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Investigation of Earthworms from the Chernobyl NPP Exclusion Zone and Fukushima NPP 100 km Area: Uptake of ^{137}Cs and Comparison of Ecological Groups

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Summary

Earthworm species inhabit the soil profile, burrowing and feeding on the soil. In the case of a nuclear accident, such as the Chernobyl Nuclear Power Plant (ChNPP) accident in 1986 and the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident in 2011, the soil is contaminated with the resulting deposition.

Earthworm species can be categorised into three different ecological groups, Epigeic, Endogeic and Anecic, based of burrowing a feeding behaviour at different depths of the soil profile. In this study Epigeic and Endogeic earthworms were investigated to determine if these differences have any effect of the uptake of Cs-137. Samples were collected by the COMET, 2017, project as part of the project: "COMET - Initial Research Activity on transgenerational effects and role of epigenetics: Results and Impact", from both the ChNPP Exclusion Zone and the FDNPP 100 km Area. In addition to this samples were also tested for evidence of uptake of Cs-137 from the gut contents into the tissue.

A Mann-Whitney non-parametric test was applied to the ecological groups from the ChNPP Exclusion Zone, which resulted is the difference between the Epigeic and Endogeic earthworm groups not being statistic significant, therefore, it cannot be concluded that there is a difference in the uptake of Cs-137 between the two ecological groups. However, very few sample sites had examples of samples from both ecological groups, therefore It was not possible to eliminate the factor of some sites having a higher activity than other sites, this was a problem for the Endogeic earthworm group which had one sample much higher than the rest, resulting in the mean being skewed.

The uptake in the skin never exceeded 10% of the gut contents, however there was evidence that there was a small amount of uptake into the tissues from both the samples at the FDNPP 100 km Area and the ChNPP Exclusion Zone. Many of the skin samples of earthworms collected from the ChNPP Exclusion Zone did not exceed the L_f and therefore were collectively measured using the Ge-detector. A reason for not as much activity in the skin of samples from this sample location as over time Cs-137 has been bound to clay minerals, preventing the bioavailability of the Cs-137 for uptake.

Introduction

The effects of very high doses as a result of nuclear accidents on soil invertebrates are observed to be detrimental to populations in most areas contaminated by radionuclides. Doses of 30 Gy resulted in the mortality of eggs and immature invertebrates leading to a decline in biodiversity in affected areas as well as causing reproductive failure in adults (Beresford et al, 2016, and Krivolutsky et al, 1999). Within the first two months following the Chernobyl Nuclear Power Plant (ChNPP) accident in 1986 invertebrate populations inhabiting leaf litter in forests five to seven km were reduced by a factor of 30. Within one-year post-accident reproduction of invertebrates continued, in part due to migration into the area from adjacent areas with less contamination. which was only able to recover nine years after the ChNPP accident (Krivolutsky et al, 1999).

After the accident at the ChNPP in 1986, in the initial deposition of fallout, the daily does on the soil surface was recorded to be more than five to seven Gy. Up to 50% of an earthworm's dose is from the penetration through the cuticle, the outermost layer above the epidermis, with the remaining 50% from the soil they ingest. within a radius of 2-7 km from the site, in the first few months following the ChNPP accident, up to 90% of soil dwelling biota, including earthworms died, the majority of which was the death of egg clutches and young hatchlings, (Zaitsev et al, 2014).The juvenile stages of earthworm species exhibit the same LD_{50} as mice , despite this, adult earthworms are one of the most radioresistant multicellular animals (Geras'kin et al, 2008, and COMET, 2017).Earthworms and other detritivores consistently have the highest activity concentrations in comparison to other invertebrate species in radionuclide contaminated environments (Copplestone et al 1999).

The International Commission on Radiological Protection (ICRP) (2008) has recognised the importance of protecting the environment against the detrimental effects of ionising radiation. In order to create a numerical reference between exposure, dose, effects and consequences a set of reference animals and plants were created. This can be used to aid in the use of dose conversion factors for the calculation for internal or external dose of 75 radionuclides. These parameters are based on the previously defined "Reference Man" (ICRP, 1975), later extended to the reference person (ICRP, 2007), taking into account factor such as: the quality of data available, representation of morphology and taxonomy including reproduction biology and lifecycles. The Earthworm was chosen to represent as the reference for a terrestrial annelid, assumed to have the characteristics as from the family Lumbricidae, produce five cocoons per week hatching after four weeks, hatchling taking 10 weeks at reach sexual maturity and to live for up to four years.

Aims and Objectives

This study aims to explore the uptake of radionuclides in earthworm species collected from the ChNPP Exclusion Zone and the FDNPP 100 km Area. Focusing on the differences between earthworm ecological groups and the distribution through the body, comparing the skin and the gut contents. Key research of the effects of radionuclides and earthworm species displays a bias towards representing Epigeic earthworms, especially *Eisenia fetida,* and many studies use laboratory experiments. This study aims to investigate samples taken from areas that had experienced a nuclear accident and investigate if the findings of laboratory studies apply to these in-situ samples.

Objectives of this study are:

- The objective of this study is to identify if there is a significant difference between the activity and the ecological groups of earthworms: Epigeic and Endogeic.
- Additionally, to determine if the activity measured was due to the gut contents or if there was transfer to the skin.

Literature Review

The FDNPP and ChNPP accidents have been extensively studied to understand the dynamics and effects of the resulting fallout. As Earthworms are soil invertebrates their habitat and food source are within the soil matrix. The deposition and migration of radionuclides through the soil profile results in an external and internal interaction with earthworm species. However, as a result of the behaviour of the earthworm ecological groups, the depth of the radionuclides may limit the extent of their interactions. The study of earthworm anatomy was necessary to understand the uptake of radionuclides from the consumption of contaminated soil, through the digestive tract, potentially resulting in the skin and organs. The review of the previous literature in all areas of radionuclides, earthworms and soil interactions gives insight into the mechanisms at work.

Distribution of Radiocaesium in Soil Over Time

In cases such as the ChNPP accident and the FDNPP Accident the fallout immediately processing the event would settle on the surrounding area. Over time it is expected that in this area mostly devoid of human activity would see a migration of the fallout contents over time through natural mechanisms such as gravity, transport via rain, runoff, and surface water, and disturbances from wildlife. After the events of both accidents a number of studies have investigated the migration of radionuclides in the soil matrix and the factors that impact this.

In coniferous forests within the FDNPP exclusion zone, 10 months post-accident, it was found that 99% of the Radioactivity in samples was from within the upper 10 cm, of which had an organic matter content higher than 10% (Teramage et al, 2014). In addition to this 52% of the Cs-137 that was attributed to the FDNPP accident fallout was found within the raw organic layers, litter and layer of decomposing organic matter, with the remaining portion below the layer of decomposing organic matter. Accumulation of Cs-137 in the layer of decomposing organic matter made up 47% of the soil inventory of samples, which retarded subsequent migration through the soil profile. However, some of the Cs-137 that was attributed to the FDNPP accident fallout was observed at a depth of 16 cm which could be attributed to infiltration of radiocaesium contaminated rainwater that may have penetrated to a deeper depth in the soil profile before adsorption started. This deeper contamination is not representative of long-term migration dynamics, but however shows the effects of the initial fallout (Teramage et al, 2014). This effect continued to be observed in samples taken up to 18 months after the FDNPP accident. Between 51% and 92% of radioactivity was detected in the litter and layers of decomposing organic matter, with variation attributed to root uptake due to the season at which the samples were taken. By the time of the sampling 18-months post-accident Teramage et al, 2016, theorised that the radioceasium has been fixed by the clay minerals in the soil matrix as a largely irreversible sorption equilibrium resulting in the radionuclide being unavailable to other environmental compartments, and that future decrease in levels in the ecosystem would be determined by the physical radioactive decay (Teramage et al, 2016).

