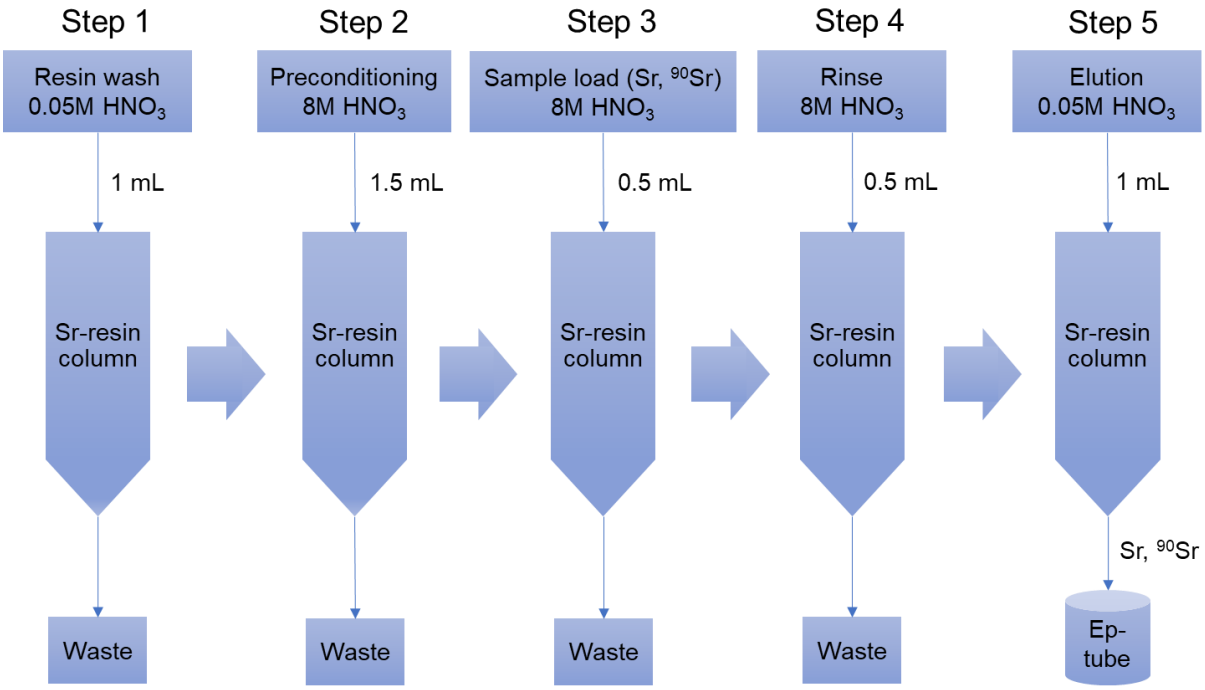

Figure 2.9: Stepwise overview of EXC with Sr-resin packed microcolumns.

The column was prepared for Sr separation by washing the resin bed with 1 mL 0.05M HNO_3 in case of contamination of strontium (Figure 2.9, step 1). Subsequently, the column was preconditioned with 1.5 mL 8M HNO_3, the same acid concentration as the sample, to make the resin ready and available for strontium binding (Figure 2.9, step 2).

The sample, contained in an Eppendorf tube, was loaded onto the column with a clean syringe (Figure 2.9, step 3). The Eppendorf tube and syringe were washed with 0.5 mL 8M HNO_3 to reduce sample loss. Acid wash was transferred to the column with the same syringe as the sample and used as rinse to decontaminate the resin from interfering zirconium and other matrix elements (Figure 2.9, step 4).

In the last separation step, stable and radioactive Sr was eluted with 1 mL 0.05M HNO_3 and collected in an Eppendorf tube (Figure 2.9, step 5).

2.7 Digital autoradiography

Prior to microwave acid digestion and ICP-MS analysis, digital autoradiography (DA) was performed on the Chernobyl arthropods. Digital autoradiography is a technique used to record radiation by exposure of a radioactive source to a sensitive medium. The sensitive medium is a specifically manufactured phosphor screen, also known as a photo imaging plate, which is

sensitive to all kinds of radiations. The imaging plate contains photostimulable crystals, for example BaFBr: Eu^{2+} phosphor crystals. When exposed to ionizing radiation, the europium is ionized to metastable Eu^{3+} and the radiation energy is stored in the crystal until scanned with a laser beam. The imaging plate scanner will excite Eu^{3+} with a laser beam to an unstable state, resulting europium going back to its initial state of Eu^{2+} and release energy as a photon. This photon is collected into a photomultiplier tube and converted to electrical signals. The information from the signals is transformed into a digital image by a computer (Fichet et al., 2016).

The method can give information on where radionuclides are accumulated in tissues and a quantification of how much radioactivity the tissue contains. However, the method is not qualitative and will neither tell what type of radiation has been emitted or which radioactive isotope has been exposing the imaging plate. The quality of the image after scanning the imaging plate depends on the radiation source and its activity, and the exposure time may wary due to this. Weak signals from a small activity sample can be amplified and may become more detectable if the exposure time increases from days to weeks (Pettersen, 2021).

Arthropods were removed from preservation in ethanol and each arthropod sample was dried on top of a filter in room temperature for 72 h. A filter was put on top of each sample to prevent the arthropods from blowing away and keep them separated from each other. If not dry, the moisture from the arthropods might have destroyed the phosphor crystals in the imaging plate. To avoid arthropods with higher levels of radioactivity interfering with the imaging plates' exposure from arthropods with lower activity, arthropods with similar size were grouped together on cardboard plates. Two 0.5 Bq droplets of ^{90}Sr and ^{134}Cs was added onto each cardboard plate to compare the detection between the two isotopes, but also to compare this activity to the arthropods. To ensure the same conditions between the added droplets and the arthropods, these spots of activity were also covered with a filter.

Each cardboard plate was placed within a protective cassette with an imaging plate on top of the arthropods. This was done with the lights off to reduce contamination from light radiation on the imaging plates. The cassette prevents exposure from light radiation. Additionally, the cassettes were stored in a dark room, covered in lead to reduce exposure from background radiation. To ensure good detection and collection of exposure, the imaging plates were exposed for three weeks. The imaging plates were scanned using a HD-CR 35 NDT Imaging Plate Scanner (DÜRR NDT), converting the stored information into digital images.

The DA images were converted to 16-bit images and were edited using Fiji Image J software (imagej.net, (Schindelin et al., 2012)) to better visualize the exposure from the arthropods. Originally, the DA images were in different shades of grey, where the black areas in the images indicated the presence of radioactive materials. The level of grey in a particular pixel is indicated by the grey value. In a 16-bit image, the grey value is between 0 (black) and 65535 (white). To better visualize the amount of radiation shown in the images, the shades of grey was converted to a shade of purple, resulting in black was displayed as white, and white was displayed as black. In the edited DA images, pixels with grey value 0 and 65535 are shown as white and black, respectively.

2.8 ERICA Assessment Tool

The ERICA project (Environmental Risk from Ionising Contaminants: Assessment and Management) was carried out between 2004 and 2007 and was concluded with the publication of two significant outputs: the ERICA Integrated Approach and the ERICA Assessment Tool. The basis of the Integrated Approach is the quantification of environmental risk. The Approach combines data on environmental transfer and dosimetry to provide a measure of wildlife exposure which is compared to exposure levels at which detrimental effects are known to occur. These data sets are used in calculations supported by the software programme, ERICA Assessment Tool, to estimate risks to selected animals and plants (Brown et al., 2008; Brown et al., 2016).

Absorbed dose rates from a selection of the analysed Chernobyl arthropods were estimated using a tier 2 model in version 1.3.1 of the ERICA Assessment Tool software. Organism models of the arthropods were made based on the arthropods' geometry, i.e., mass and size measurements. Because of a predefined mass range when creating an organism model in the software, arthropod models cannot weigh less than 1 mg. All of the arthropods, except scarab beetle (E17/17-Scarab-004), was set with an "on-soil" occupancy factor of 1. The Scarab beetle was set with an "in-soil" occupancy factor of 1 to compare the impact on dose rates. The occupancy factor explains how long a given organism spends at a location in its given habitat, whether it is in soil, on soil or in air.

Soil activity measurements of ^{90}Sr from a Chernobyl earthworm study (Newbold et al., 2019) was used in the dose rate calculations. Average soil concentrations at Glubokya Marsh within the Chernobyl Exclusion Zone were used since the site had the highest soil activity

concentrations and was located in the vicinity of the Chernobyl arthropods sampling sites C1-C4 (see Table B.1 in Appendix B). The soil activity, together with the determined ^{90}Sr activity concentrations in the Chernobyl arthropods measured on the ICP-QQQ, were used to calculate concentration ratios, which is a key parameter for the derivation of dose rates. The concentration ratio is a transfer parameter used to quantify transfer in the environment, and is defined as the ratio of the activity concentration in whole organism to that in a media, e.g., soil or water (Brown et al., 2016):

Eq. 5 $$CR = \frac{Activity\ concentration\ in\ organism\ (\frac{Bq}{kg})}{Activity\ concentration\ in\ soil\ (\frac{Bq}{kg}),\ water\ or\ air\ (\frac{Bq}{m^3})}$$

2.9 Data analysis

The results from the ICP-QQQ measurements were corrected based on analysis of the internal standards. In addition to this, one of the calibration standards was used as a drift check and quantified periodically during the analysis and subsequently used to correct the results for instrument drift. Drift in the analytical instrument may occur during a long session of analysis, which can result in temporary variations in signal intensity.

The five replicates of the spiked ^{90}Sr in-house standard were used to evaluate the method's precision and accuracy, while the control samples were used for determination of detection and quantification limit for ^{90}Sr in the method.

Limit of detection (LOD) and quantification (LOQ) for ^{90}Sr were determined from the ^{90}Sr concentrations in the control arthropod samples, collected in Norway. Norwegian arthropods were used because they contain the same interferences as the Chernobyl arthropods, but with non-detectable amounts of ^{90}Sr.

A 200 µg/L Zr solution was analysed to illustrate the interference from Zr on ^{90}Sr during the ICP-QQQ analysis when the O_2 and H_2 mixture was present in the CRC. The background equivalent concentration (BEC) for the interference from Zr on ^{90}Sr was calculated based on the measurement of the 200 µg/L Zr solution.

Radiation dose rates were estimated in the ERICA assessment tool software.

2.9.1 **Corrections and calculations**

The quantified concentrations in the analysed samples were corrected by the indium IS using the Agilent ICP-MS MassHunter software. The results from the ICP-QQQ analysis were transferred to Microsoft® Excel® spreadsheets. All of the calculations were performed in Excel®. Instrument drift was detected by the periodically measured calibration standard and a factor, K, was calculated for each period (Eq. 6). These factors were multiplied with the measured concentrations within each of these periods and corrected for drift (Eq. 7). *[std]* is the known concentration of the calibration standard. *[std start]* is the measured concentration of the standard at the beginning of a period while *[std end]* is the measured value at the end of the period.

Eq. 6 $$K = \frac{[std]}{\frac{[std\ start]+[std\ end]}{2}}$$

Eq. 7 $$[corrected\ value] = [measured\ value] * K$$

All of the concentrations for the samples were obtained as weight by volume (w/V). For further treatment, the values were calculated from w/V to weight by weight (w/w). The ^{90}Sr concentrations were calculated to activity concentrations (Bq/g).

Mean values and standard deviations were calculated for the five in-house standard replicates. The precision for the method was evaluated by calculating relative standard deviations (RSD) for each element measured in the in-house standard replicates (Eq. 8).

Eq. 8 $$RSD\ (\%) = \frac{standard\ deviation}{average} * 100\%$$

The accuracy for the method is represented by the deviation between the measured ^{90}Sr concentrations in the in-house standard and the actual spiked amount of ^{90}Sr, i.e., the bias. The bias from the ^{90}Sr measurements in the in-house standard was calculated with Eq. 9:

Eq. 9 $$Bias\ (\%) = \frac{Measured\ value - True\ value}{True\ value} * 100\%$$

Limit of detection (LOD) and limit of quantification (LOQ) for ^{90}Sr were determined from the ^{90}Sr concentrations in the control arthropod samples and were calculated with equation 10 and 11. To give a better indication of the performance of the method, the limits were given in total mass and activity.

Eq. 10

$$LOD = 3 * standard\ deviation\ (\mathrm{Sr} - 90 \text{ concentrations in control arthropods n} = 23)$$

Eq. 11

$$LOQ = 10 * standard\ deviation\ (\mathrm{Sr} - 90 \text{ concentrations in control arthropods n} = 23)$$

3 RESULTS

3.1 Microcolumn separation

The majority of the results related to method development have already been presented in Chapter 2, so this section summarises key results linked to the microcolumn separation from the 58 samples analysed during this thesis.

3.1.1 **Amount of Sr-resin in microcolumns**

The microcolumns used for separation of stable and radioactive strontium in Chernobyl arthropod samples, control samples and in-house standard replicates were packed with Sr-resin by Triskem International. These microcolumns were weighed before resin were added. After EXC, the microcolumns with added resin were dried and weighed again. The resin mass mean value of n = 58 microcolumns was 48 ± 2 mg. The mass varied from 42 to 51 mg, where only four microcolumns contained a resin mass between 42 and 44 mg Sr-resin.

3.1.2 **Decontamination of interferences**

All of the samples were split into two aliquots to be measured both directly and after EXC on the ICP-MS. To observe how well calcium and zirconium were decontaminated after EXC, decontamination of Ca and Zr were calculated based on determined sample concentrations with and without EXC. The mean values for decontamination of n=58 samples for Ca and Zr were 98 ± 2% for both elements. The rinse volume of 0.5 mL HNO_3 used during the EXC was adequate and resulted in the removal of large amounts of Ca and Zr from the samples with high efficiency.

3.1.3 **Sr recovery**

Stable strontium was used as yield monitor to correct the measured ^{90}Sr in the samples. For the recovery of Sr, one of the 58 samples was excluded due to possible contamination of Sr. The recovery mean value of stable strontium obtained in the elution step during EXC after n = 57 separation samples was 93 ± 8%. The four samples with the lowest recovery of stable strontium were the control samples K7 (81%), K11 (78%), K15 (62%) and K17 (72%).

3.2 Analytical results

3.2.1 Limit of detection and quantification

Control arthropods, collected in Norway, were analysed to calculate limit of detection (LOD) and limit of quantification (LOQ) for ^{90}Sr. The control arthropod samples went through the same proceedings as the Chernobyl arthropod samples and were determined for ^{90}Sr concentrations. ^{90}Sr measurements in n=23 control arthropods were used to estimate the LOD and LOQ values. The control arthropod samples were diluted in 0.6 mL. Because of a wide range of control arthropod mass (from 0.005 to 0.125 g), the median mass of 0.024 g were used in the calculations. The limits were given in total mass and activity to give a better indication of the performance of the method. Equations used for the calculation of LOD and LOQ can be found in section 2.9.1 (Eq. 10 and 11).

The LOD and LOQ for ^{90}Sr in the method were calculated to be 1.1 fg (5.5 mBq) and 3.6 fg (18 mBq), respectively.