Soil samples collected in 2012 and 2013 from various locations within the 20 km of the FDNPP site, (the designated exclusion zone). The samples were collected from forest and grassland sites that were in the direction of the plume, therefore the sample sites have moderate to high contamination levels. Analysis of the samples showed that empirical migration velocities of Cs-134 and Cs-137 were found to be between 0.9 and 3.5 cm y^{-1}, with slower migration in grassland compared to forest soil. This study also indicated that the migration velocity of Cs-134 and Cs-137 increased with increasing organic matter content. Compartment models also found similar results; however, the results of these models did not prove to be statistically significant and could benefit from further study. (Mishra et al, 2018).

Previous land use before the deposition of fallout and contamination of the soil can affect the pattern of migration of Cs-137. Forested areas experience secondary deposition from contaminated leaves falling in the autumn, causing more retention of radionuclides in the leaf litter. Grassland areas however do not experience the same effect and vertical migration from the leaf litter to the surface soil was much faster than forested sites (Takahashi et al, 2015). After two years the Cs-137 inventory in the leaf litter in previously agricultural fields, tobacco and paddy fields, was reduced to less than 3.4% compared to 18-41% in the forested sites. The continued downward migration of Cs-137 to the subsurface soil layer was observed in Paddy fields, which two years post-accident 25.5% of total Cs-137 was observed to a depth of 5-10cm (Takahashi et al, 2015). With migration of the majority of total Cs-137 inventory, as a result of the FDNPP accident fallout, from the leaf litter within two years continuous research is needed to understand and predict the migration of Cs-137 long-term. By 2017, six years post-accident, 80-95% of deposited Cs-137 in forested areas had migrated and distributed to the mineral soil layers (Takahashi et al, 2018).

From maps created of the Fukushima Prefecture before the FDNPP accident (MLIT, 1971) the area can mostly be identified as brown forest soils, which care either Cambisols or Stagnosols, however the area is also urbanised and previously used for agriculture which can affect how homogenous the soil profile is with depth. When the soil has a homogenous profile the location of 90% of the radicaesium inventory can be as deep as 17 cm just one-year post accident, compared to an average of depth of 90% of the radiocaesuim of all sample site of 3.01 cm (Matsuda et al, 2015).

In the initial few years after the ChNPP accident the rate of migration if Cs-137 was 0.3 cm/h through each organic horizon of the soil of forests and 0.2 cm/h for grassland soils (at the time of this study the migration had not exceeded the O_r horizon for forest soils and A_h) (Schimmack et al, 1989). A 25 year long monitoring study of the deposition and migration of Cs-137 in the ChNPP Exclusion Zone soils. In the initial months after the ChNPP accident nearly all of the deposition was contained within the leaf litter however in the following years the Cs-137 migrated to the soil profile at various rates depending on the physio-chemical properties and ecosystem features such as the thickness of the leaf litter. After four to five years less than 1% of the Cs-137 was contained within the leaf litter and after 25 years 50% had moved to the deeper layers of the soil however only up to a depth of 5 cm (Shcheglova et al, 2014). Autoradiography conducted by Korobova et al, 2014, has also been applied to soils from the ChNPP Exclusion Zone. This displayed a patchy distribution of Cs-137 in the upper 5cm. The distribution of Cs-137 followed that patters of the distribution of organic matter. The vertical migration of Cs-137 in the ChNPP Exclusion Zone may be delayed due to fixation in the podzolic sandy soil containing K feldspars (Korobova et al, 2014).

However, distribution of the fallout from the accident can also be resuspended as secondary airborne contamination due to natural process and anthropogenic activity, such as agricultural activity and timber processing (Hollände and Junker, 1995).

Earthworm activity may also be a factor in the migration of radionuclides in the soil profile through burrowing and feeding activities. Within five to 20 years earthworms have the ability to turn the topsoil of a grassland environment once and potentially transport 2 kg m^{-2} $year^{-1}$ of dry matter from the deep soil to the topsoil layer, creating a more homogenous mixing of the soil profile. This effect is particularly noted from earthworm species of the Anecic ecological group due to the behaviour of using vertical casts, consuming matter from topsoil and leaf litter layers and redepositing in the deep soil layers. With the soil and organic matter that is consumed, transported and deposited in radionuclide contaminated areas the transport of these radionuclides will be transported and mixed throughout the soil profile with this process (Müller-Lemans and von Dorp, 1996). Modelling of bioturbation of Cs-137 in Sweden concluded that biological transport mechanisms were the dominant factor effecting the downward migration of radiocaesium in that sample site. the study found that deeper penetration of Cs-137 was a result of non-local mixing through endogeic and Anecic earthworm species engaging in vertical burrowing activities deeper into the soil profile (Jarvis et al, 2010). Additionally, burrowing activity of moles can also disturb the vertical migration of radionuclides. In the ChNPP Exclusion Zone between 2010 and 2016 a comparison of a plot disturbed my moles and an undisturbed control plot the centre of the Cs-137 migration was deeper by a factor of three (Ramzaev and Barkovsky, 2018).

Factors Affecting Uptake from the Soil Medium

Transfer from the soil into the organism can be affected by various factors that prevent or enhance the uptake by Earthworm.

Initially following the ChNPP accident earthworms and invertebrates inhabiting a depth of below 5 cm depth in the soil profile were not observed to have the catastrophic mortality observed in soil invertebrates inhabiting leaf litter of forested areas. This difference of mortality rates was due to the shielding protection the soil gave from the β-radiation, of which would have been 94% of the total dose. (Krivolutsky et al. 1999). This highlights that shielding is a factor that prevents initial exposure: Endogeic earthworms have limited interactions with the surface soil and leaf litter, in the initial deposition this lack of interaction would influence the exposure through diet. This effect would be negated by the movement of radionuclide through the vertical soil profile over time.

Uptake of radiocaesium can be influenced by the analogue potassium due to their chemical similarities allowing for uptake at sites in place of the other, as well as competition between the two elements. In an environment with a deficiency of potassium by weight, radiocaesium would receive preferential uptake by the organism in place of the analogue (Whicker, 1983).

In addition to this radiocesium is easily bound to clay, such as the common micaceous clay mineral illite, and the longer the sorption time the less reversible the sorption process

(Gobran et al (ed.), 2000).The Cs is trapped in the illite layers as a result of the collapse of the frayed edges of the sorption sites, this results in the Cs ions becoming immobile and preventing the Cs from being bioavailable (Fuller et al, 2015). This effect was observed by Hasegawa et al, 2013 with found that earthworm species dwelling in and consuming soil, such as the Endogeics and Anecic groups, may be prevented from assimilating C-137 into the body due to the binding of the Cs-137 with the clay minerals in the soil (Hasegawa et al, 2013).

Distribution of Radiocaesium Within the Body (Earthworm)

Several studies have investigated the distribution of radioactivity and radionuclides throughout the body of an earthworm sample, with the comparison between the earthworm samples and soil samples, and also between the skin and the gut.

Detritivore invertebrates, such as earthworms, exhibit consistently higher Cs-137 in comparison to all other groups of invertebrates, this increased level of Cs-137 was observed in the Fukushima area in the following years after the accident (Hasegawa et al, 2015).

A study by Fijiwara et al, 2015, investigated the uptake and retention of radiocaesium in Epigeic (*Eisenia fetida*) earthworms cultured in a laboratory by Prof. Gamou, Kyorin University exposed to soil samples collected from within the Fukushima Prefecture. The earthworm samples were exposed to the soil samples for between 1 to 36 days, removed in intervals of either 1, 2, 6, and 36 days or 1, 2, 4, 7, 14, and 22 days, depending of the soil sample. Some earthworms were transferred to soil containing no radiocaesium after one week of exposure in soil containing radiocaesium. After measurement of earthworm samples on a p-type high-purity germanium detector results showed that uptake was initially high however after a period of culturing the concentration ratio stabilised at approximately 0.02–0.06. Autoradiography results showed that the majority of the radioactivity was coming from the gut contents, with no accumulation in specific tissues. After transfer to soil containing no radiocaesium the detection of radiocaesium in the earthworm sample's bodies did not meet the detection limit even after one day of culturing, reflecting the speed of digestion and metabolism in the *Eisenia fetida* (Fujiwara et al, 2015).