3.2.2 Method's precision and accuracy

Due to the lack of a CRM for arthropods, an in-house standard was made from various digested arthropods collected in Norway and spiked with a ^{90}Sr concentration of 144.2 pg/L (44 Bq/g), which is traceable to a certified source solution (Eckert & Ziegler, 2017). The in-house standard was used to evaluate the precision and accuracy in the method and control the calibration on the instrument. Five replicates of the in-house standard, with the same sample preparation as the analysed Chernobyl arthropods, were measured on the ICP-MS. In Table 3.1, mean values with standard deviations of the in-house standard replicates for Ca, Sr, Zr and ^{90}Sr are given. Relative standard deviation (RSD) of the replicate measurements has been calculated to give a measure of precision of the method.

Table 3.1: Mean values and RSD, given in dry weight, of n=5 replicates of the in-house standard for Ca, Sr, Zr and ^{90}Sr. The theoretical mass of each arthropod in the in-house standard was set to 0.010 g. All replicates were diluted in 0.6 mL.

Analyte	Mean ± SD		RSD (%)
Ca	1.28 ± 0.012	g/kg	1.01
Sr	9.8 ± 0.12	mg/kg	1.3
Zr	450 ± 5.6	µg/kg	1.3
^{90}Sr	9.6 ± 0.29	ng/kg	3.1
^{90}Sr	48 ± 1.5	Bq/g	3.1

Table 3.1 shows the mean values for the measured analytes in the in-house standard. The RSD values indicates good precision as the standard deviations are relatively small compared to the mean values for all of the analytes (1-3%). However, the RSD value for ^{90}Sr is somewhat higher than the other analytes. The determined ^{90}Sr concentrations in the in-house standard showed accuracy for ^{90}Sr to be within 10% of added activity concentration.

3.2.3 **Removal of ^{90}Zr in the collision-reaction cell**

The Zr solution with a concentration of 200 µg/L was measured on the ICP-QQQ to observe how much ^{90}Sr was determined. The response from the Zr solution was measured as 80 pg/L ^{90}Sr. The background equivalent concentration (BEC) calculated from the measurement was:

$$BEC = \frac{0.4\ ppq\ 90Sr}{ppb\ Zr}$$

This implies that the remaining Zr in the samples were removed during the triple quadrupole ICP-MS analysis.

3.3 ^{90}Sr activity in Chernobyl arthropods

In order to measure ^{90}Sr levels and natural levels of Ca, Sr, and Zr in individual Chernobyl and control arthropods, digested arthropods were split in two aliquots. One aliquot was measured for ^{90}Sr levels using ICP-QQQ following EXC with the application of a Sr-selective resin. The second aliquot was measured directly on ICP-QQQ for natural levels. The analysed Chernobyl arthropods are listed in Table 3.2, which in addition to concentrations of Ca, Sr, and Zr, provides the activity concentrations of ^{90}Sr. Based on measurements of the in-house standard, measurement uncertainties are estimated at ca. 1% for Ca, Sr and Zr, and 3% for ^{90}Sr (Table 3.1). A full overview of analysed Chernobyl arthropods, including taxonomy of the arthropods and samples below LOQ, can be found in Table C.1 in Appendix C.1. For comparative purposes, results of Chernobyl arthropods measured for ^{90}Sr without EXC in the previous pilot study can be found in Appendix D.1 (Table D.1) (Jensen, 2020). In Table 3.3, natural levels of Ca, Sr and Zr in the analysed control arthropods are presented.

Table 3.2: Activity concentrations of ^{90}Sr and concentrations of Ca, Sr, and Zr determined in selected Chernobyl arthropods. All arthropod samples were diluted in 0.6 mL. 'Mass' refers to mass weighed after drying in room temperature for 72 h, prior to digestion. Two different weighing scales were used, one to +/- 0.001 g and the other to +/- 0.01 mg. In the table, the masses have been rounded to the last significant figure. The concentrations are presented with two significant figures, due to the uncertainty of the arthropod masses after drying.

Site	Sample code	Family	Common name	Trophic Group	Mass (mg)*	Ca (g/kg)	Sr (mg/kg)	Zr (µg/kg)	^{90}Sr (ng/kg)	^{90}Sr (Bq/g)
C4	NA-1.1	vrac	Diptera	Mixed feeder	5	10	18	70	8.0	41
	NA-1.2	vrac	Diptera	Mixed feeder	7	11	20	60	10	53
	NA-1.3	vrac	Diptera	Mixed feeder	4	7.6	20	83	5.7	29
	E17/17-Opilio-001	Opiliones	Harvestman	Insect predator	2	21	21	80	15	74
	E17/17-Arane-001	Araneae	Spider	Insect predator	7	10	18	26	20	100
	E17/17-Trich-001	Trichoptera	Caddisfly	Mixed feeder	18	8.2	18	110	11	56
C3	E17/17-Staph-pred, lake 10.10.16	Staphylinidae	Rove beetle	Predator	20	3.6	5.2	16	3.8	19
	E17/17-Form-003	Formicidae	Ant	Mixed feeder	1.96	12	20	74	27	140
C2	NA-2.1	Brachycera	Fly	Mixed feeder	6	5.7	11	69	0.89	4.6
	NA-2.2	Brachycera	Fly	Mixed feeder	2	14	27	130	6.7	34
	E17/17-Opilio-002	Opiliones	Harvestman	Insect predator	14	9.2	18	130	12	60
	NA-3 [a]	Larva	Beetle	?	1.01	10	20	$18 \cdot 10^3$	12	60
	E17/17-Paras-002	Parasitica	Wasp	Parasitoid/ predator	1.44	15	13	180	4.6	23
C1	E17/17-Syrph-001.1	Syrphidae	Diptera	Mixed feeder	14	6.6	18	200	1.0	5.2
	E17/17-Syrph-001.2	Syrphidae	Diptera	Mixed feeder	4	9.1	28	240	1.9	10
	E17/17-Opilio-003.1	Opiliones	Harvestman	Insect predator	7	11	32	$4.9 \cdot 10^3$	15	79
	E17/17-Opilio-003.2	Opiliones	Harvestman	Insect predator	6	11	36	270	10	53
	E17/17-Opilio-003.3	Opiliones	Harvestman	Insect predator	2	8.3	25	160	6.6	34
	E17/17-Coleo	Coccinellidae	Ladybug	Predator	1.61	19	29	470	9.6	49

	E17/17-Form-004	Formicidae	Ant	Mixed feeder	0.26	21	53	700	37	190
I1	E17/17-Scarab-004	Scarabaeidae	Scarab beetle	Detritivore	171	1.4	5.3	320	0.91	4.6
	E17/17-Opilio-004	Opiliones	Harvestman	Insect predator	7	10	25	66	4.8	25
I2	E17/17-Brachy-010.1	Brachycera	Fly	Mixed feeder	6	4.0	8.7	71	1.2	5.9
	E17/17-Brachy-010.2	Brachycera	Fly	Mixed feeder	1	3.5	9.5	160	5.0	25
	E17/17-Opilio-010.1	Opiliones	Harvestman	Insect predator	4	12	16	110	5.3	27
	E17/17-Opilio-010.2	Opiliones	Harvestman	Insect predator	5	10	14	110	5.0	26
	E17/17-Allep-004 [a]	Lepidoptera	Butterfly	Leaf chewer	20	2.8	4.3	32	1.2	6.1
	E17/17-Curcu	Curculionidae	True weevil	Leaf chewer	3.12	6.1	21	240	5.7	29

[a] Larva

The analysed arthropods, which had a variety of mass from 0.26 to 171 mg, show a range of ^{90}Sr activity concentration from 4.6 to 190 Bq/g (Table 3.2). Three of the arthropods exceed a ^{90}Sr activity concentration of 100 kBq/kg. The highest result, ant from site C1 (E17/17-Form-004), contained 190 Bq/g. Two other samples with concentrations over 100 Bq/g were spider from C4 (E17/17-Arane-001) with 100 Bq/g and ant from C3 (E17/17-Form-003) containing 140 Bq/g.

Two arthropods had Zr levels that stood out compared to the rest. Beetle larva from C2 (NA-3) and harvestman from C1 (E17/17-Opilio-003.1) contained 18 mg/kg and 4.9 mg/kg of Zr, respectively. These levels may be the result of soil in the samples. However, since the removal of Zr was so effective in the CRC, these levels are unlikely to have impacted on the measurement of ^{90}Sr. Two samples, E17/17-Heter-001 and E17/17-Acari-002, were not included in Table 3.2 due to ^{90}Sr levels lower than LOQ of 3.6 fg (0.018 Bq) (Table C.1 in Appendix C.1). Comparing these results with data from the pilot study (Table D.1 in Appendix D.1), shows that a number of samples contained higher levels of ^{90}Sr than the arthropods from this study. The two millipede samples (Millipede from C4 and E17/17-Myriapoda from I2) contained the highest levels of ^{90}Sr with 2900 Bq/g and 330 Bq/g. Additionally, many other mixed feeders, detritivores and herbivores exceeded ^{90}Sr levels of 100 Bq/g, including

Chilopoda, *Staphylinidae*, *Curculionidae* and *Lepidoptera*. However, in general, the range of ^{90}Sr concentrations was of a similar order of magnitude to those from the pilot study.

The ^{90}Sr activity concentration determined for each arthropod is shown in Figure 3.1.

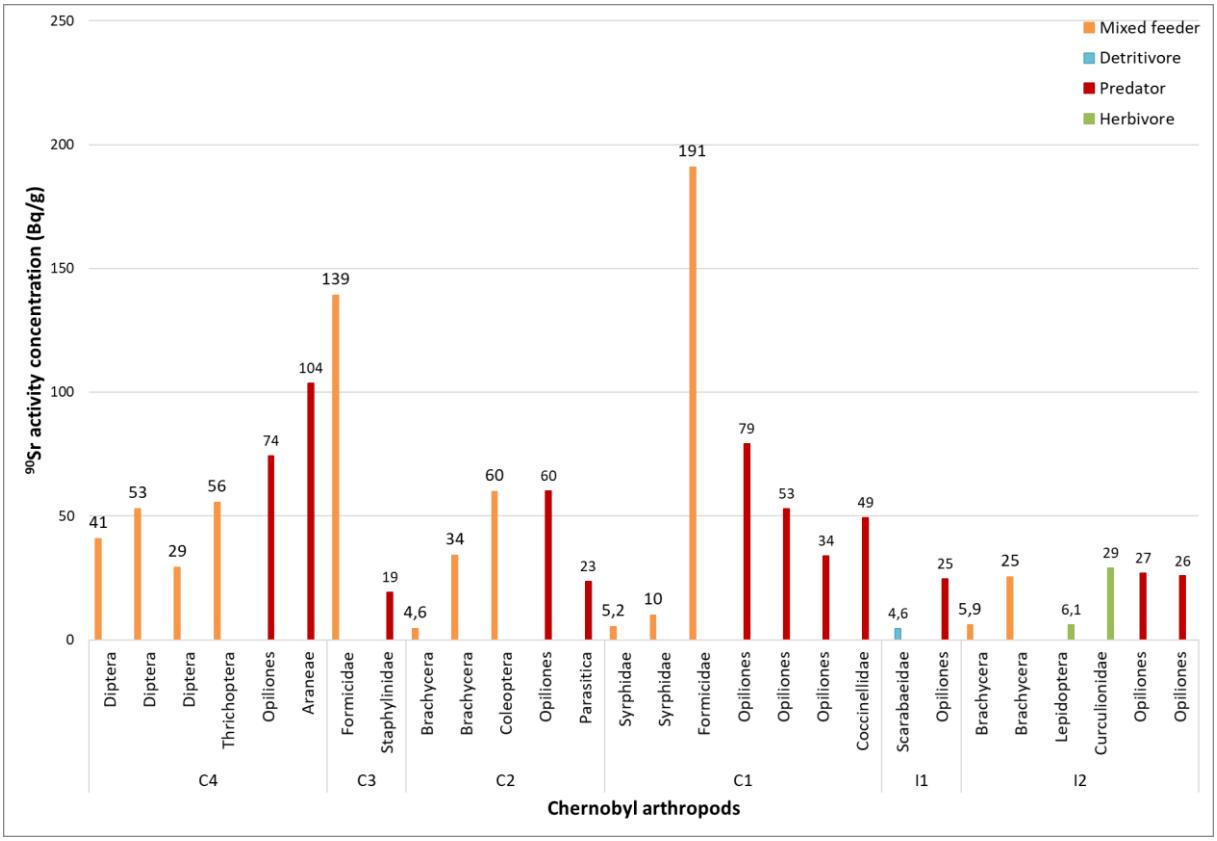

Figure 3.1: Strontium-90 activity concentrations determined in analysed Chernobyl arthropods compared between collection site and trophic level. Mixed feeder (orange), Detritivore (blue), Predator (red) and Herbivore (green).

The majority of the analysed arthropods are either mixed feeders or predators (Figure 3.1). The activity concentrations determined in the arthropods collected in site I1 and I2 are lower compared to the sites with higher soil activity (C1-C4).

Arthropods high in calcium were additionally high in stable strontium content. However, some arthropods with lower content of calcium had relative high concentrations of strontium. Ant from C1 (E17/17-Form-004) had both the highest concentrations of calcium and stable strontium, 21 g/kg and 53 mg/kg, respectively. This insect had the lowest mass of all the samples: 0.26 mg. The scarab beetle from I1 (E17/17-Scarab-004) and the butterfly larva from I2 (E17/17-Allep-004) were the arthropods with the highest mass but with lowest concentrations of calcium and stable strontium.

Stable strontium was plotted against calcium and radioactive strontium to show correlation between Sr-Ca and Sr-^{90}Sr uptake in the analysed Chernobyl arthropods. Figure 3.2 shows the relationship between the Sr and Ca concentrations. Since Sr tends to follow Ca biologically, the Ca content of arthropods can give an indication of how much Sr they contain.