Whilst Fujuwara et al, 2015, exposed laboratory bred earthworms to soil samples collected from the field, Tanaka et al, 2018, sampled both soil and earthworms from a site 40.1 km from the FDNPP. Soil samples were collected to a depth of 5cm and Epigeic earthworms, identified and from the Megascolecidae family, were collected from the leaf litter from 2014, 2015, and 2016. Samples were also measured in a high-purity germanium detector and autoradiographic images were taken. Similar to Fujumara's results the autoradiography images showed that the distribution of the radioactivity was contained within the gut, with post dissection radiographic images combined with quantitative measurements from the germanium detector showed that 95% of the Cs-137 was from within the intestines (Tanaka et al, 2018).

The factors controlling uptake of radionuclides into the tissue of the earthworm may be influenced by the source of food consumed, which would be decided by the ecological group to which the species belonged. Assimilation of radiocaesium (Cs-134 and Cs-137) in Aporrectodea longa, an Anecic species, fed on contaminated soil and apple leaves (Brown and Bell, 1995). Retained and assimilated radioactivity was found to be 5 - 25% for soil fed worms and 55 – 100% for leaf fed worms before the gut contents was eliminated. Dry weight transfer factors (Concentration in worm tissue/ Concentration in substrate), post gut clearance, showed little variation between the two feed sources: 0.04 and 0.04 for worms fed on radiocaesium contaminated soil, and 0.03 and 0.05 for worms fed on radiocaesium contaminated apple leaves. The transfer factor results show a negligible difference between the two feeds, however the higher assimilation of the organic matter in the apple leaves may increase the transfer of radiocaesium to predators of earthworms (Brown and Bell, 1995).

In a study by Sheppard et al, 1997, following the initial quick depuration by gut clearance of the earthworm samples, radiotracers: I-125, Cs-134, Mn-54, Zn-65, and Cd-109, were measured for the physiological depuration. The mean half-time for gut clearance for all radiotracers was 1.4 days, in contrast the physiological depuration from the tissue took longer, up to 210 days for I-125. Despite the short half time associated with the loss of Cs-134 from tissues, just 24 days, after 80 days 20% of the total concentration still remained present in the tissues of *Lumbricus terrestris* (epi-Endogeic species).

Effects of Radiation to Earthworms

Comparisons of the acute lethal dose range (Gy) and taxonomic groups has been compared by Whicker and Schultz, 1982, in Figure 1. It is clear in this graph that the most resistant mammal, the highest point of the acute lethal dose range (Gy) for this group, is less resistant than the least resistant insect, the lowest point of the acute lethal dose range (Gy) for this group, and all taxonomic groups higher than this: Moss, Lichen, Algae, Bacteria, Protozoa, Molluscs and Viruses. Whicker and Schultz, 1982 noted that humans were among the most sensitive mammals and therefore makes them one of the most sensitive organisms.

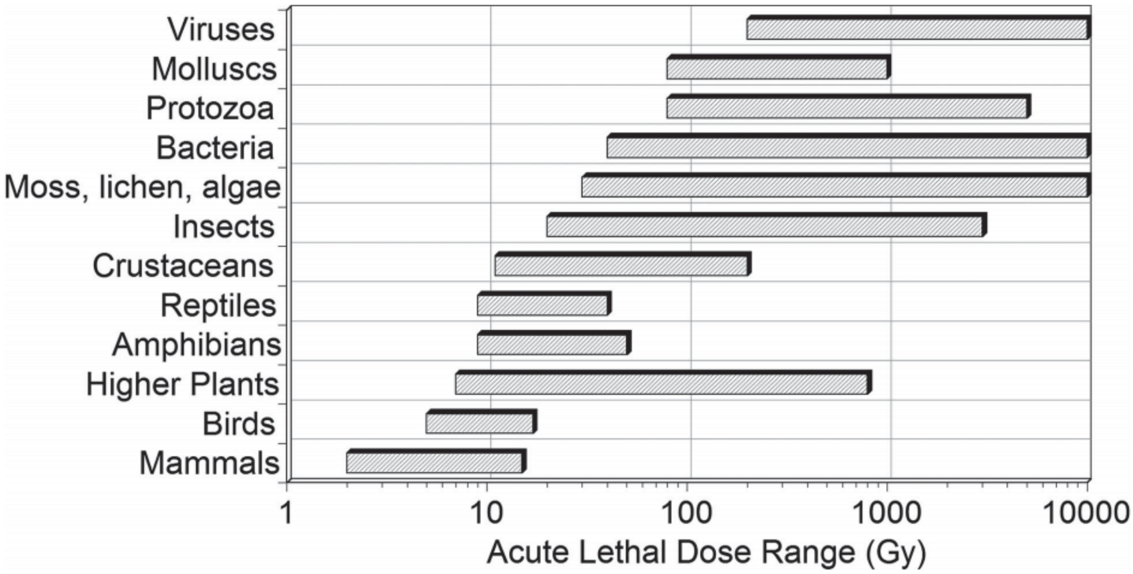


Figure 1, " Acute dose ranges that result in 100% mortality in various taxonomic groups. Humans are among the most sensitive mammals and, therefore, among the most sensitive organisms” (Whicker and Schultz 1982).

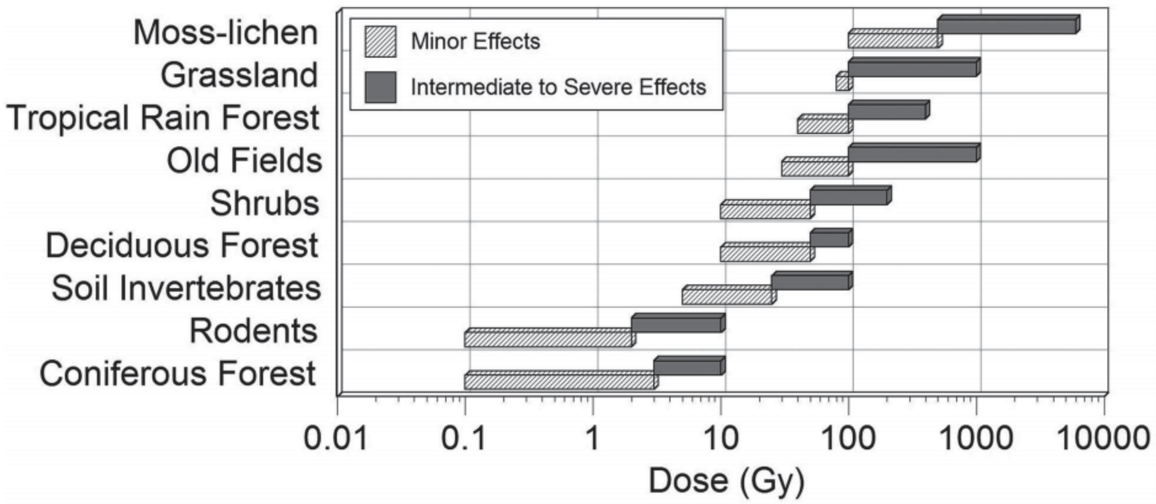


Figure 2, " Range of short-term radiation doses (delivered over 5 to 60 d) that produced effects in various plant communities, rodents and soil invertebrates. Minor effects include chromosomal damage, and changes in productivity, reproduction and physiology. Intermediate effects include changes in species composition and diversity through selective *mortality. Severe effects (massive mortality) begin at the upper range of intermediate effects" (Whicker and Fraley 1974; Whicker 1997).*

Whicker continued to study this in 1997, and with Fraley in 1974, subdividing soil invertebrates into their own category in Figure 2. This graph shows the range of dose (Gy) required for minor effects, including, changes in productivity, chromosomal damage, changes to reproduction, and physiological changes, and intermediate and severe effects, including ultimately death. In the ICRP, 2008, document: Environmental Protection - the Concept and Use of Reference Animals and Plants (RAPs) the observations on morbidity of earthworms included: posterior regeneration was inhibited at 200 Gy, growth was inhibited at 100 Gy, the epidermal cells and reductions in testicular cells showed signs of affect at 5-20 Gy (although recovery was possible over time even with chronic exposure) and abnormalities in earthworm juveniles were observed at 264 mGy/day. Observation of mortality of adult earthworms noted that the $LD_{50/30}$ was at 650-680 Gy and hatching success was affected when cocoons were exposed to 264 mGy/day or when the adults were exposed to 20 Gy before eggs were laid. For reference the $LD_{50/60}$ for Human is a midline dose of just 4 Gy.