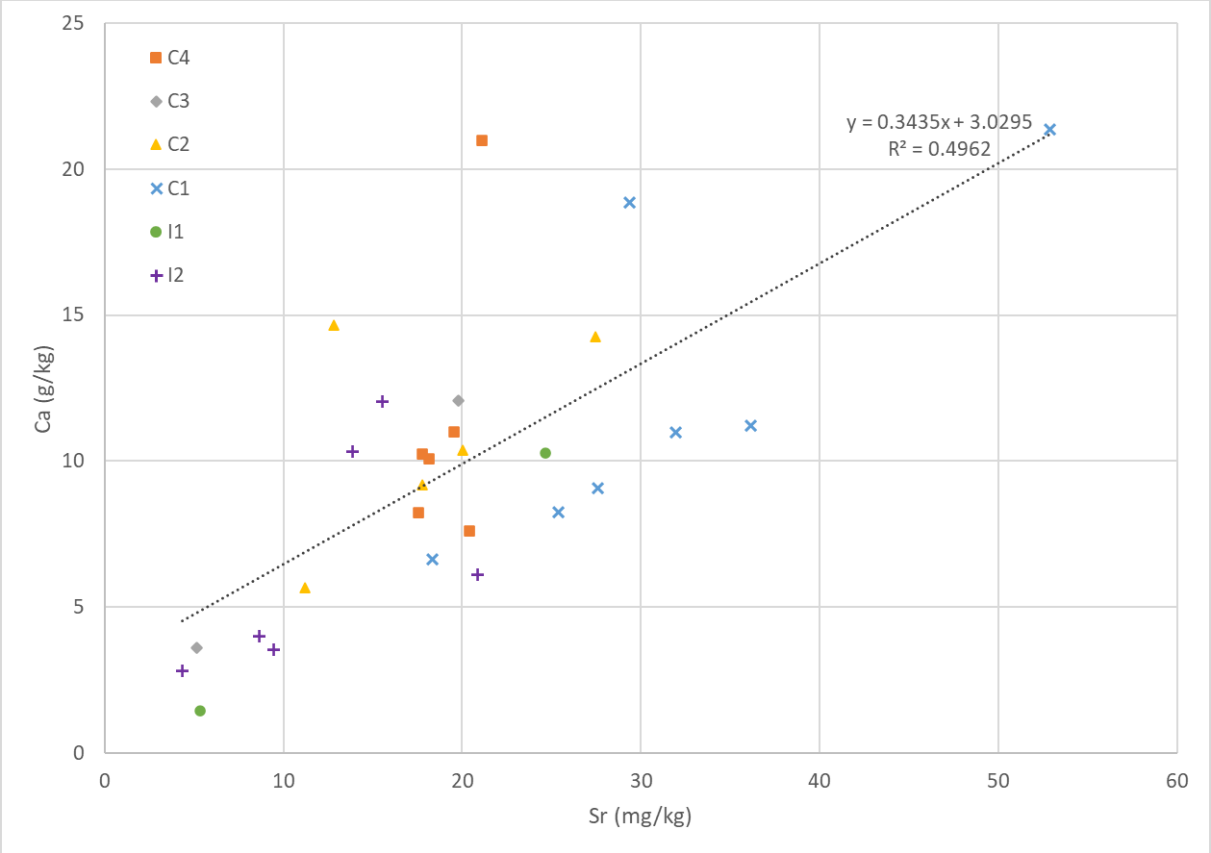

Figure 3.2: Scatter plot of the Sr vs. Ca concentrations of the analysed Chernobyl arthropods from six sites. Site C4 (orange), C3 (grey), C2 (yellow), C1 (blue), I1 (green) and I2 (purple).

According to the plot shown in Figure 3.2, there is a correlation between the Sr and Ca concentrations in the analysed Chernobyl arthropods (R^2 = 0.50). The plot shows there is variation in total amounts of Ca and Sr between sites, perhaps due to different types of soil. Furthermore, the data points from the same site are relatively close to each other. Arthropods within same family collected at the same site seem to have similar Ca and Sr content.

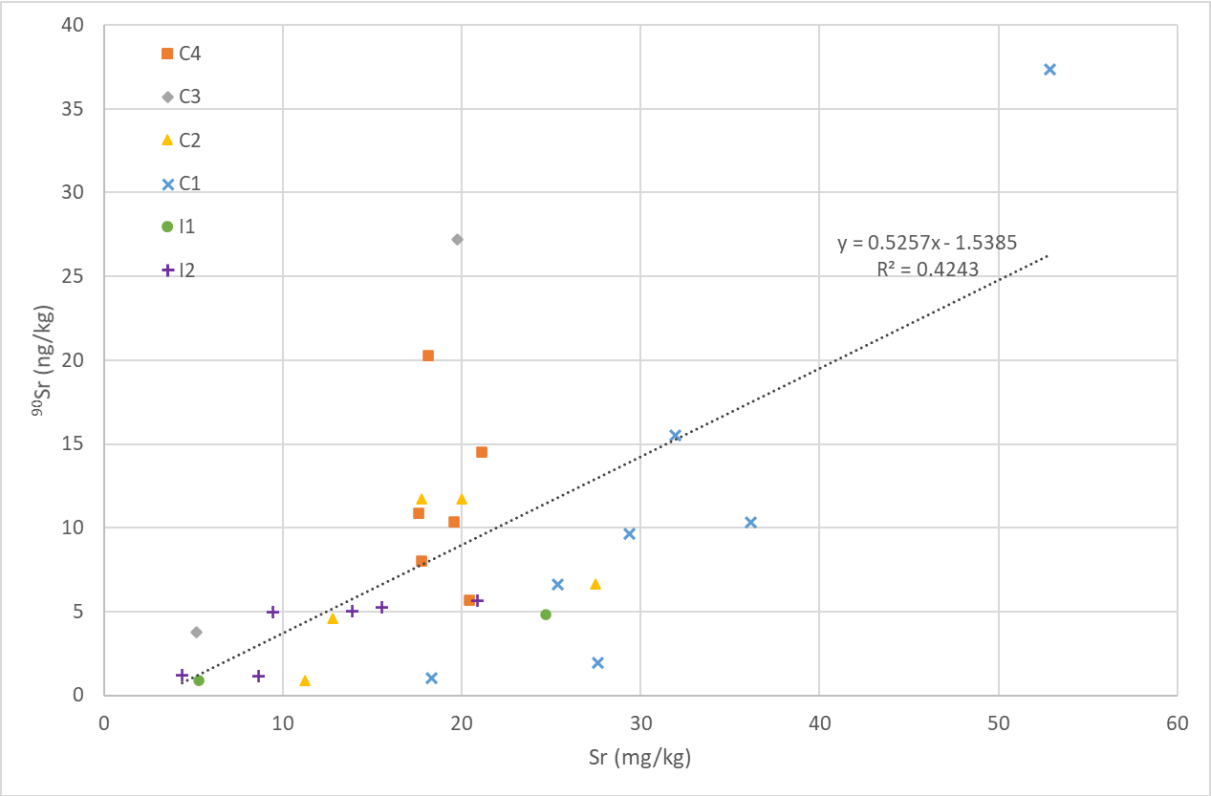

Figure 3.3: Scatter plot of the Sr vs. ^{90}Sr concentrations of the analysed Chernobyl arthropods from six sites. Site C4 (orange), C3 (grey), C2 (yellow), C1 (blue), I1 (green) and I2 (purple).

Figure 3.3 shows the relationship between stable Sr and ^{90}Sr in the analysed Chernobyl arthropods. The scatter plot shows that there is a reasonable correlation between Sr and ^{90}Sr (R^2 = 0.42), and that the variation in ^{90}Sr is bigger than stable Sr. Arthropods collected at sites with high soil activities seems to have higher levels of ^{90}Sr (Site C1-C4) compared to arthropods at the intermediate sites (I1 and I2). This may indicate higher uptake of ^{90}Sr in areas with higher soil activity.

Natural levels of Ca, Sr, and Zr in Norwegian control arthropods are presented in Table 3.3 to check and compare natural levels in control arthropods to Chernobyl arthropods.

Table 3.3: Concentrations of Ca, Sr, and Zr, given in dry weight, determined in Norwegian control arthropods. All control arthropod samples were diluted in 0.6 mL. Mass refers to mass weighed after arthropods has been dried at 35-40 °C for 72 h. The masses was weighed on a scale with the uncertainty of +/- 0.001 g and have been rounded to the last significant figure. The concentrations are presented with two significant figures, due to the uncertainty of the arthropod masses after drying.

Sample code	Family	Common name	Mass (mg)	Ca (g/kg)	Sr (mg/kg)	Zr (mg/kg)
K1	Diplopoda	Millipede	24	87	550	4.8
K3	Diplopoda	Millipede	38	66	640	0.82
K4	Diplopoda	Millipede	14	130	610	0.57
K5	Diplopoda	Millipede	26	95	570	0.81
K7	Chilopoda	Centipede	22	1.7	9.0	0.75
K8	Chilopoda	Centipede	24	1.4	5.8	0.21
K9	Chilopoda	Centipede	16	1.6	6.2	0.38
K10	Curculionidae	True weevil	26	1.0	66	0.32
K11	Carabidae	Ground beetle	29	0.89	3.3	0.11
K12	Geotrupidae	Dung beetle	125	0.27	2.2	0.23
K13	Geotrupidae	Dung beetle	79	0.27	2.0	0.29
K14	Araneae	Spider	77	1.6	9.9	0.15
K15	Araneae	Spider	9	1.3	7.2	0.062
K16	Brachycera	Fly	14	0.83	2.6	0.043
K17	Brachycera	Fly	5	1.4	7.1	0.57
K18	Brachycera	Fly	11	0.51	1.8	0.49
K19	Brachycera	Fly	20	1.7	5.0	0.13
K20	Brachycera	Fly	18	1.4	6.5	0.071
K21	Vespidae	Wasp	27	0.73	2.4	0.14
K22	Vespidae	Wasp	26	0.52	1.6	0.12
K23	Vespidae	Wasp	22	0.76	4.4	0.19
K24	Vespidae	Wasp	25	0.84	3.3	0.82
K25	Vespidae	Wasp	24	0.94	4.0	0.54

The concentrations of Ca and stable Sr contained in control arthropods, shown in Table 3.3, are considerably lower than the levels contained in the Chernobyl arthropods (Table 3.2). The exception is the millipedes (K1-K5), which contained substantially high Ca (66 - 130 g/kg) and Sr levels (0.55 - 0.64 g/kg). Of the remaining arthropods other than the millipedes, the true weevil (K10) contained relatively high concentration of Sr (66 mg/kg). Sample K1 (millipede) contained the highest level of Zr (4.8 mg/kg), possibly from soil. Furthermore, the control arthropods had a higher mass compared to the Chernobyl arthropods.

Stable strontium was plotted against calcium to observe if there was a correlation in Sr-Ca uptake in the Norwegian control arthropods. Figure 3.4 and 3.5 shows the same plot with and without outliers to illustrate the change in correlation between the two plots.

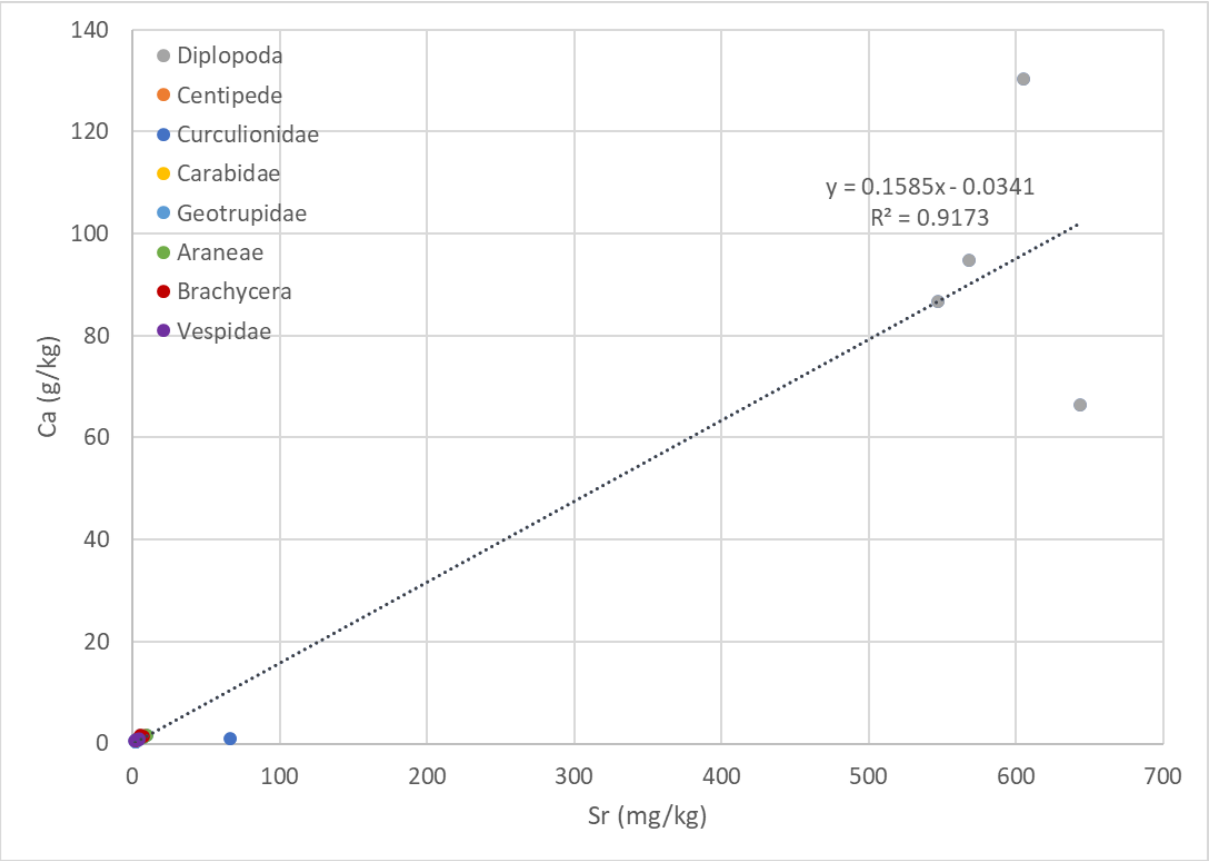

Figure 3.4: Scatter plot of the Sr vs. Ca concentrations of the analysed control arthropods. The data are categorized with arthropod family. Diplopoda (grey), Centipede (orange), Curculionidae (dark blue), Carabidae (yellow), Geotrupidae (light blue), Araneae (green), Brachycera (red) and Vespidae (purple).

Figure 3.4 illustrates that the control samples are divided into two groups, the millipedes (*Diplopoda*) and the samples with lower Ca and Sr concentrations. The millipede samples contributes to an artificially high R^2 value of 0.92 due to their high Sr and Ca content. The true weevil's (*Curculionidae*) high Sr concentration makes it an outlier.

The relationship between the Sr and Ca concentrations for the control arthropods without the Diplopoda and Curculionidae samples are shown in Figure 3.5.