Ecological Groups

While collectively referred to as Earthworms, a system of has been created to categorise species into ecological groups that reflect feeding and burrowing behaviours.

Earthworms belong to the order of Oligochaeta, a subclass under the Phylum Annelida, which includes more than 8000 species from approximately 800 genera. Earthworms are found across the globe in most habitats other than extreme environments, such as deserts and permanent snow and ice. Earthworm species belonging to the family Lumbricidae, commonly found throughout Europe, are extremely invasive and wide spread, often outcompeting indigenous earthworm fauna. (Edwards (ed.), 2004). Of the 77 identified species of earthworms in Japan the majority are from the family Megascolecidae, 52 species, compare to just 14 species from the family Lumbricidae (Blakemore, 2003).

The division of earthworm species into groups based on behaviour and habitat has been discussed and debated over time, starting with the recognition between species living and feeding above soil in the leaf little and species living below the litter layer, in the top soil and subsoil. In 1971 and 1977 Bouché divided and categorized earthworm species with the terms Epigeic, Anecic and Endogeic. These categories have been widely accepted by the literature (Edwards and Bohlen, 1996).

Epigeic earthworm species dwell within the upper layer of the mineral soil, the soil surface and within the leaflitter. Inhabiting the organic horizon these species will mainly consume decaying organic matter, including animal debris and agricultural produce. Compared to species of other ecological groups, Epigeic earthworms are usually smaller and have a darker pigment. Species that fall within this group have a high reproduction rate and grow rapidly; these features allow for the adaption to dramatic changes in their environment. (Edwards and Bohlen, 1996; Ray (ed.), 2018).

Anecic earthworm species inhabit permanent vertical burrow systems extending up to several meters in depth into the soil profile enabling them to traverse though out both shallow and deeper depths. At night these species will emerge from their burrows to feed primarily on leaflitter, manure and organic matter in the beginning stages of decomposition. This action is an important process in the mixing and incorporation of organic matter into the deeper soil profile, this is significant pedological process contributing to nutrient cycling and soil formation. Anecic earthworms are characteristically large in size and darker in colour, compared to the other earthworm ecological groups.

Endogeic earthworm species live and feed within the soil, they are unpigmented and feed on decomposing roots and soil organic matter. These earthworms' species can be further divided into polyhumics, mesohumics and oligohumics (Lavelle and Spain, 2005). Polyhumic species ingest high organic content soil in the upper organic soil layers, despite 75% of soil organic matter is bound to clay particles. Mesohumic indiscriminately consume both mineral and organic particles in the upper 10-12 cm of the soil profile. Oligohumic species, found in tropical environments, feed on poor quality organic matter in the deep horizons of the soil profile, depths of about 30-40cm (Lavelle, 1988).

Earthworm Anatomy

Although earthworm species have been categorised into separate ecological groups based on habitat behaviours, however, earthworms have a simple body structure will little variation between different earthworm species.

The anatomy of earthworms is thoroughly described by Berman, 1985. An earthworm has a dorsal side (the upper side) and ventral side (the lower side), these can be identified by colouring as the dorsal side is a darker colour and the ventral side has bristles (setae) along the length of the body, used for movement, grip and aid in burrowing. There are four pairs on each segment of the body with the exception of the first and last segment. Additionally, on the ventral side there are small pores along the body wall which are also used for breathing, keeping the skin moist and waste excretion. These are positioned on all segments other than the first three and last segment along the body. Each segment is separated by a thin wall called the septum. Closer to the anterior of the body of a sexually mature earthworm is the clitellum used in reproduction where a cocoon is formed for the development of offspring. Earthworms are hermaphrodites as they produce both sperm cells and egg cells by the respective male and female sex organs however require another member of the same species to reproduce with (Berman, 1985).

The digestive tract of earthworm species is a simple structure, the alimentary canal passes through the length of the body from mouth to the terminal region without coiling. In this system the first four body segments are the pharynx, from which for the next nine to thirteen sections are the oesophagus, after which the intestine section of the gut system starts. In most families of earthworms there will be a gizzard (a thick muscular section of the digestive tract used to grind food), however in the Lumricidae family, common in Europe, this feature is missing instead replaced by a crop and a muscular gizzard before the start of the intestine, this is displayed in Figure 3 as an illustration of the cross section of the anterior region of the gut system of Lumbricus terretstris (Sims and Gerald, 1985)

Like all animals' earthworms excrete three types of wastes, liquid wastes, gaseous wastes and solid matter, all but solid matter waste is excreted through the previously mentioned pores whereas solid matter waste is passed via the anus. This system is called the Nephridia of which each segment of the earthworm contains a pair, as seen in Figure 4 as an illustration of a cross section of the body of an earthworm (Berman, 1985).