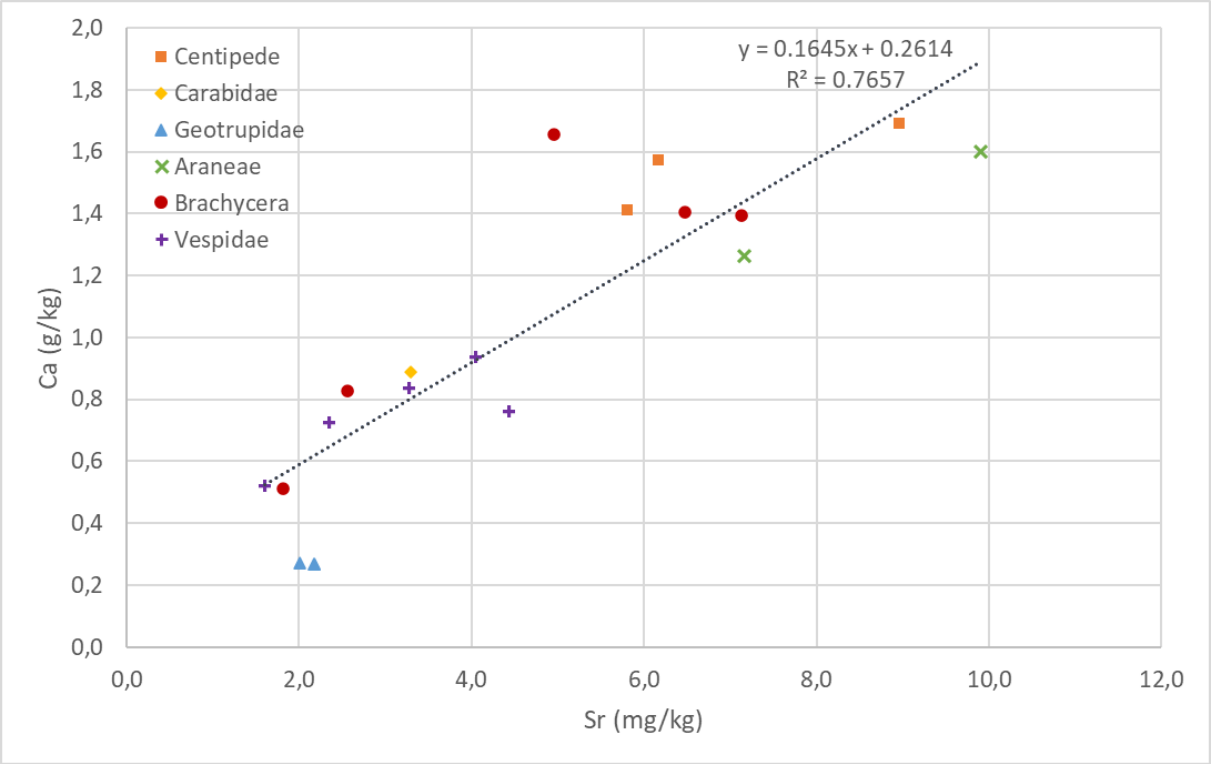

Figure 3.5: Scatter plot of the Sr vs. Ca concentrations of the analysed control arthropods. The data are categorized with arthropod family. Centipede (orange), Carabidae (yellow), Geotrupidae (light blue), Araneae (green), Brachycera (red) and Vespidae (purple).

The scatter plot in Figure 3.4 shows a better correlation between stable Sr and Ca when millipede and weevil samples has been excluded from the plot (R^2 = 0.77). Again, the Ca and Sr content for the control samples are significantly lower than the Chernobyl arthropods. The arthropods' mass can be a reason for this, since the control samples had a bigger mass then most of the Chernobyl arthropods. In addition to this, the soil type may differ between Norway and Chernobyl where the arthropods were collected.

3.4 Estimated dose rates

ERICA Assessment Tool was used for estimation of radiation dose rates on models based on selected Chernobyl arthropods. In Table 3.4, the dose rates for the different arthropods are given, which is based on the measured ^{90}Sr activity concentration of each arthropod and the ^{90}Sr soil activity (see section 2.8 for more details). Estimated radiation dose rates from ^{137}Cs, ^{90}Sr, ^{241}Am and Pu for other Chernobyl and reference arthropods from previous work can be found in Appendix D.2 (Ormaasen, 2020).

Table 3.4: Estimated dose rates for selected Chernobyl arthropods exposed to ^{90}Sr.

Site	Sample code	Family	Common name	Trophic Group	Mass (mg)	90Sr (Bq/g)	External Dose rate (µGy h^{-1})	Internal Dose Rate (µGy h^{-1})	Total Dose Rate (µGy h^{-1})
C4	NA-1.2	vrac	Diptera	Mixed feeder	7	53	$3.5 \cdot 10^{-6}$	9.5	9.5
	E17/17-Arane-001	Araneae	Spider	Insect predator	7	100	$3.5 \cdot 10^{-6}$	19	19
	E17/17-Trich-001	Trichoptera	Caddisfly	Mixed feeder	18	56	$3.5 \cdot 10^{-6}$	13	13
C3	E17/17-Staph-pred, lake 10.10.16	Staphylinidae	Rove beetle	Predator	20	19	$3.5 \cdot 10^{-6}$	4.3	4.3
	E17/17-Form - 003	Formicidae	Ant	Mixed feeder	1.96	140	$3.5 \cdot 10^{-6}$	19	19
C2	E17/17-Opilio-002	Opiliones	Harvestman	Insect predator	14	60	$3.5 \cdot 10^{-6}$	13	13
	E17/17-Paras-002	Parasitica	Wasp	Parasitoid/ predator	1.44	23	$3.5 \cdot 10^{-6}$	2.8	2.8
C1	E17/17-Syrph-001.1	Syrphidae	Diptera	Mixed feeder	14	5	$3.5 \cdot 10^{-6}$	1.1	1.1
	E17/17-Coleo	Coccinellidae	Ladybug	Predator	1.61	49	$3.5 \cdot 10^{-6}$	6.3	6.3
	E17/17-Form-004	Formicidae	Ant	Mixed feeder	<1	190	$3.5 \cdot 10^{-6}$	21	21
I1	E17/17-Scarab-004	Scarabaeidae	Scarab beetle	Detritivore	171	5	$4.4 \cdot 10^{-6}$	1.6	1.6
	E17/17-opilio-004	Opiliones	Harvestman	Insect predator	7	25	$3.5 \cdot 10^{-6}$	4.5	4.5
I2	E17/17-Brachy-010.1	Brachycera	Fly	Mixed feeder	6	6	$3.5 \cdot 10^{-6}$	1.0	1.0
	E17/17-Brachy-010.2	Brachycera	Fly	Mixed feeder	1	25	$3.5 \cdot 10^{-6}$	3.1	3.1
	E17/17-Allep-004 [a]	Lepidoptera	Butterfly	Leaf chewer	20	6	$3.5 \cdot 10^{-6}$	1.4	1.4
	E17/17-Curcu	Curculionidae	True weevil	Leaf chewer	3.12	29	$3.5 \cdot 10^{-6}$	4.4	4.4

[a] Larva

The estimated dose rates presented in Table 3.4 show that the uptake of ^{90}Sr has a greater impact on the internal dose rate compared to what ^{90}Sr exposure from soil has on the external dose rate. The internal doses ranged from 1.0 to 21 µGy h^{-1} and, as expected, the arthropods that contained the highest activity concentrations in Table 3.2 had the highest dose rates. Arthropods with dose rates above 10 µGy h^{-1} are above the ERICA dose rate screening value. The external dose rate for the scarab beetle (E17/17-Scarab-004) is slightly higher than the other arthropods because of an "in-soil" occupancy factor of 1 (3.5 pGy h^{-1} "on-soil" and 4.4 pGy h^{-1} "in-soil"). This illustrates that the dose rate is higher when an organism is in soil due to exposure from all sides while organisms on soil are exposed to ^{90}Sr from one side. Comparing samples of different masses and similar activity concentrations, illustrates that mass has a slight impact on absorbed dose rate, in that the smaller arthropods have a lower absorbed dose than larger arthropods. This reflects a greater proportion of the ^{90}Sr "escaping" from the organism. For example, the *Scarabaeidae* has a mass of 171 mg while the *Lepidoptera* has a lower mass of 20 mg and they have the ^{90}Sr concentrations of 5 and 6 Bq/g, respectively. Yet, the *Scarabaeidae* has a slightly higher dose rate (1.6 µGy h^{-1}) than the *Lepidoptera* (1.4 µGy h^{-1}). Comparisons of ^{90}Sr dose rates with those from other radionuclides are discussed in section 4.3.

3.5 Digital autoradiography results

Digital autoradiography was performed on the Chernobyl arthropods to visualize how much radioactivity they contained before further analysis was utilized. Arthropods with similar size were grouped together on cardboard plates and each plate was added two 0.5 Bq droplets of ^{90}Sr and ^{134}Cs to compare the activity from the arthropods. The arthropods were contained in a protective cassette, exposing the imaging plates for three weeks. The produced DA images were edited using Fiji Image J software. A map with pictures taken of each arthropod sample before DA together with the edited DA images is shown in the figures 3.6, 3.7, 3.8 and 3.9. The colour scale bars in the DA images has the unit, grey value, which is a number used to specify the level of grey of a pixel (see section 2.8 for more information). Digital autoradiograph of Chernobyl arthropods performed in previous pilot work is shown in Appendix D.3, Figure D.1 (Pettersen, 2021).

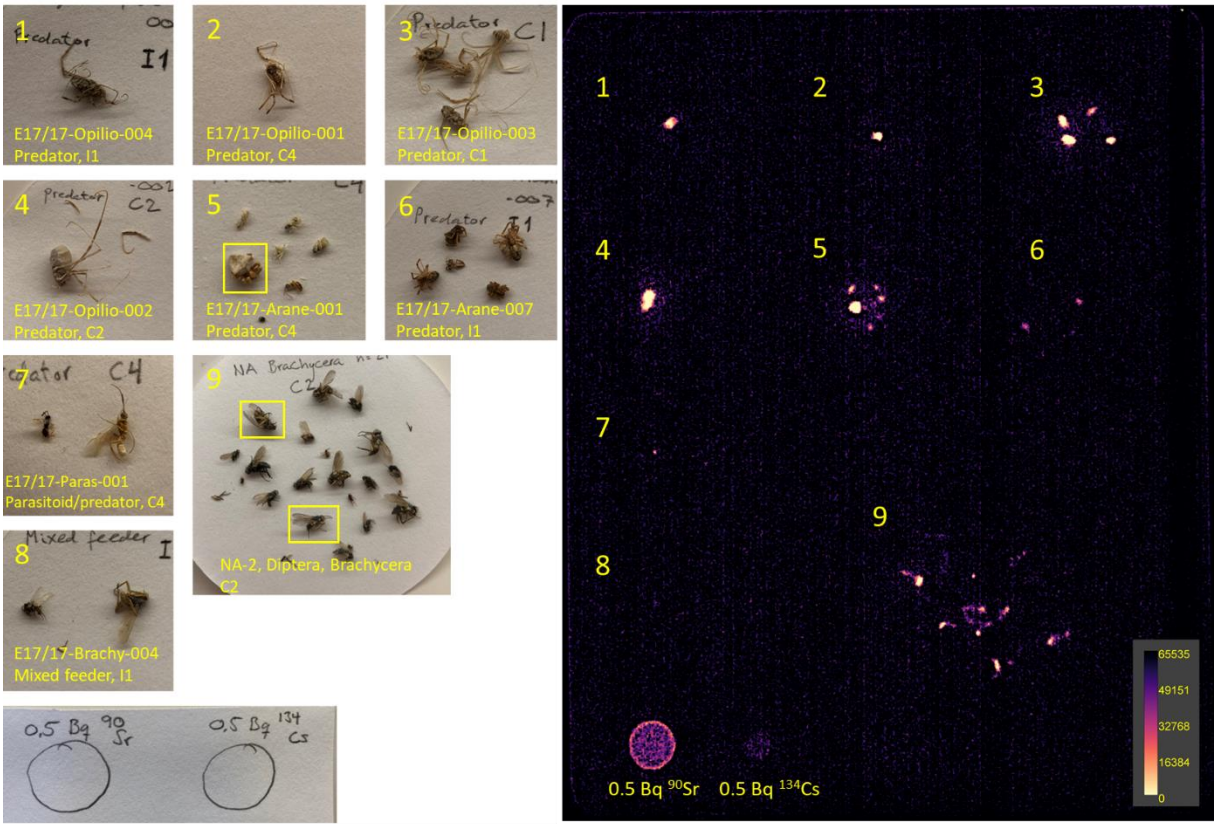

Figure 3.6: Autoradiograph of batch 1 of Chernobyl arthropods (right), as depicted from arthropod map (left), obtained with an exposure time of three weeks of a photo imaging plate. The unit of colour scale bar for DA image is grey value. Spots with grey value 0 (shown as white) contains higher amounts of radioactivity.

Figure 3.6 shows that radiation has been detected in all samples in batch 1, but little in samples 6, 7 and 8. The detection varies between arthropods in samples with more than one arthropod, e.g., sample 5 and 9. By comparing the two droplets of ^{90}Sr and ^{134}Cs, radiation from ^{90}Sr is better detected than ^{134}Cs, even though the activity is the same. This implies that ^{90}Sr in the arthropods contribute more to the total emitted radiation than radiocaesium. A selection of arthropods with high detection of radiation in the DA image was selected for further ^{90}Sr analysis. The selected arthropods from batch 1 were from sample 1-5 and 9. The marked arthropods from sample 5 and 9 were selected.

In Figure 3.7, DA image of the largest Chernobyl arthropods are shown.

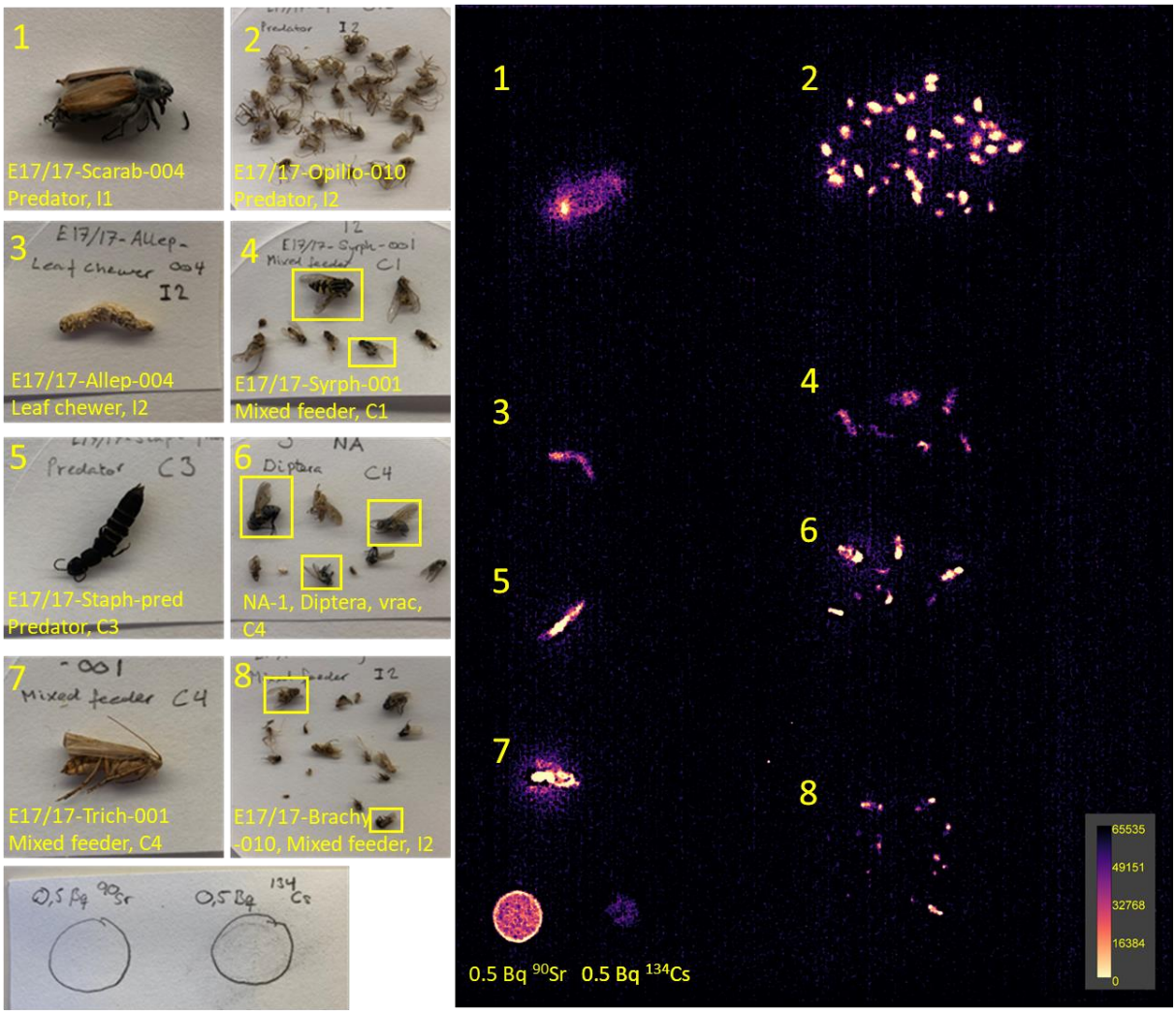

Figure 3.7: Autoradiograph of batch 2 of Chernobyl arthropods (right), as depicted from arthropod map (left), obtained with an exposure time of three weeks of a photo imaging plate. The unit of colour scale bar for DA image is grey value. Spots with grey value 0 (shown as white) contains higher amounts of radioactivity.