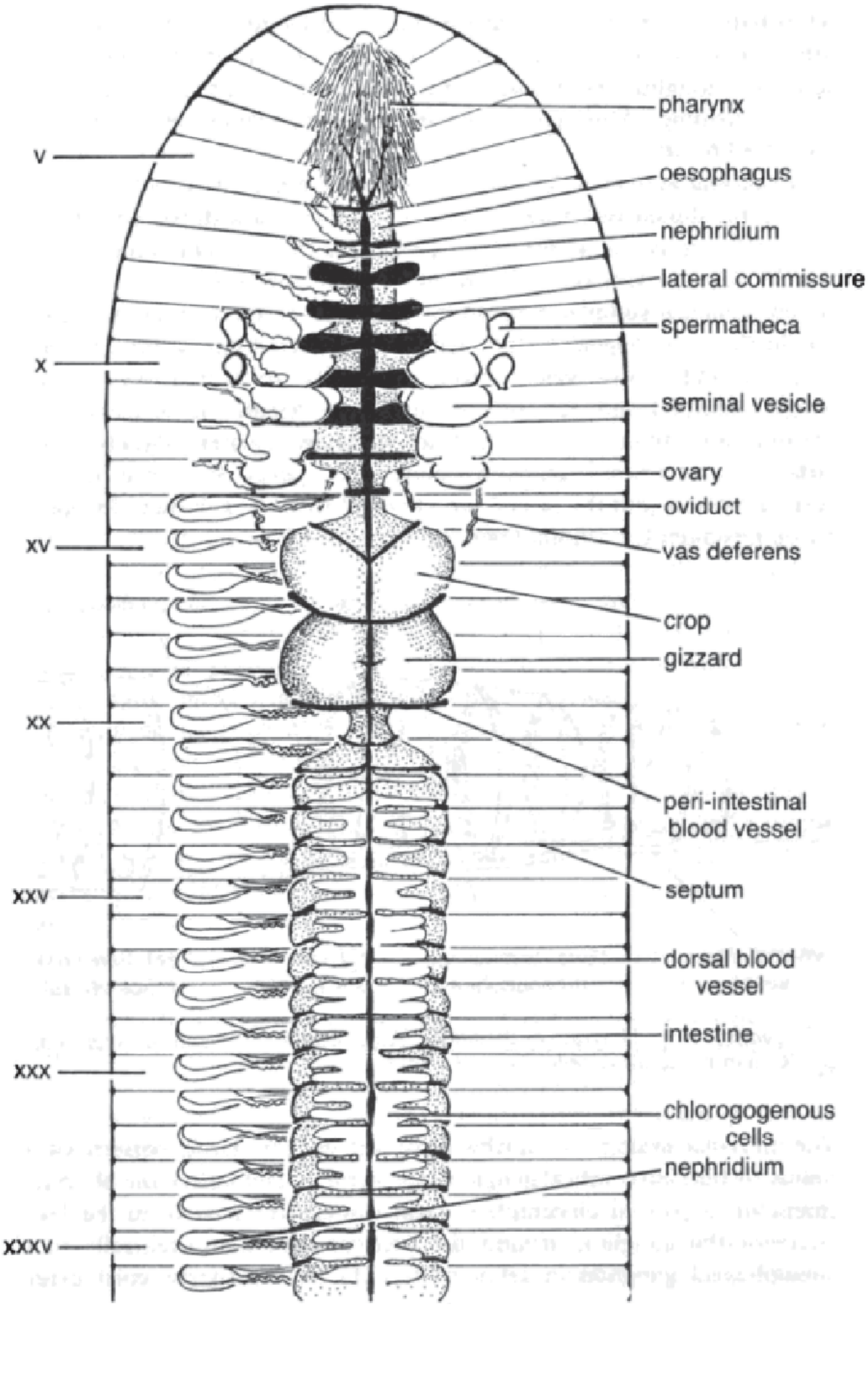


Figure 3, "Lumbricus terretstris. Internal morphology. General dissection of the anterior region, dorsal view. (Nephridia not shown on the right side.) Roman numerals denote segment numbers." (Sims and Gerald, 1985)

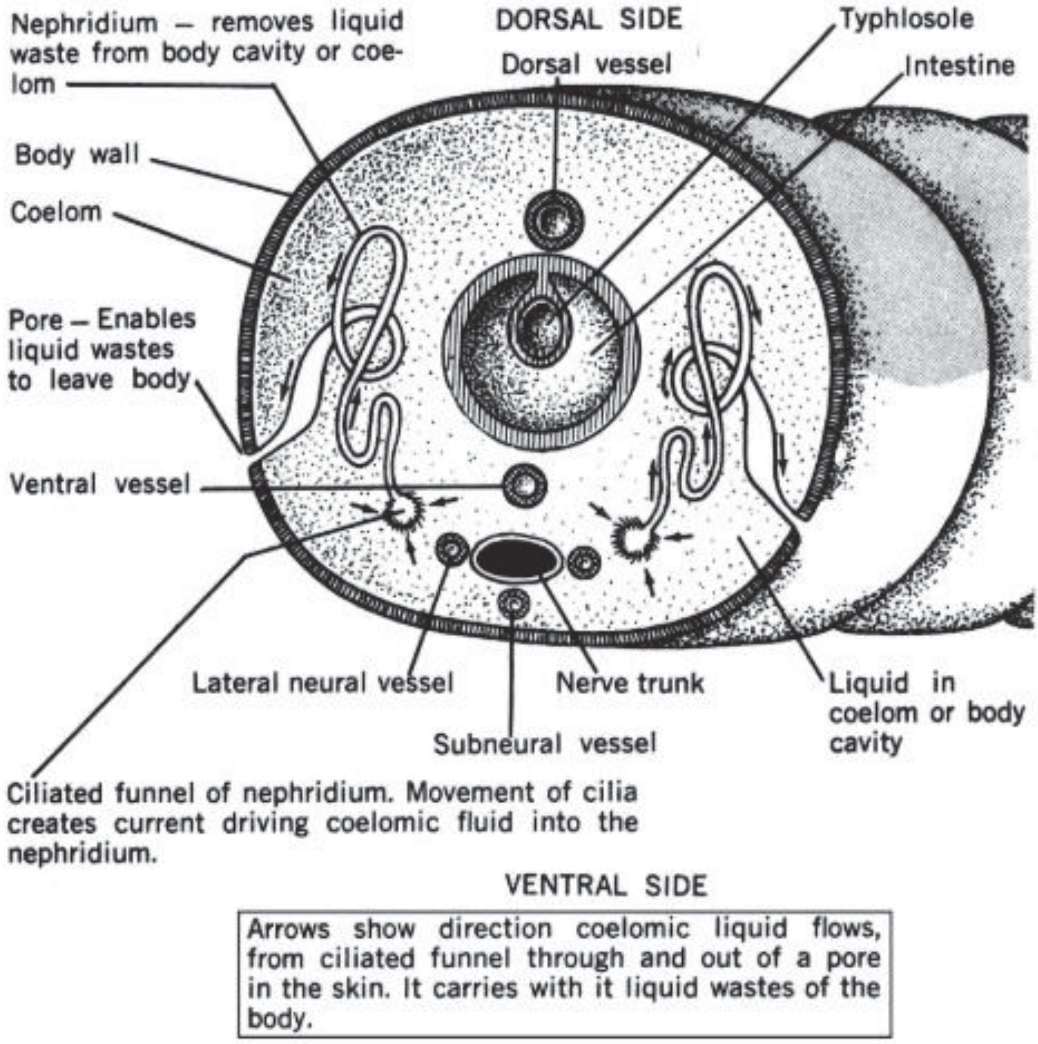


Figure 4, "Cross section of an Earthworm through the intestinal region emphasising the Nephridia" (Berman, 1985).

Limitations of Previous Studies

Despite the recognized importance of earthworm species, and their position as the dominant macrofauna in many ecosystems globally, there is an under representation of these species in literature. The majority of studies focusing on the effects of radiation on earthworm species use the species *Eisenia fetida*, a common species of earthworm in every continent. the Organisation for Economic Co-operation and Development (OECD) recommends this species for toxicology studies. However, the limiting factor of using this species is that *Eisenia fetida* inhabits the leaflitter as it is an Epigeic species, therefore there is an oversight of representation of Endogeic and Anecic species which interact more with the mineral soil.

Another issue of the literature concerning Earthworms and the effects of radiation is the fact that many of the studies have been published in, for example, Russian, which prevents access to resources that could have helped in this study. Many times, the abstract was published in English however the language barrier prevented full access to the source. This is an issue that creates a barrier between discourse in the scientific field.

Earthworm ecological groups are identified through their habitat and behaviour in the soil profile however the literature commonly fails to identify any interactions between earthworm ecological groups. There are many studies observing the interactions between earthworms and other species such as with nematodes which was studied by Dionísio et al, 2018, which mentioned the different ecological groups of earthworm species however only compared this feature to their ecological and functional interactions with another species. There is a limited number of studies which will include examples from multiple earthworm ecological groups and do not usually compare interactions between them.

Materials and Methodology

Earthworm Sample Collection

COMET Project Background

The earthworm samples used in this study were initially collected as part of a larger project called "*COMET - Initial Research Activity on transgenerational effects and role of epigenetics: Results and Impact*", (final report published on 14th April 2017), (COMET, 2017). This project was co-funded by the European Commission under the Seventh Euratom Framework Programme for Nuclear Research & Training Activities, starting on the 1st July 2013 for a duration of 48 months. The objectives of the COMET (Coordination and implementation of a pan-Europe instrument for radioecology) project included studies to understand the effects of chronic low-dose exposure to ionising radiation and the underlying molecular mechanisms, potentially contributing to effects that govern the activation or repression of the epigenome, specifically DNA methylation. Samples for this project included field samples of wildlife taken from the contaminated areas of the ChNPP Exclusion Zone and the FDNPP 100 km area, included in these wildlife samples were the earthworm samples used in this study.

Earthworm samples were selected to be a part of this COMET project as they are one of the RAPs selected by the ICRP (ICRP 2008), as well as proving in previous studies an adaptation response to other environmental toxins (Kille et al, 2013).

COMET Project Methodology – ChNPP Exclusion Zone.

An initial survey for COMET was carried out in 2014, 28 years after the ChNPP accident. This survey was conducted as a collaboration between Norges Miljø- og Biovitenskapelige Universitet (NMBU), The International Radioecology Laboratory (IRL) and The Institute for Radiological Protection and Nuclear Safety (IRSN). In order to identify the species that are present at various site locations a global assessment of earthworm communities was conducted in order to determine the best format for the assessment design. To represent the types of dominant habitats, 20 sites were selected, including: grassland, woodlands, and wetland. This survey resulted in qualitative data collection of all earthworm species found in a total of two hours over a 50 m diameter.

This survey was followed up by another field trip to the ChNPP accident involving members from NMBU, IRL and The Natural Environment Research Council (NERC) 2 years later in October 2016. This field trip included the collection of earthworms from areas identified as a range of expected levels of exposure, from background radiation to relatively high contamination. The survey in 2014 highlighted wetland habitats as having the highest density of earthworms, which due to the pattern of deposition following the accident also has the highest measurement of radionuclide activity.

Over nine different sites six earthworm species were identified and collected, these included; *Eisenia fetida, Eiseniella tetraedra, Octolasion lacteum, Lumbricus rubellus, Aporrectodea caliginosa* and *Aporrectodea rosea (Table 1).* The species *Octolasion lacteum* and *Aporrectodea caliginosa* were present at multiple sites with a range of exposure levels and in a high abundance. This was not the case for species such as *Eisenia fetida*, *Lumbricus rubellus* and *Aporrectodea rosea,* which were only present at one or 2 sites. Although a large number of individuals of *Lumbricus rubellus* were found at a site described as medium level of radionuclide contamination, individuals of both *Eisenia fetida* and *Aporrectodea rosea* were found only at control locations.

Eiseniella tetrahedra – Epigeic

Octolasion lacteum – Endogeic

Lumbricus rubellus – Epigeic

Aprorrectodea caliginosa – Endogeic

Apporrectodea rosea – Endogeic

Eisenia fetida – Epigeic

(Information on these species ecological groups from: The Earthworm Society of Britain, https://www.earthwormsoc.org.uk/earthworm-ecology)

As a part of COMET, 2017, these samples were initially weighed, some taken for further analysis and were dissected into head and gut samples, and a few of the remaining individuals were measured for radionuclide analysis and dosimetry assessment. At each site soil samples were also taken and measured for activity of Cs-137, Sr-90, Am-241, Pu-isotopes, as well as to identify the soil microbial activity.

Table 1 Description in the COMET report: " Earthworm numbers collected from 9 sampled sites in the CEZ during the October 2016 Field visit". COMET, 2017, 22.

Site number	Habitat	Reading soil surface (uSv/hr)	Total number of worms collected	Total number of species	*Eiseniella tetrahedra*	*Octolasion lacteum*	*Lumbricus rubellus*	*Aporrectodea caliginosa*	*Aporrectodea rosea*	*Eisenia fetida*
Control 1 (4)	Pond	0.12	45	5	15	2	0	2	5	6
Control 2 (6)	Garden	0.2	33	2	0	0	0	32	1	0
Control 3 (7)	Upper bank	0.12	21	4	0	1	0	20	0	0
Control 4 (9)	Marsh/wetland	0.34	13	2	3	10	0	0	0	0
Medium 1 (1)	Pond	4	0	0	*0*	*0*	*0*	*0*	*0*	*0*
Medium 2 (2)	Pond	4.5	61	3	4	37	0	20	0	0
Medium 3 (3)	Pond	8.2	32	3	4	9	10	8	0	0
Medium 4 (5)	Pond	5	28	3	1	0	17	0	0	0
High 1 (8)	Marsh	12	?	2	?	20	0	0	0	0

Sample Sites Data– ChNPP Exclusion Zone

The parameters of the sample sites for the collection of samples in the ChNPP Exclusion Zone are displayed in Tables 2 and 3. Maps of the location of the sample sites and their relative location within Ukraine were created using the GPS co-ordinates, these can be seen can be seen in Figures 5 and 6. For reference of the Cs-137 deposition soon after the ChNPP Accident Figure 7 can be compared with Figure 5 to relate the kBq/m^2 to the sample sites, however it should be noted that ambient air dose does not give an accurate estimate of the dose received by an earthworm.

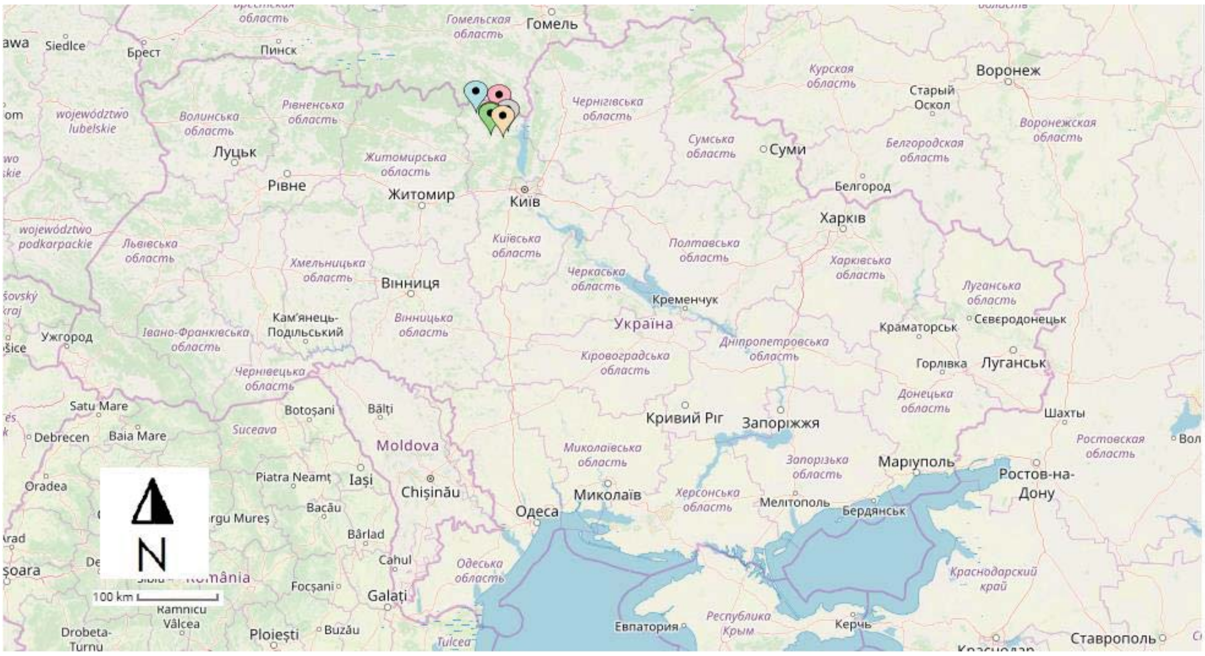
Details of the sampling sites is provided from data presented in COMET ,2017, which includes a description of the site by name (name of the closest location or description of the location if the sample was not collected close to a named town), GPS location and a description of the site, to show the variety of habitats covered in the sample site location selection. Table 3 combines this information with details on the quantity of earthworms collected at each site along with the ecological group to which the group has been assigned.

Table 2, Sample site name, GPS Co-ordinates of sample sites, and sample site description for the samples collected in the ChNPP Exclusion Zone. Glinka is considered as the Control site. Original data from COMET, 2017.