Radiation in all of the arthropods in batch 2 was detected in the DA image in Figure 3.7. The figure shows especially high detection in Sample 2 and 7. The scarab beetle in sample 1 is observed with highest accumulation of radioactivity close to its abdomen. Because of high detection of radiation, arthropods from all the samples were used for further analysis of ^{90}Sr. The arthropods marked in sample 4, 6 and 8, were selected from these samples.

Figure 3.8 illustrates the amount of radioactivity in the smallest Chernobyl arthropods by DA.

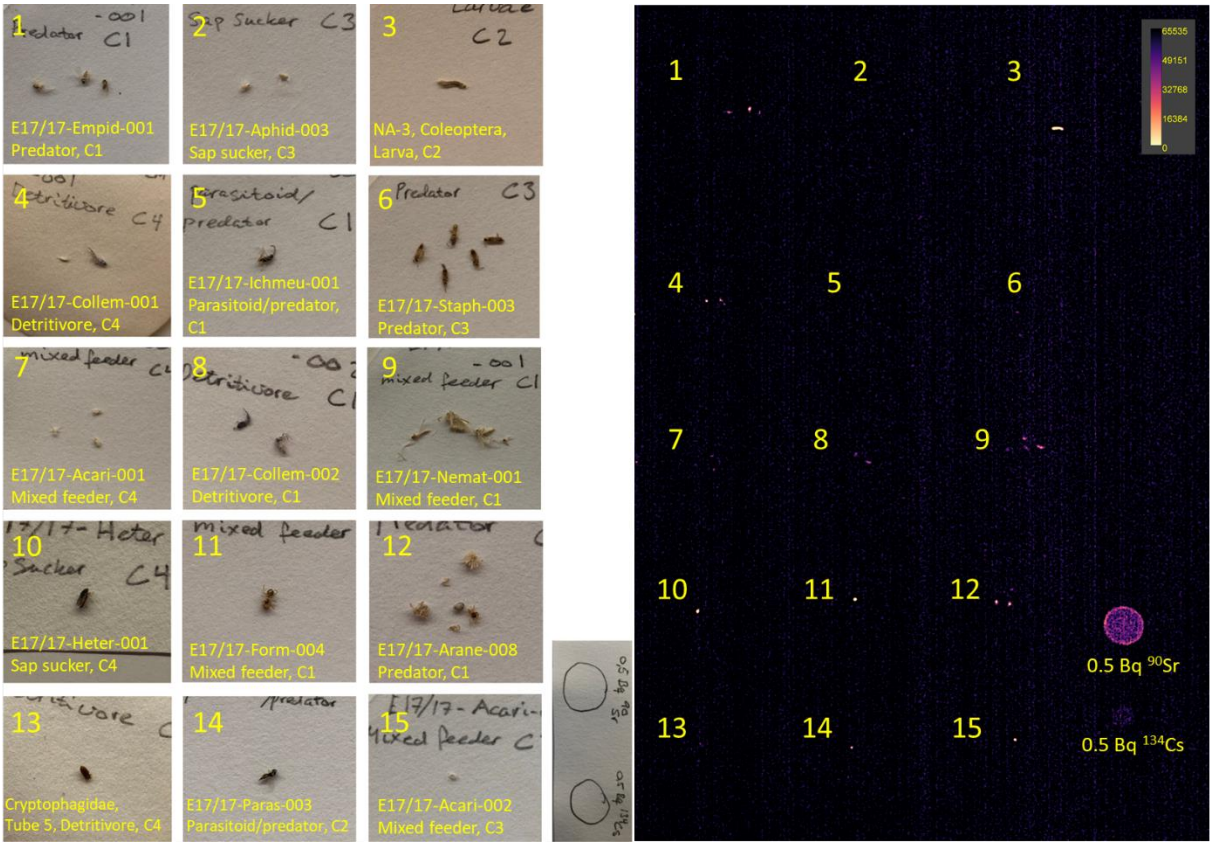

Figure 3.8: Autoradiograph of batch 3 of Chernobyl arthropods (right), as depicted from arthropod map (left), obtained with an exposure time of three weeks of a photo imaging plate. The unit of colour scale bar for DA image is grey value. Spots with grey value 0 (shown as white) contains higher amounts of radioactivity.

Figure 3.8 shows the DA image of batch 3, the smallest arthropods collected. Due to the fact that these arthropods were quite small, radiation has been detected in all of the samples, except sample 2, 5, 6 and 13. Arthropods in sample 10, 11, 15, and especially sample 3 stands out, and were selected for further analysis.

The last batch of Chernobyl arthropods using DA to quantify the amounts of radionuclides are shown in Figure 3.9.

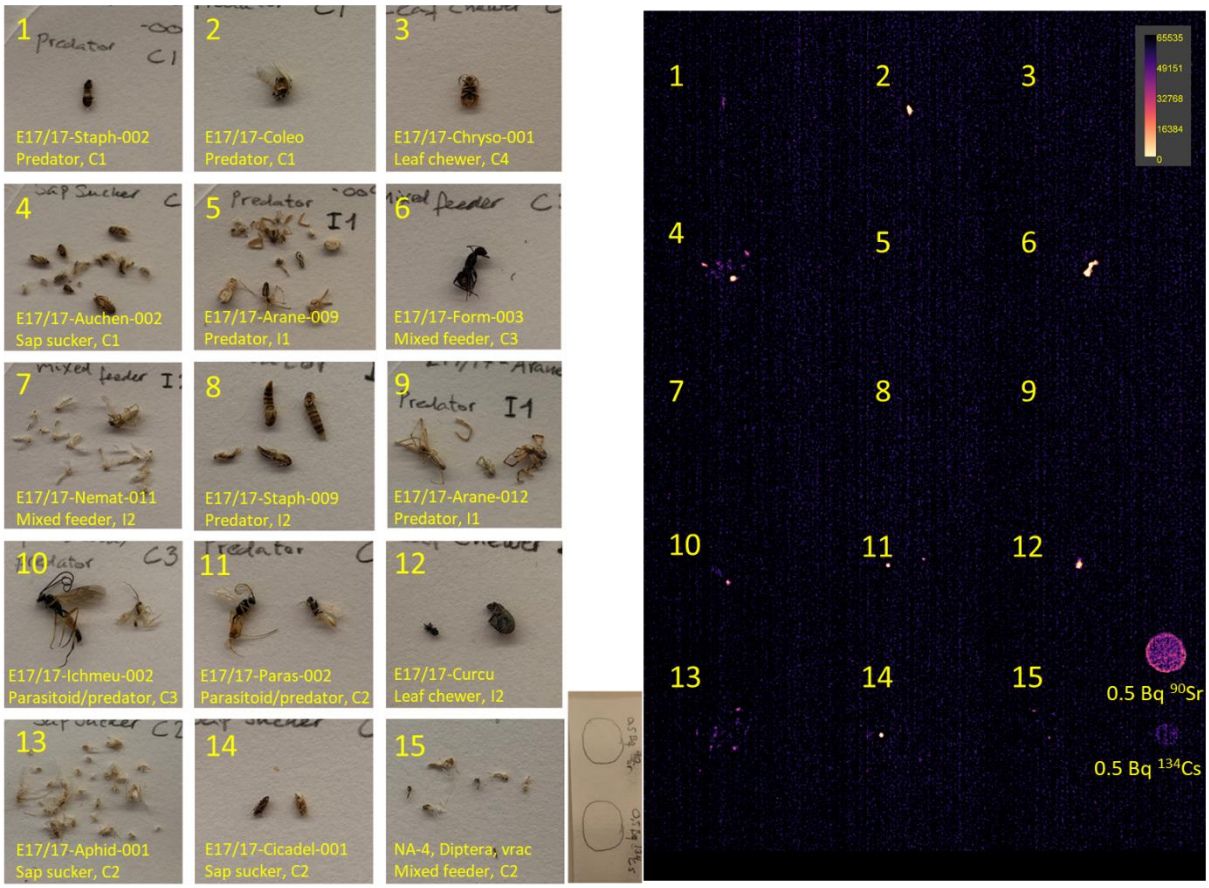

Figure 3.9: Autoradiograph of batch 4 of Chernobyl arthropods (right), as depicted from arthropod map (left), obtained with an exposure time of three weeks of a photo imaging plate. The unit of colour scale bar for DA image is grey value. Spots with grey value 0 (shown as white) contains higher amounts of radioactivity.

The DA image of the arthropods in batch 4, shown in Figure 3.9, shows variation of detection between samples. The arthropods in sample 5 and 7-9 are not visible and all are from site I1 or I2, locations with intermediate contamination of radioactivity. Insects from sample 2, 6, 11 and 12 were selected for further analysis due to low grey values.

4 DISCUSSION

4.1 Quality of method

To summarise, the EXC step using Sr-resin resulted in a very good recovery for Sr (93 ± 8%) and a highly effective removal of Ca and Zr (98 ± 2%). The microcolumns were packed with a resin mass that ranged from 42 to 51 mg (48 ± 2 mg). Columns packed with less resin did not result in a lower recovery of Sr. Samples that ended up with lower yield of Sr could be due to the effect of air bubbles sent through the column during EXC, causing Sr to elute early.

The remaining Zr in the arthropod samples after EXC reacted close to 100% with a mixture of O_2 and H_2 in the CRC of the ICP-QQQ. The main product of the reaction was the cluster $^{90}Zr(OH)_3(H_2O)_2^+$, which had a mass of 177 amu. The highly efficient removal of ^{90}Zr in the CRC indicates that 98% decontamination of ^{90}Zr during EXC is sufficient.

Based on 0.6 mL sample size (median mass of 0.024 g), the method's detection, and quantification limits for ^{90}Sr were 1.1 fg (5.5 mBq) and 3.6 fg (18 mBq), respectively. Measurements of the five in-house standard replicates showed a very good method precision for Ca, Sr, Zr and ^{90}Sr (1-3% RSD) and an acceptable method accuracy for ^{90}Sr of within 10% of added activity concentration.

Both indium and rhodium were added as IS to all of the analysed samples, but the quantified concentrations were only corrected with the use of In. Rhodium (7.46 eV) has a higher first IE than Sr (5.69 eV). This means more Sr will be ionized in the ICP torch compared to Rh if ionization effects are present. Whereas In (5.79 eV), which has a more similar first IE to Sr, is a more suitable internal standard for Sr, with the best correction for loss of analyte and matrix effects during sample preparation and analysis. Since Rh was added to the samples prior to microwave acid digestion, whereas In was added either after digestion or after EXC, Rh was used as IS for Ca and Sr to observe potential sample loss between digestion and EXC. Large decreases in Rh recovery was not observed during ICP-QQQ analysis, indicating little sample loss.

This project focused on the development of a simple and efficient method to measure ^{90}Sr by ICP-QQQ in small biota, such as arthropods. The resultant method enabled measurement of individual arthropods with a mass range of 0.26 to 171 mg, with determined ^{90}Sr activity concentrations from 4.6 to 190 Bq/g. Other studies have reported determination of ^{90}Sr in

different environmental samples by ICP-QMS analysis, which is equipped with a CRC followed by a single quadrupole (Feuerstein et al., 2008; Taylor et al., 2006). Chemical separation using resin was applied in both methods prior to analysis to separate Sr from the matrix, combined with instrument-based separation. The reported detection limits were 0.2 pg/g (1 Bq/g), 0.1 pg/g (0.5 Bq/g), 0.04 pg/g (0.2 Bq/g) and 3 pg/L (5 Bq/g) in soils, sediments, plants, and water, respectively. The recovery for stable strontium was comparable to this thesis but was somewhat lower in plants. Taylor et al. (2006) employed a two-stage EXC using Sr-resin because of the high Zr concentrations in the environmental samples and both studies reported effective removal of Zr with the use of O_2 in the CRC. Taylor et al. (2006), however, observed formation of new interferences with m/z = 90 during analysis. This is not a problem when using an ICP-QQQ, which combines two quadrupoles before and after the CRC. Before entering the CRC, the Q1 will remove all ions with mass not equal to 90 amu when Q1 is set to m/z = 90. This provides a greater control over the reaction chemistry that occurs in the cell and prevents the formation of polyatomic oxides that could overlap with the ^{90}Sr signal (Russell et al., 2017).

Other studies have measured ^{90}Sr in samples of contaminated soil (500 g), following acid digestion and determined by ICP-QQQ without preceding chemical separation. For the suppressing of ^{90}Zr, oxygen gas was introduced in the CRC. The detection limit was found to be 0.2 Bq/mL (0.04 pg/mL) (Amr et al., 2016; Bu et al., 2016). Another method using ICP-QQQ, determined ^{90}Sr in ashed fish bones (49-476 mg) from fish caught in the ChEZ (Reinoso-Maset et al., 2021). Here, samples were chemically separated using EXC with Sr-resin after microwave acid digestion and measured directly in an ICP-QQQ, where ^{90}Zr was removed by the combination of O_2 and H_2 in the CRC. The quantification limit achieved were reportedly 3 pg/L (~1 Bq/g). This is lower than what was observed without separation (Amr et al., 2016), but comparable to the limit achieved in this thesis. Therefore, EXC with Sr-resin in combination with ICP-QQQ based separation to remove interferences provides lower detection limits compared to methods that only use instrument-based separation. The MS methods mentioned above had all relatively short sample preparation and analysis time, i.e., within a couple days. It should, however, be noted that these methods analysed samples with significantly higher mass compared to the arthropods and the samples were usually concentrated by evaporation to 1 mL or more prior to analysis. The mass of fish bone samples measured by Reinoso-Maset et al. (2021) were closest to the mass of the arthropod samples.

The only previous papers measuring ^{90}Sr in arthropods used either LSC (Mietelski et al., 2004; Mietelski et al., 2010) or beta spectrometry (Beresford et al., 2020a). In these papers, each

sample was comprised of multiple arthropod individuals. To measure ^{90}Sr in the arthropods, Beresford et al. (2020) used a beta spectrometer with a thin-filmed (0.1 mm) plastic scintillator detector, which perform a non-destructive, direct measurement of the samples without any radiochemical pre-treatment. The spectrometer in the study was developed to measure ^{90}Sr content in thick-layered samples that do not exceed a ^{137}Cs/^{90}Sr ratio of 30:1 (Gaschak et al., 2011). The use of beta spectrometer has also been performed on ashed fish bone (Reinoso-Maset et al., 2021). In the study by Mietelski et al. (2010), LSC was used to determine ^{90}Sr in arthropods collected at ChEZ, preceded by radiochemical separation using Sr-resin and ^{85}Sr as a chemical yield tracer. Each sample was ashed in a muffle furnace prior to analyses. It took 2 weeks to establish the ^{90}Sr-^{90}Y secular equilibrium and each sample was measured for 13 h. The minimum detection limit for ^{90}Sr in 1 g sample mass and full recovery was estimated to be 0.017 Bq/g (Mietelski et al., 2010). On the contrary, the method used in this project took only a few days, including sample preparation and EXC of individual arthropods, with a significant shorter analysis time of ~3 min/sample on the ICP-QQQ and lower LOD.

The recovery determined by measuring the ^{85}Sr tracer on a gamma spectrometry in Mietelski et al. (2010), led to varied means of recovery in the different arthropod samples (47-93%). The variation in recovery for Sr could have been a consequence of analyte loss due to many steps and separations in the method. Because of measurements on LSC, the samples were separated twice due to retention of ^{210}Pb and its nuclide daughters in the Sr-resin, which can interfere with the ^{90}Sr signal by emitting beta and alpha particles (Gaca et al., 2006; Mietelski et al., 2010). In comparison, the method presented in this thesis achieved a good recovery for Sr, perhaps due to fewer steps in the method and because an additional chemical separation was made unnecessary by the *m*/*z* separation in the ICP-QQQ.

The fact that the samples in this thesis were separated for Sr and matrix concentrations were reduced, makes the determined ^{90}Sr values more accurate than the values reported in the pilot study on the Chernobyl arthropod collection. High matrix concentrations in the samples may have increased the production of matrix effects during ICP-MS analysis. Nevertheless, the data from the pilot study can give a good indication of expected ^{90}Sr levels in the measured arthropods (Table D.1 in Appendix D.1), and for most of the samples the levels of Zr would not be expected to lead to overestimates of ^{90}Sr concentrations.

All the separated samples were redissolved with a volume of 0.5 mL prior to ICP-QQQ analysis to have the option of measuring the samples twice. Lower detection limits for ^{90}Sr could have been achieved by redissolving the samples with a lower volume. For example, a volume of 0.2

mL should be sufficient because each sample had a total acquisition volume of 186 µL during the analysis. With 'pre-emptive rinse' enabled, even lower detection limits can be achieved by concentrating the samples to a volume as low as 100 µL.

To conclude, the method presents quick and simple sample preparation and precise measurements with ICP-QQQ analysis with short analysis time. Additionally, the use of microcolumns allowed the use of small volumes of sample and acid throughout the method and lower detection limits could have been achieved by concentrating the samples even more after separation. Finally, the use of a micro autosampler enabled a total acquisition volume of <0.2 mL for each sample, resulting in little waste during analysis.

4.2 Quantified ^{90}Sr activity concentrations

The arthropods analysed in this work ranged from 4.6 to 190 Bq/g dry weight and were mostly mixed feeders and predators (Table 3.2 and Figure 3.1). Many of these mixed feeders and predators had elevated levels of ^{90}Sr, especially species within the class of *Arachnida* and the *Formicidae* family. Mietelski et al. (2010) observed ^{90}Sr levels ranging from 0.19 to 395 Bq/g ash weight (a.w.). Seven results exceeded 100 Bq/g, which consisted mostly of millipedes, ants, and larvae of *Coleoptera*. The activity concentrations of ^{90}Sr and ^{137}Cs in the study by Mietelski et al. (2010) were comparable, but lower levels of ^{137}Cs compared to ^{90}Sr was observed in millipedes. In comparison, results from Beresford et al. (2020a) determined significantly lower ^{90}Sr levels in different species of beetles and bees, ranging from 0.26 to 2.40 Bq/g fresh mass. For a majority of the samples, ^{90}Sr activity concentrations were comparable to ^{137}Cs, but ^{137}Cs tended to be higher. The highest levels was observed in *Tropinota* spp., a scarab beetle, where the activity concentrations of ^{90}Sr and ^{137}Cs were 2.40 Bq/g and 29 Bq/g, respectively (Beresford et al., 2020a). Whereas the measured arthropods in this thesis and the pilot work (Table D.1) showed ^{90}Sr levels that were an order of magnitude greater than ^{137}Cs in the same arthropods, as reported by Renkas (2019).

Comparing the analysis in this thesis with the data reported from the pilot study (Table D.1 in Appendix D.1), this thesis showed similar or higher levels of ^{90}Sr in mixed feeders and predators from the same site. The pilot study had a greater proportion of detritivores and herbivores in the sample, a number of which had higher levels of ^{90}Sr than the arthropods measured in this work. Renkas (2019) observed, similar to the pilot study, elevated ^{137}Cs levels in detritivores, such as millipedes and scarab beetles. Many of the sampled arthropods are

ground living animals or live their lives among the litter. Additionally, larval and pupal stages of many different arthropod species (e.g., beetles, flies, and moths) may spend time close to the soil layer and the forest litter where radionuclides are known to be accumulated (Møller & Mousseau, 2009; Shcheglov et al., 2014). A Japanese study on radiocaesium transfer in the forest insect food web after the Fukushima accident (Ishii et al., 2017) showed high concentrations in carnivores and omnivores, but detritivores species, that feed on forest litter and fungi, were found to have the highest levels. That study also confirmed that contaminated forest litter and other forest components such as fungi, decaying wood and lichens are important sources of ^{137}Cs transfer into the forest arthropod community. Species of mixed feeders and detritivores who decompose litter and other forest components are therefore likely to have an increased uptake of radionuclides and the results from this thesis and the pilot study does suggest high levels in these groups.

The Millipedes used as control samples all showed elevated levels of calcium and stable strontium. This correlates well with the determined levels in the millipede samples in the pilot work (Table D.1) and is a probable reason for high ^{90}Sr uptake. Millipedes are very important decomposers and live mostly on dead plant matter. Unlike most other terrestrial arthropods, millipedes have reinforced their exoskeleton of chitin by calcification (Borrell, 2004). Therefore, they are often found in places with access to lime, especially in humid deciduous forests (Djursvoll, 2015). Mietelski et al. (2010) reported several millipedes exceeding ^{90}Sr levels of 100 Bq/g (a.w.), which correlates well with the quantified ^{90}Sr levels in the pilot work. Furthermore, Mietelski et al. (2010) observed that millipedes had a relatively low ^{137}Cs:^{90}Sr ratio and a relatively high ^{90}Sr:$^{239+240}$Pu ratio, indicating a greater transfer of Sr in millipedes than Cs and Pu. This correlates with the findings by Renkas (2019), who determined ^{137}Cs level in the same millipede sample (229 Bq/g; site C4) which was determined for ^{90}Sr in the pilot work. Because Sr is a chemical analogue of Ca, it may appear that ^{90}Sr is taken up by millipedes by eating decaying leaves and litter and is regulated by the same mechanisms that calcify the species' exoskeleton. To conclude, Mietelski et al. (2010) stated that high levels of radiostrontium can be expected in arthropods living within the forest litter, having mixed trophic habits and relatively long lifespans, e.g., millipedes.

The three samples from the *Curculionidae* family in this research and the pilot study (Table D.1) had some of the highest accumulation of ^{90}Sr of all the collected herbivores. All three samples have been collected at different sites (I2, C3, C4) and the accumulated ^{90}Sr levels in the samples (29 to 220 Bq/g) correlates with the measured ^{90}Sr values at the collection sites.

The same applies to the larva and imago samples of *Lepidoptera* (Table 3.2 and D.1). The activity concentrations of ^{137}Cs in herbivore, measured by Renkas (2019), was very low in comparison. According to Yoschenko et al. (2019), still after 30 years since the deposition, more than half of the ^{90}Sr inventory in forests within the ChEZ can be found in the aboveground forest biomass or accumulated in litter (Yoschenko et al., 2019). Some species of *Curculionidae* feeds on the bark of small conifer plants and they dig down to the roots of these trees to lay eggs, while many species of *Lepidoptera* are known to be voracious eaters of leaves and burgeons as caterpillars (larval stage) (Krokene, 2020). Additionally, Beresford et al. (2020) observed the activity concentration of ^{90}Sr was an order of magnitude higher than ^{137}Cs in the trunk wood of *Pinus sylvestris* (Scots pine). Concentrations in branches and needles were comparatively high, whilst for cones the concentration of ^{137}Cs were an order of magnitude higher than ^{90}Sr. The high levels of ^{90}Sr in these herbivore insects may give an indication of present plants with high ^{90}Sr content located at the sites with high contamination and, as a result, an efficient source of ^{90}Sr uptake in herbivores. Considering the distribution of radionuclides in *P. sylvestris*, how much and which radionuclides are transferred to arthropods can depend on their diet, specifically which trees, and plants they feed on.

Natural levels of Ca and Sr was measured to evaluate a possible correlation between Ca-Sr and Sr-^{90}Sr uptake in the analysed arthropods. When comparing the Ca-Sr uptake in Chernobyl arthropods (Figure 3.2) with the control samples (Figure 3.5), the arthropods collected at ChEZ have significantly higher levels of Ca and Sr than the Norwegian arthropods. This may indicate that the areas around Chernobyl, where the arthropods have been collected, have greater access to exchangeable forms of Ca and Sr, which can be dependent on soil type. It has previously been said that soil-to-plant transfer of ^{90}Sr will decrease when exchangeable forms of Ca in soil increases (Yoschenko et al., 2019), but a high level of Ca and Sr in the arthropods could be associated with a higher ^{90}Sr uptake. The Norwegian arthropods had a significant larger mass and size compared to those from Chernobyl, and lower levels of Ca and Sr were also observed in the Chernobyl arthropods with larger mass. So, differences could also be influenced by mass. The scatter plots (Figure 3.2 and 3.3) indicate that there is a reasonable correlation between uptake of Ca and Sr (R^2=0.50) and between Sr and ^{90}Sr (R^2=0.42), but it is difficult to say with certainty due to few analysed samples. The Norwegian arthropods, however, showed a stronger correlation between Ca and Sr (R^2=0.77) despite lower levels, indicating that calcium may have some impact on Sr uptake for these samples.

Although the Chernobyl arthropods had in general higher levels of Ca and Sr than the control samples, the natural Sr levels in the millipede control samples were more in line with the millipede samples measured in the pilot study. The calcium levels, however, were significantly high in comparison to the pilot samples. The soil type at site C4 may have been poorer in calcium content compared to the Norwegian millipede collection site, causing lower competition for Sr uptake in plants and millipedes.

Digital autoradiography was performed to give an indication of how much radioactivity the arthropods contained before further analysis. Based on the added 0.5 Bq droplets of ^{90}Sr and ^{134}Cs, beta radiation emitted from ^{90}Sr was better absorbed on the DA imaging plates than the emitted gamma radiation. This suggests that ^{90}Sr, and not ^{137}Cs, contributed more to the emitted radioactivity from each arthropod sample shown on the DA images. In some of the arthropods, the radioactivity was more homogenously distributed. Perhaps these arthropods have Ca rich tissues in their entire bodies where Sr will be distributed, unlike in vertebrates, where Ca and Sr are mostly taken up in bones. Another reason can be that these arthropods were smaller in size or perhaps more radioactive than the other arthropods contained in the same cassette. Possibly they should have had a shorter exposure time or put in a cassette with more similar sized samples. Nonetheless, several of the arthropods showing high autoradiography levels were determined to have high ^{90}Sr activity concentrations, including the ant (*Formicidae*) and the spider (*Aranea*) samples. In other arthropods the radioactivity was distributed heterogeneously, which made it possible to observe which tissues radionuclides had accumulated in. It was observed that the scarab beetle had a high accumulation of radioactivity close to its abdomen, which potentially could have been a radioactive particle the beetle had ingested. This was also observed in the scarab beetles, including the larva, in Figure D.1 (Appendix D.3). However, the imago beetles were some of the samples with the lowest ^{90}Sr activity concentrations (5 to 15 Bq/g), while the larva contained 88 Bq/g. Apparently, radiostrontium were accumulated in their abdomen, most likely from food, and were not distributed homogenously throughout their bodies.

Overall, the determined ^{90}Sr levels by ICP-QQQ was in good agreement between and DA. The determined levels in the pilot work was also in good agreement with the DA image (Figure D.1 in Appendix D.3). In hindsight, the arthropods in the pilot work, especially the millipedes and the weevils, should have been measured individually instead of together because of the high levels of ^{90}Sr which was determined by ICP-QQQ and observed on the autoradiograph. By

measuring them individually, the determined activity concentrations would have been more accurate according to each arthropod's mass.

4.3 Radiation dose rates

The calculated radiation dose rates absorbed by the Chernobyl arthropods (Table 3.4), indicates that high uptake of ^{90}Sr can lead to high internal radiation dose rates (1.0 - 21 µGy h^{-1}), in many cases exceeding the ERICA dose rate screening value of 10 µGy h^{-1}. Compared with the external dose rates, the internal dose rates are of 5 to 6 orders of magnitude greater. Comparing the dose rates from ^{90}Sr with dose rates from ^{137}Cs (Table D.2 in Appendix D.2), shows a similar order of magnitude for total doses for the two radionuclides. This means that arthropod exposure would be underestimated if ^{90}Sr exposures were not accounted for. The external dose rates received from ^{137}Cs (10-18 µGy h^{-1}) are significantly higher than the external dose rates received from ^{90}Sr. The accumulated ^{137}Cs gives a slightly elevated internal dose (0.3-3.5 µGy h^{-1}). Dose rates from ^{137}Cs are more dependent on what the soil activity is compared to what the arthropods have accumulated. Whereas the dose rates from ^{90}Sr are more dependent on the internal accumulation of ^{90}Sr. The radioactive ^{137}Cs isotope is a gamma emitter, and gamma radiation is much more penetrating than alpha and beta radiation, resulting in a significant external dose.

The low external dose rates for ^{90}Sr are expected due to the fact that ^{90}Sr is a pure beta emitter, which is quickly stopped in most media. It has been suggested that shortly after the Chernobyl accident, the soil fauna were affected less than the litter fauna, because soil had a shielding effect against the beta radiation (Krivolutzkii & Pokarzhevskii, 1992). Energy can be deposited if beta radiation has been emitted within short range of the organism. However, beta emitters have to be swallowed or inhaled to have significant energy depositions internally, which increases the internal dose and the potential of causing severe damage to cells and DNA. The internal dose rates reflect this.