Site Name	GPS Co-ordinates	Site Description
Glinka	51.24129, 29.90569	Forested pond bank
Zamoshnya	51.23637, 29.8982	Forested pond bank
Glyboky Lake	51.44502, 30.06361	Forested marsh
Road Near Ukraine – Belarus Border	51.474772, 29.633966	Forested stream bank
Uzh River	51.20546, 30.12853	River bank
Chernobyl City	51.27936, 30.21294	Garden soil

Glinka	51.24129, 29.90569	Road Near Ukraine – Belarus Border	51.474772, 29.633966
Zamoshnya	51.23637, 29.8982	Uzh River	51.20546, 30.12853
Glyboky Lake	51.44502, 30.06361	Chernobyl City	51.27936, 30.21294

Figure 5, Map of the location of the sample sites in the ChNPP Exclusion Zone. Created using: *https://www.mapcustomizer.com/map/Chernobyl%20NPP%20Exclusion%20Zone%20sample%20site%20locations*



(red marker)	Glinka	51.24129, 29.90569	(blue marker)	Road Near Ukraine – Belarus Border	51.474772, 29.633966
(green marker)	Zamoshnya	51.23637, 29.8982	(orange marker)	Uzh River	51.20546, 30.12853
(pink marker)	Glyboky Lake	51.44502, 30.06361	(grey marker)	Chernobyl City	51.27936, 30.21294

Figure 6, Map of the location of the sample sites in the ChNPP Exclusion Zone showing the location within Ukraine. Created using: *https://www.mapcustomizer.com/map/Chernobyl%20NPP%20Exclusion%20Zone%20sample%20site%20locations*

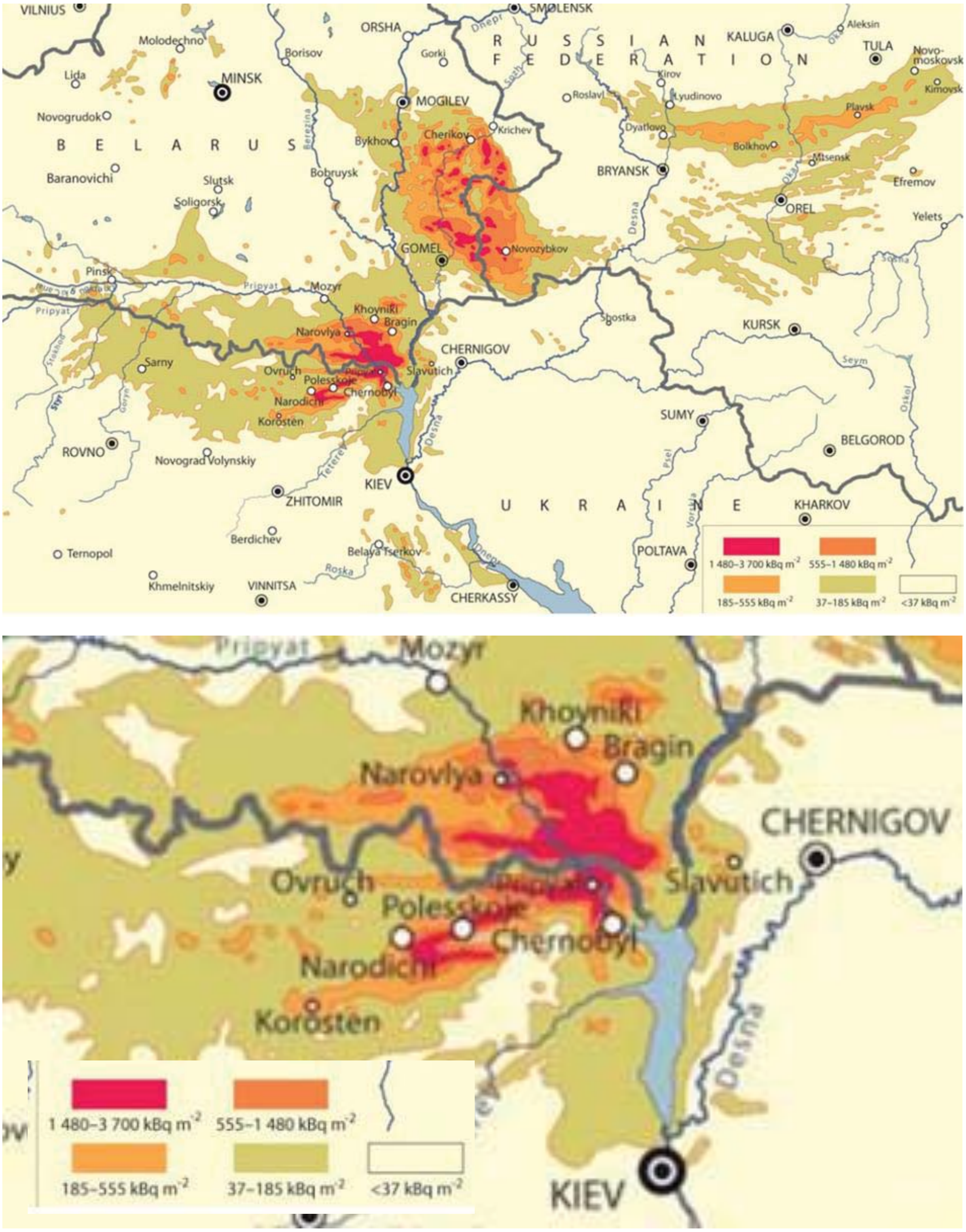


Figure 7, "Map of Cs-137 Deposition levels in Belarus, the Russian Federation and Ukraine as of December 1989" (IAEA, 1991)

Table 3, Site Name corresponding with the quantity and Ecological group that the earthworm's samples were identified as for samples collected from the ChNPP Exclusion Zone. Original data from COMET, 2017.

Site Name	Quantity of Earthworms	Earthworm ecological group
Glinka	16	Epigeic
Glinka	3	Endogeic
Glinka	1	Hydromorphic
Zamoshnya	21	Epigeic
Glyboky Lake	5	Endogeic
Road Near Ukraine – Belarus Border	1	Epigeic
Road Near Ukraine – Belarus Border	16	Endogeic
Uzh River	20	Aquatic
Chernobyl City	9	Epigeic
Chernobyl City	5	Endogeic

COMET Project Methodology – FDNPP 100 km Area

Following the sampling methodology in the ChNPP Exclusion Zone an initial survey was conducted in the Fukushima Prefecture and adjacent control sites, in September 2016, 5 years after the FDNPP accident. The objectives of this initial survey were: to identify the species diversity and an overview of the contamination, to verify if the presence of species in a control site can also be identified in a contaminated site, and to detect differences in bioaccumulation between species that share the same habitat and level of contamination. In total the survey identified 15 site locations including: forest soils, lakesides, stream banks and mountain slopes, gardens, and lakesides and roadsides with herbaceous vegetation. The contamination at these sites ranged from (<2 µGy/h – 100 µGy/h).