The highest soil activity measured at Glubokya Marsh by Newbold et al. (2019), were used for the estimation of external radiation dose rates in the Chernobyl arthropods (Table B.1 in Appendix B). The external doses have higher uncertainty than the internal doses due to high variation of activity concentrations in soil. Therefore, the external doses should be considered as the maximum dose, whereas the internal doses, which are based on determined levels in arthropods, are more reliable dose estimates.

The increase in external dose when the arthropods live in soil is a consequence of radiation exposure from all sides. On the other hand, arthropods who just live on soil or partially in the air will be less exposed to radiation and only from one side. For ^{90}Sr, the change in external dose is not significant whether the organism is living in or on soil, while this is more dependent for ^{137}Cs.

In previous research, radiation dose rates for reference arthropods have been calculated (Table D.3 in Appendix D.2). The reference organisms are based on reference woodlouse and bee. The internal exposure from ^{241}Am shows a higher internal dose than ^{90}Sr, due to its emitting alpha particle. However, ^{90}Sr is of greater concern because of higher amounts in soils and chemical similarity to Ca. When summarising the dose rates into total dose rate per organism, the dose rates becomes relatively high for each of the two reference arthropods (30 and 46 $\mu Gy\ h^{-1}$ for Flying insect and Arthropod (detritivore), respectively). Additionally, the risk will increase when several stressors are considered. The calculated dose rates in Table 3.3 and D.2 have been accounted for only one type of radioactive stressor, either ^{90}Sr or ^{137}Cs. There are multiple radioactive stressors combined with other stressors inside ChEZ that can affect organisms. When considering the different radionuclides that can affect the Chernobyl arthropods, one can assume that the total dose rate per organism would increase, but that the external and internal dose from ^{137}Cs and ^{90}Sr, respectively, would be the main contributors.

The radiation exposure that causes high calculated radiation doses absorbed by the arthropods indicates an increased risk and possibility of DNA damage. However, a few grasshopper studies states that radiation can have caused arthropods to adapt to the elevated radioactivity. A Danish study by Mortensen (2013), documented significantly less DNA damage in grasshoppers from ChEZ compared to an artificially irradiated Danish population and an untreated Danish population of grasshoppers. The study concluded that the chronic radiation in ChEZ has caused adaptation of grasshoppers to survive high radioactivity. The adaptations may have occurred as a consequence of a more efficient protective and repair mechanism to maintain DNA integrity and perhaps reducing the probability of generating mutations (Mortensen, 2013). A study by Bonisoli-Alquati et al. (2018) states something similar, that grasshopper parents that are exposed to ionizing radiation in highly contaminated areas in ChEZ do not affect DNA damage in their offspring possibly due to adaptation. However, DNA damage was observed in offspring that reached maturity at a younger age (Bonisoli-Alquati et al., 2018).

Studies on the effects of radionuclide contamination on leaf litter decomposition within ChEZ have shown more contradictory conclusions. Mousseau et al. (2014) reported that radioactive

contamination has reduced the litter mass loss rate, resulting in an increase of accumulated litter on the forest floor (Mousseau et al., 2014). Another study, by Bonzom et al. (2016), registered the opposite, indicating that decomposers increase litter mass loss when the leaf litter has increasing dose rates (Bonzom et al., 2016). More research and better dose estimates are needed to investigate controversial effects, including how radio-contamination affects ecosystem processes and if decomposition of forest litter contribute to high external and internal dose rates in arthropods.

5 CONCLUSION AND FURTHER RESEARCH

The developed method presented in this thesis has shown a very good recovery for Sr and a highly effective reduction of interfering Ca and Zr in individual, low volume arthropod samples, using developed microcolumns designed for chemical separation with Sr-resin. Subsequently, the ICP-QQQ analysis resulted in low detection limits, precise determination of ^{90}Sr in low volume arthropod samples and efficient removal of isobaric ^{90}Zr interference. Due to rapid sample preparation and short analysis time, the samples were analysed within days.

By testing the method, arthropods showed a wide range of ^{90}Sr activity concentrations, which were in good agreement with the conducted digital autoradiography. The results showed highest levels of ^{90}Sr in mixed feeders and predators. A correlation between ^{90}Sr and stable Sr and Ca levels in the measured arthropods was observed, indicating that Ca may provide a good indicator of stable and radioactive Sr uptake in arthropods.

Dose rate estimations based on the determined ^{90}Sr levels in the analysed Chernobyl arthropods were found to have high internal dose rates, as a consequence of ^{90}Sr uptake. Whereas ^{90}Sr caused considerably low external dose rates, the results showed that the gamma radiation emitted by ^{137}Cs gave significantly high external dose to arthropods in highly ^{137}Cs contaminated soils. Consequently, the estimations showed that ^{90}Sr and ^{137}Cs are the main contributors to the total dose rates in arthropods, and that not accounting for internal ^{90}Sr dose could result in an underestimation of exposure.

The project provided a method that can determine ^{90}Sr in arthropods. However, more work remains to achieve a complete understanding of radionuclide transfer and cycling in ecosystems. More measurements of stable Ca and Sr in soils and plants should be made in future research to better understand the transfer of these elements to arthropods, but also how they influence the transfer of ^{90}Sr. In addition, better dose estimates are needed to investigate the previously reported controversial effects and how chronic exposure affects arthropods.

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Appendix

Appendix A: Product ion scan

These figures is referred to in section 2.4.2. Product ion scan (PIS) for both Sr and Zr were performed to check possible reactions when a mixture of O_2 and H_2 were in the CRC. The product ion scans were performed on an ICP-QQQ. Figure A.1 shows the PIS result performed for Sr.

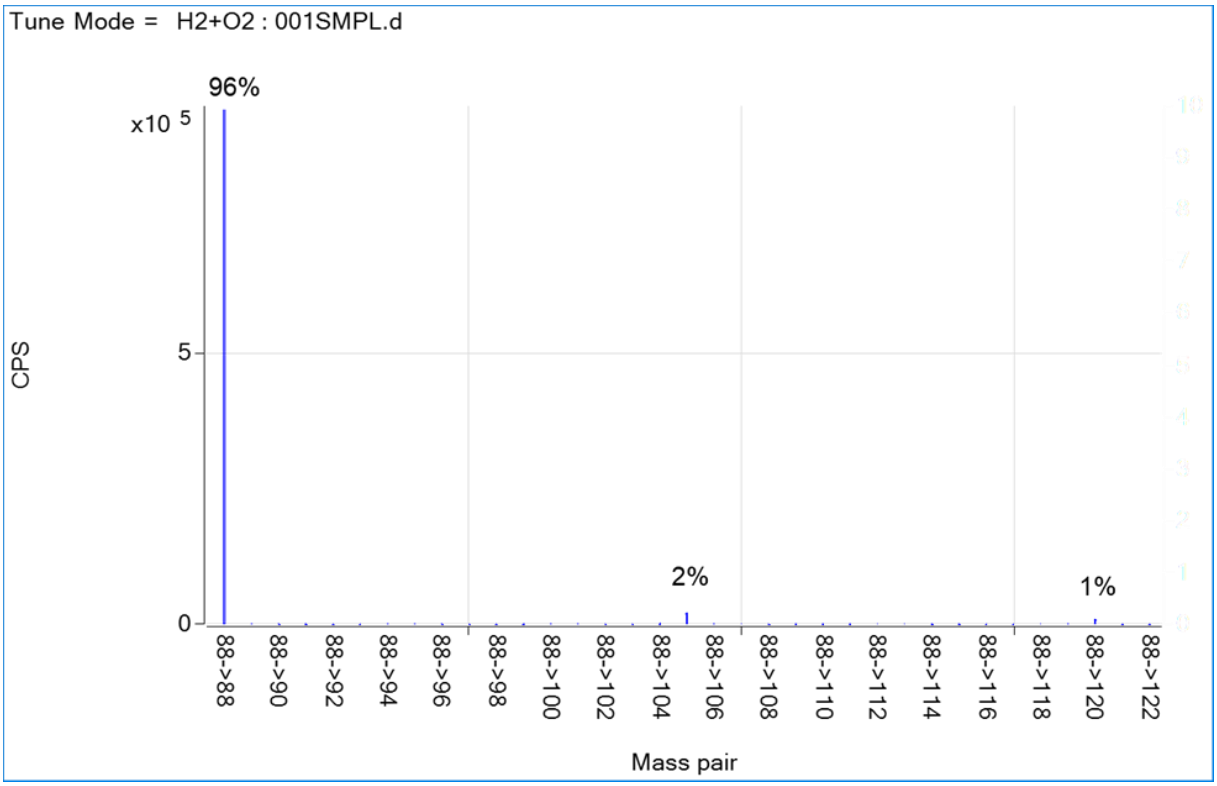

Figure A.1: Possible reactions between Sr and a mixture of O_2 and H_2. The x-axis shows the possible masses the reaction can produce while the y-axis shows the response given in CPS.

The result obtained from the PIS of Zr is shown in Figure A.2.

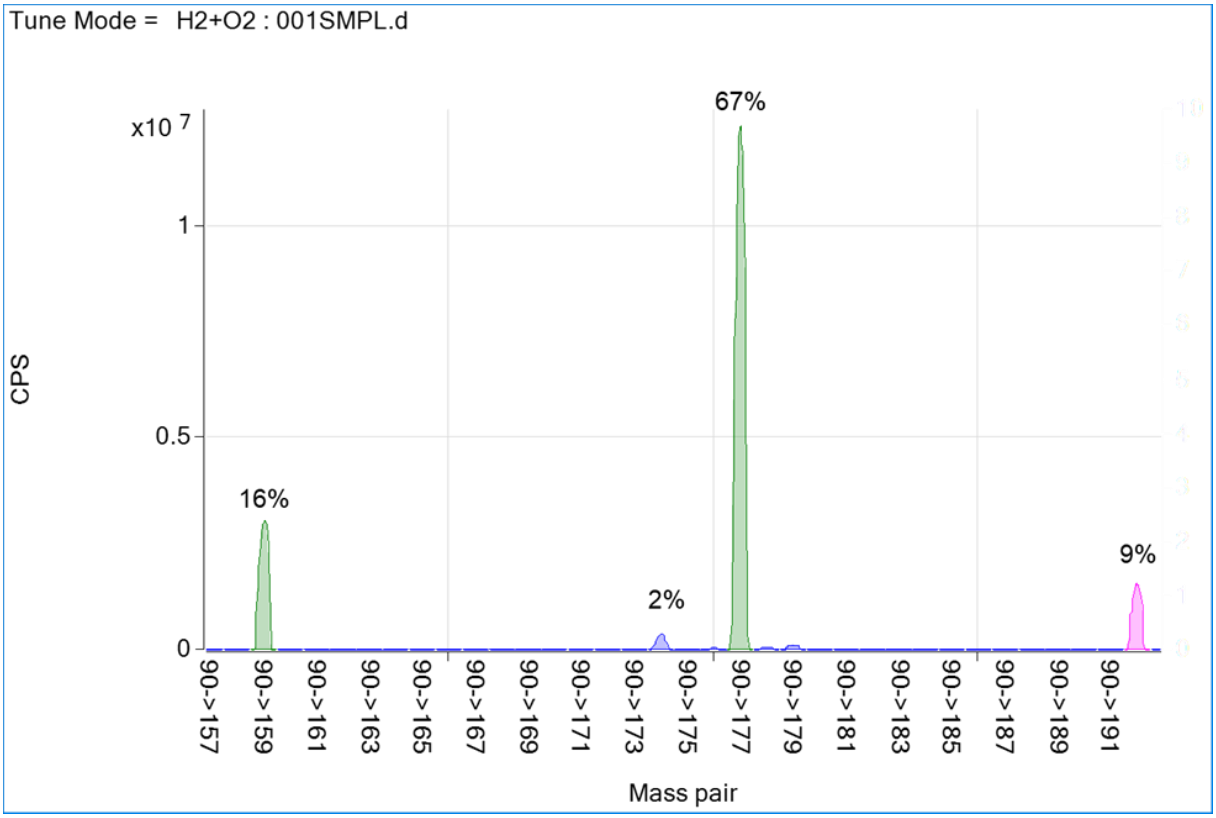

Figure A.2: Possible reactions between Zr and a mixture of O_2 and H_2. The x-axis shows the possible masses the reaction can produce while the y-axis shows the response given in CPS. Colours indicate detector mode. Blue: pulse counting. Green: analogue counting. Pink: mixed analogue and pulse counting.

Appendix B: Soil activity concentrations

This table is referred to in section 2.8. The information given in Table B.1 is obtained from a Chernobyl earthworm study (Newbold et al., 2019) and shows measured soil activity concentration collected at the site Glubokya Marsh, within the Chernobyl Exclusion Zone. The highest soil activity for ^{90}Sr measured at this location was used for the estimation of radiation dose rates in the Chernobyl arthropods. Additionally, the soil activity for ^{137}Cs, ^{90}Sr, ^{241}Am and Pu were used for the estimation of dose in previously measured Chernobyl arthropods and reference arthropods (Appendix D.2). The soil data obtained from Glubokya Marsh was selected because the measured soil concentrations from this area was the highest and in near vicinity of where the Chernobyl arthropods were collected.

Table B.1: Soil activity measured at Glubokya Marsh. Data was obtained from (Newbold et al., 2019).

Site	Northing	Easting	Distance to Power Plant (km)	Habitat	Sample date	^{137}Cs (Bq/g)	^{90}Sr (Bq/g)	^{241}Am (Bq/g)	Pu-total (Bq/g)
H1 Glubokya Marsh	51.445025	30.063611	6.5	Marshland	08.10.2016	86	28	4.1	2.3

Appendix C: Complementary materials

C.1: ^{90}Sr activity in Chernobyl arthropods

Table C.1 is referred to in section 3.3 and is an overview of the taxonomy, including weight and absolute values for all of the Chernobyl arthropods analysed for ^{90}Sr.

Table C.1: Taxonomy, weight, and absolute values of analysed Chernobyl arthropods. All samples were diluted in 0.6 mL. 'Mass' refers to mass weighed after drying in room temperature for 72 h, prior to digestion. Two different weighing scales were used, one to +/- 0.001 g and the other to +/- 0.01 mg. In the table, the masses have been rounded to the last significant figure.

Site	Sample code	Class	Order	Family	Common name	Trophic Group	Mass (mg)*	^{90}Sr (fg)	^{90}Sr (Bq)
C4	NA-1.1	Insecta	Diptera	vrac	Diptera	Mixed feeder	5	38	0.19
	NA-1.2	Insecta	Diptera	vrac	Diptera	Mixed feeder	7	67	0.34
	NA-1.3	Insecta	Diptera	vrac	Diptera	Mixed feeder	4	22	0.11
	E17/17-Opilio-001	Arachnida	Opiliones	Opiliones	Harvestman	Insect predator	2	32	0.16
	E17/17-Arane-001	Arachnida	Araneae	Araneae	Spider	Insect predator	7	140	0.69
	E17/17-Trich-001	Insecta	Trichoptera	Trichoptera	Caddisfly	Mixed feeder	18	190	0.99
	E17/17-Heter-001 [b]	Insecta	Hemiptera	Heteroptera	Heteroptera	Sap sucker	0.24	<3.6	<0.018
C3	E17/17-Staph-pred, lake 10.10.16	Insecta	Coleoptera	Staphylinidae	Rove beetle	Predator	20	76	0.39
	E17/17-Form-003	Insecta	Hymenoptera	Formicidae	Ant	Mixed feeder	1.96	53	0.27
	E17/17-Acari-002 [b]	Arachnida	Acari	Acari	Mite/tick	Mixed feeder	0.04	<3.6	<0.018
C2	NA-2.1	Insecta	Diptera	Brachycera	Fly	Mixed feeder?	6	5.0	0.026
	NA-2.2	Insecta	Diptera	Brachycera	Fly	Mixed feeder?	2	15	0.078

	E17/17-Opilio-002	Arachnida	Opiliones	Opiliones	Harvestman	Insect predator	14	170	0.85
	NA-3 [a]	Insecta	Coleoptera	larva	Beetle	?	1.01	12	0.061
	E17/17-Paras-002	Insecta	Hymenoptera	Parasitica	Wasp	Parasitoid/ predator	1.44	6.6	0.034
C1	E17/17-Syrph-001.1	Insecta	Diptera	Syrphidae	Diptera	Mixed feeder	14	14	0.074
	E17/17-Syrph-001.2	Insecta	Diptera	Syrphidae	Diptera	Mixed feeder	4	7.5	0.038
	E17/17-Opilio-003.1	Arachnida	Opiliones	Opiliones	Harvestman	Insect predator	7	100	0.51
	E17/17-Opilio-003.2	Arachnida	Opiliones	Opiliones	Harvestman	Insect predator	6	60	0.31
	E17/17-Opilio-003.3	Arachnida	Opiliones	Opiliones	Harvestman	Insect predator	2	15	0.078
	E17/17-Coleo	Insecta	Coleoptera	Coccinellidae	Ladybug	Predator	1.61	16	0.079
	E17/17-Form-004	Insecta	Hymenoptera	Formicidae	Ant	Mixed feeder	0.26	9.6	0.049
I1	E17/17-Scarab-004	Insecta	Coleoptera	Scarabaeidae	Scarab beetle	Detritivore	171	160	0.79
	E17/17-opilio-004	Arachnida	Opiliones	Opiliones	Harvestman	Insect predator	7	34	0.17
I2	E17/17-Brachy-010.1	Insecta	Diptera	Brachycera	Fly	Mixed feeder	6	6.6	0.034
	E17/17-Brachy-010.2	Insecta	Diptera	Brachycera	Fly	Mixed feeder	1	6.5	0.033
	E17/17-Opilio-010.1	Arachnida	Opiliones	Opiliones	Harvestman	Insect predator	4	22	0.11
	E17/17-Opilio-010.2	Arachnida	Opiliones	Opiliones	Harvestman	Insect predator	5	26	0.13
	E17/17-Allep-004 [a]	Insecta	Lepidoptera	Lepidoptera	Butterfly	Leaf chewer	20	24	0.12
	E17/17-Curcu	Insecta	Coleoptera	Curculionidae	True weevil	Leaf chewer	3.12	18	0.090

[a] Larva

[b] Samples below LOQ for ^{90}Sr

Appendix D: Supplementary materials

D.1: Pilot study - ^{90}Sr activity in Chernobyl arthropods

Previous pilot work on determination of ^{90}Sr in Chernobyl arthropods collected simultaneously as the arthropods in this thesis, has been performed (Jensen, 2020). However, the arthropods did not go through a clean-up step using Sr-resin after digestion but was instead analysed directly by ICP-QQQ. Isobar ^{90}Zr was eliminated by using a mixture of O_2 and H_2 in the CRC. Activity concentrations of determined ^{90}Sr in the arthropods are shown in Table D.1. Several of the arthropod samples consisted of more than one arthropod and was acid digested together.

Table D.1: Determined concentrations of Ca, Sr, Zr and ^{90}Sr in Chernobyl arthropods analysed directly on ICP-MS. 'N' refers to number of individuals.

Site	Sample code	Class	Order	Family	Trophic Group	N	Mass (g)	Dilution (mL)	Ca (g/kg)	Sr (mg/kg)	Zr (µg/kg)	^{90}Sr (Bq/g)
C4	E17/17-Curcu-001	Insecta	Coleoptera	Curculionidae	Herbivore	4	0.0263	0.2	0.69	4.5	17	220
	E17/17-Form-001	Insecta	Hymenoptera	Formicidae	Mixed	5	0.0275	0.2	1.1	4.8	12	43
	Centipede	Chilopoda	Chilopoda	Chilopoda	Detritivore	2	0.0148	0.2	5.1	34	22	100
	E17/17-Staph-001	Insecta	Coleoptera	Staphylinidae	Predator	11	0.0012	0.1	11	30	74	61
	E17/17-Allep-001	Insecta	Lepidoptera	Lepidoptera	Herbivore	2	0.006	0.1	3.5	10	17	130
	E17/17-Aphid-005	Insecta	Hemiptera	Aphid	Herbivore	19	0.0025	0.1	12	37	22	47
	NA	Insecta	Diptera	vrac	Mixed/ Detritivore?	9	0.0026	0.1	15	35	600	20
	Tube 5 C4-12/10/16	Insecta	Coleoptera	Cryptophagidae	Fungivore	5	0.0018	0.1	11	13	69	67
	Millipede	Diplopoda	Diplopoda	Diplopoda	Detritivore	8	0.0445	0.5	17	420	53	$2.9 \cdot 10^3$

	E17/17-Scarab-001	Insecta	Coleoptera	Scarabaeidae	Detritivore	1	0.3706	10	0.55	1.7	6.3	11
	E17/17-Scarab-002	Insecta	Coleoptera	Scarabaeidae	Detritivore	2	0.2246	5	0.84	2.0	90	15
C3	E17/17-Arane-006	Arachnida	Araneae	Araneae	Predator	10	0.0244	0.2	3.3	13	16	40
	E17/17-Scarab-003 [a]	Insecta	Coleoptera	Scarabaeidae	Detritivore	1	0.0202	0.2	4.6	11	530	88
	E17/17-Nemat-002	Insecta	Diptera	Nematocera	Mixed	5	0.0014	0.1	13	26	36	24
	E17/17-Allep-002	Insecta	Lepidoptera	Lepidoptera	Herbivore	1	0.0219	0.2	2.5	6.6	27	6.9
	E17/17-Curcu-002	Insecta	Coleoptera	Curculionidae	Herbivore	7	0.0406	0.5	1.4	8.0	24	170
	E17/17-Brachy-003	Insecta	Diptera	Brachycera	Mixed	12	0.0526	0.5	2.1	7.8	22	23
C2	E17/17-Form-002	Insecta	Hymenoptera	Formicidae	Mixed	17	0.127	5	0.88	5.2	8.7	27
C1	E17/17-Aphid-002	Insecta	Hemiptera	Aphid	Herbivore	33	0.0028	0.1	13	49	170	22
	E17/17-Brachy-002	Insecta	Diptera	Brachycera	Mixed	31	0.4539	10	0.93	7.5	21	6.5
I2	E17/17-Collem-006	Entognatha	Collembola	Collembola	Detritivore	7	0.0015	0.1	13	38	93	12
	E17/17-Aphid-010	Insecta	Hemiptera	Aphididae	Herbivore	30	0.0039	0.1	7.8	21	34	4.0
	colleo-009	Insecta	Coleoptera	Staphylinidae	Mixed	50	0.0216	0.2	0.52	1.3	$1.7 \cdot 10^3$	160
	E17/17-Myriapoda	Diplopoda	Diplopoda	Diplopoda	Detritivore	3	0.0929	2.5	22	100	20	330
	E17/17-Arane-017	Arachnida	Araneae	Araneae	Predator	26	0.0818	2.5	2.2	5.6	27	11

[a] Larva

D.2: Estimated dose rates

Chernobyl arthropods analysed for ^{90}Sr (Table 3.2 and Table D.1) have previously been analysed for ^{137}Cs (Renkas, 2019). In previous work, estimation of dose rates in a selection of these Chernobyl arthropods exposed to ^{137}Cs were performed in ERICA assessment tool, as well as dose rates in reference organisms based on measured soil activity in Chernobyl Exclusion Zone (Ormaasen, 2020). The dose rates of the Chernobyl arthropods are presented in Table D.2. Information on soil activity, which is used to calculate CR, can be found in Appendix B.

Table D.2: Estimated dose rates in Chernobyl arthropods exposed to ^{137}Cs. For some of the created arthropod models, the weight is based on a collection of several arthropods.

Site	Sample code	Family	Trophic Group	Weight (g)	^{137}Cs (Bq/g)	External Dose rate (µGy h-1)	Internal Dose Rate (µGy h-1)	Total Dose Rate (µGy h-1)	CR	On-soil	In-soil
C4	E17/17-Curcu-001	Curculionidae	Herbivore	0.0263	9.4	9.9	0.38	10	0.11	1.0	0
	Centipede	Chilopoda	Detritivore	0.0148	9.5	18	3.5	22	0.11	0.5	0.5
	E17/17-Trich-001	Trichoptera	Mixed feeder	0.08	4.0	9.2	0.47	9.7	0.046	1.0	0
	E17/17-Scarab-001	Scarabaeidae	Detritivore	0.3706	28	9.9	1.2	11	0.33	1.0	0
	E17/17-Scarab-002	Scarabaeidae	Detritivore	0.2246	3.0	9.9	1.2	11	0.035	1.0	0
C3	E17/17-Arane-006	Araneae	Predator	0.0244	3.0	9.9	0.38	10	0.035	1.0	0
C2	E17/17-Form-002	Formicidae	Mixed feeder	0.127	2.2	9.9	0.26	10	0.025	1.0	0

ERICA assessment tool can be used to assessing risk and calculate dose rates on models based on reference organisms. The tool have two models that represents arthropods: arthropod (detritivore) and flying insect. The arthropod (detritivore) model (0.17 g) is based on the reference organism FASSET Woodlouse and the flying insect model (0.589 g) is based on ICRP's reference bee. Settings and parameters were set on default. Soil activity of ^{137}Cs, ^{90}Sr, ^{241}Am and Pu from Table B.1 were used to calculate the dose rates. Table D.3 shows the estimated radiation dose rates for the two arthropod reference organisms.

Table D.3: Estimated dose rates for the reference organisms Arthropod (detritivore) (woodlouse) and Flying insect (bee). Occupancy factor for Arthropod (detritivore) and Flying insect were set as 1 "in-soil" and 1 "on-soil", respectively.

Isotope	**External Dose Rate (µGy h^{-1})**		**Internal Dose Rate (µGy h^{-1})**		**Total Dose Rate (µGy h^{-1})**	
	Arthropod (detritivore)	Flying insect	Arthropod (detritivore)	Flying insect	Arthropod (detritivore)	Flying insect
^{137}Cs	27	10	1.1	1.3	28	12
^{90}Sr	$4.5 \cdot 10^{-6}$	$4.5 \cdot 10^{-7}$	3.4	4.0	3.4	4.0
^{241}Am	0.025	0.011	13	13	13	13
^{238}Pu	$2.0 \cdot 10^{-4}$	$7.5 \cdot 10^{-5}$	0.94	0.94	0.94	0.94
^{239}Pu	$9.9 \cdot 10^{-5}$	$3.8 \cdot 10^{-5}$	0.89	0.89	0.89	0.89

D.3: Digital autoradiography

In the same pilot work (Appendix D.1), prior to ^{90}Sr analysis on ICP-QQQ of the Chernobyl arthropods presented in Table D.1, the arthropods were analysed using DA (Pettersen, 2021). The photo imaging plates were exposed for three weeks. Figure D.1 shows the arthropods with the highest levels of radiation.

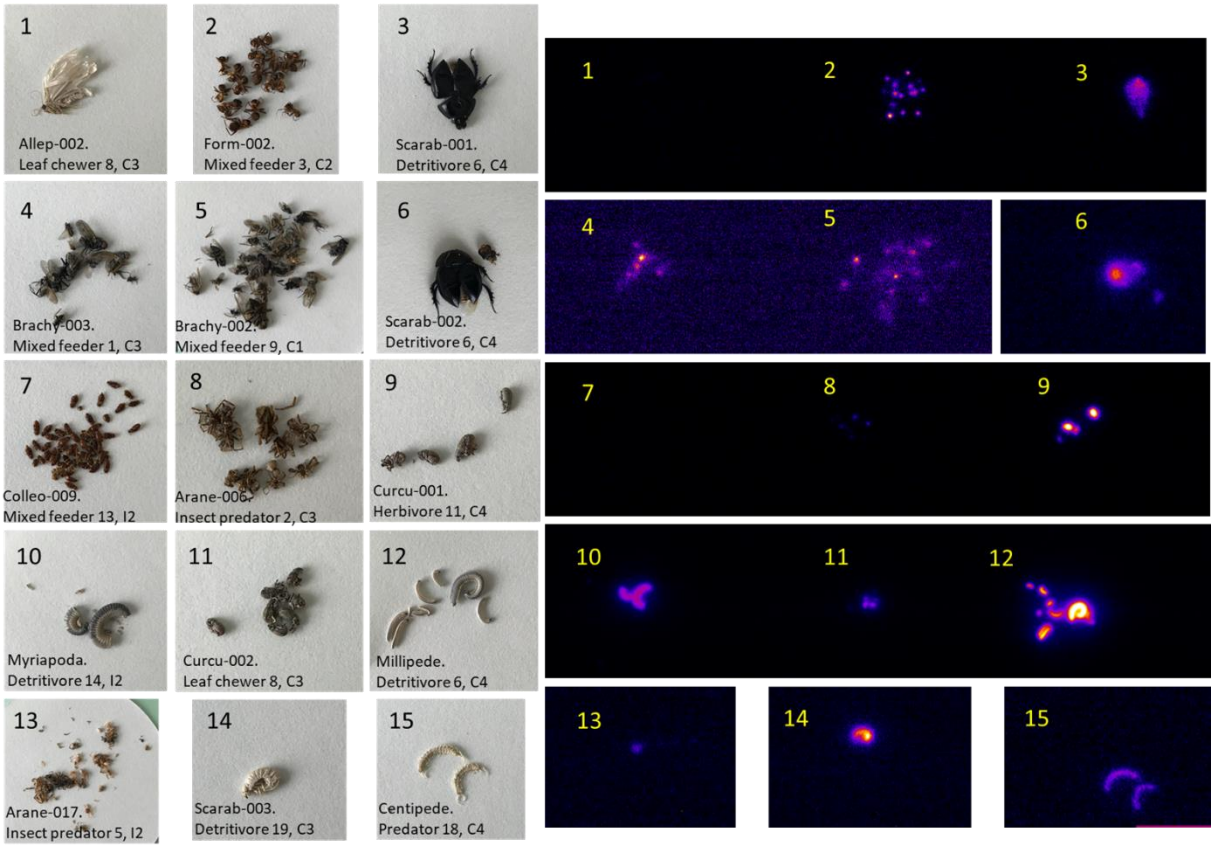

Figure D.1: Autoradiograph of Chernobyl arthropods (right), as depicted from arthropod map (left), obtained with an exposure time of three weeks of a photo imaging plate. The level of brightness indicates the amount of radioactive materials present.

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