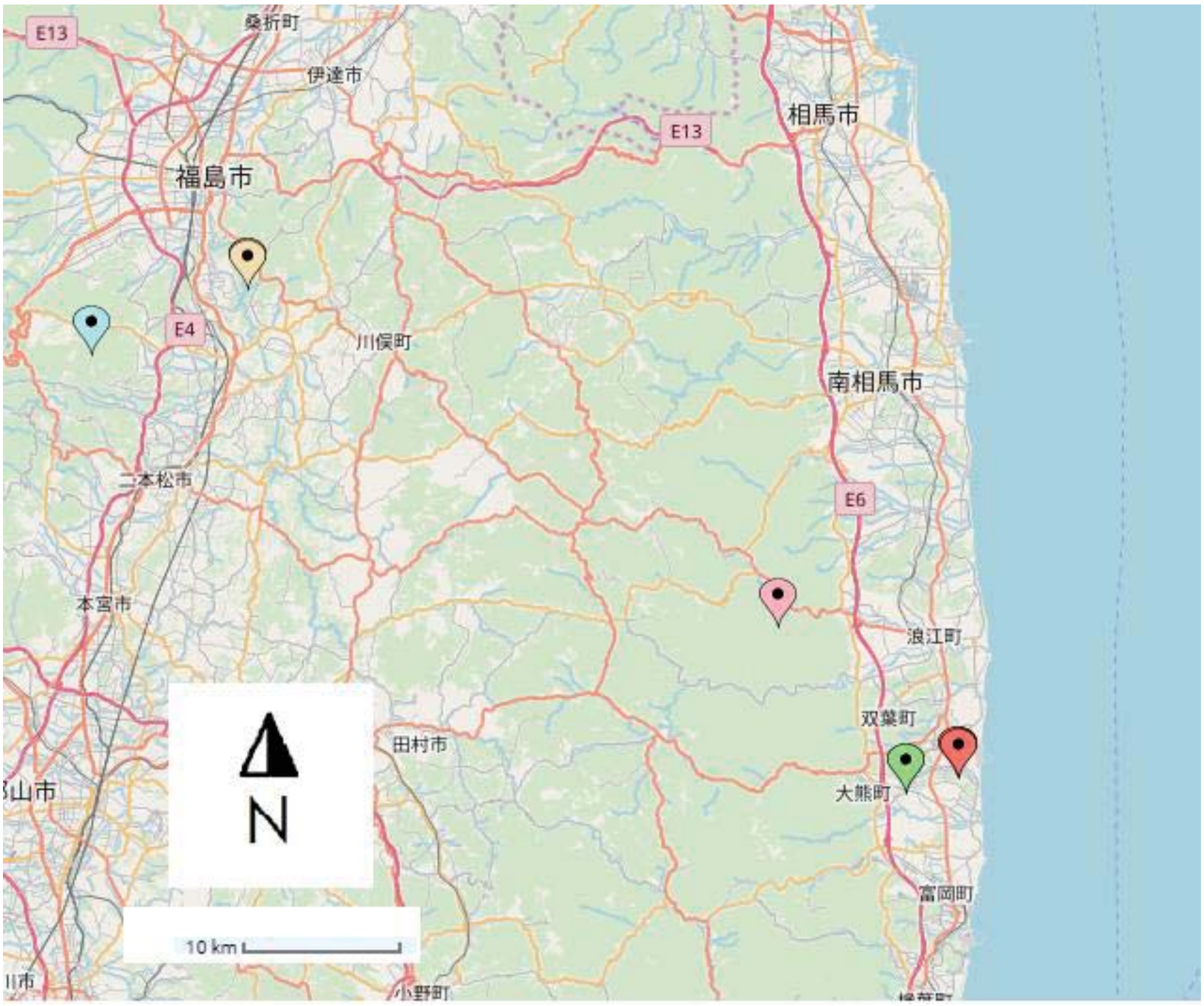
Field sampling was also conducted at this time, resulting in the collection of 147 individual earthworm specimens, from 15 identified morpho-species. All of the species of earthworms belonged to the dominant earthworm group in Asia, the Megascolecidae family.

With identification of the genus Amynthas, however identification for the COMET report remained incomplete.

As a part of COMET, 2017, a rough screening of these samples was carried out for dosimetry purposes to measure the individual or group activity, and soil samples were taken at each location. But at date no systematic analysis of the groups or individual earthworms had been carried out.

Sample Sites Data– FDNPP 100 km Area

Similarly to the Earthworm samples collected in the ChNPP Exclusion Area, the samples collected in the FDNPP 100 km Area have also been compiled into a Table to show the site name (closest named location or description of the area), GPS co-ordinates of the site, a description of the site identifying the type of habitat of the collected samples, and the quantity of earthworm samples collected at each site, as seen in Table 4. GPS co-ordinates have been input into a map programme to show their relative locations within the FDNPP 100 km Area and within Japan in Figures 8 and 9. Some sites had multiple descriptions and co-ordinates within a very small location area and therefore share the same site name. Due to no identification of the ecological groups or species identification this category was omitted and the table was compiled into just one table displaying all of the information. Some locations did not have GPS co-ordinates available: Site 3 (1&2), Forest Site (1&2) and Observatory Site (1, 2 & 3).



Inkyozaka Lake (1)	37.42523, 141.01768	Ogaki Dam (1)	37.511554, 140.886082
Inkyozaka Lake (2)	37.42610, 141.01740	Mt. Sasamori, Abukuma River tribute	37.670106, 140.384040
Inkyozaka Lake (3)	37.42535, 141.01826	No Name (1)	37.70792, 140.49927
Inkyozaka Lake (4)	37.42596, 141.01663	No Name (2)	37.70912, 140.49835
Site 2 (1)	37.41587, 140.97999	No Name (3)	37.70912, 140.49835
Site 2 (2)	37.41600, 140.97976		

Figure 8, Map of the location of the sample sites in the FDNPP 100km Area. Created using: https://www.mapcustomizer.com/map/Fukushima%20NPP%20

Inkyozaka Lake (1)	37.42523, 141.01768
Inkyozaka Lake (2)	37.42610, 141.01740
Inkyozaka Lake (3)	37.42535, 141.01826
Inkyozaka Lake (4)	37.42596, 141.01663
Site 2 (1)	37.41587, 140.97999
Site 2 (2)	37.41600, 140.97976
Ogaki Dam (1)	37.511554, 140.886082
Mt. Sasamori, Abukuma River tribute	37.670106, 140.384040
No Name (1)	37.70792, 140.49927
No Name (2)	37.70912, 140.49835
No Name (3)	37.70912, 140.49835

Figure 9, Map of the location of the sample sites in the FDNPP 100km Area showing the location within Japan. Created using:
https://www.mapcustomizer.com/map/Fukushima%20NPP%20

Table 4, Sample site name, GPS location of sampling site, sampling site description and the quantity of earthworms collected at each site for samples collected in the FDNPP 100 km Area. Original data from the COMET Report: COMET - Initial Research Activity on transgenerational effects and role of epigenetics: Results and Impact, 2017. In comparison to the data collected from the ChNPP Exclusion Zone.

Site Name	GPS Co-ordinates	Site Description	Quantity of Earthworms
Inkyozaka Lake (1)	37.42523, 141.01768	Forested lakeside	6
Inkyozaka Lake (2)	37.42610, 141.01740	Edge of forest path	9
Inkyozaka Lake (3)	37.42535, 141.01826	Forest soiil	4
Inkyozaka Lake (4)	37.42596, 141.01663	Edge of abandoned rice field	
Site 2 (1)	37.41587, 140.97999	Roadside, in vegetation	12
Site 2 (2)	37.41600, 140.97976	Roadside, in Vegetation	36
Site 3 (1)	No Co-ordinates	Roadside, in vegetation (40 µSv)	7
Site 3 (2)	No Co-ordinates	? (40 µSv)	11
Forest Site (1)	No Co-ordinates	Garden Soil	8
Forest Site (2)	No Co-ordinates	Forested stream bank	3
Ogaki Dam (1)	37.511554, 140.886082	Forested stream bank	12
Ogaki Dam (2)	37.511554, 140.886082	Forested top of slope	2
Mt. Sasamori, Abukuma River tribute	37.670106, 140.384040	Forested stream bank	6
No Name (1)	37.70792, 140.49927	Forested stream bank	3
No Name (2)	37.70912, 140.49835	Forested top of slope	2
No Name (3)	37.70912, 140.49835	Lakeside, herbaceous vegetation	12
Observatory Site (1)	No Co-ordinates	Forest soil	8
Observatory site (2)	No Co-ordinates	Forested stream bank (larger stream)	3
Observatory Site (3)	No Co-ordinates	Forested stream bank (smaller stream)	3

Comet Project - Species Identification

Before the samples taken from the ChNPP Exclusion Zone were received identification of morphospecies had been conducted, or at least to which ecological group the individual belonged to. This included species from Epigeic, Endogeic and 21 earthworm samples from aquatic morphospecies, however none of earthworm samples from the ChNPP Exclusion Zone were identified as from the Anecic ecological group. This means that the Anecic ecological group will not be represented in this study. Additionally, aquatic species are not included in this study therefore these samples were not used.

This totalled to 25 Epigeic earthworm species and 9 Endogeic earthworm species.

Samples taken from the Fukushima 100 km area were not initially identified further than an observation that all of the species belonged to a common earthworm family for the east Asian area, Megascolecidae, and possibly from the genus Amynthas.

Ordering of Samples

Containment and Preservation

The samples were separated and preserved in small scintillation vials fit for the rack for measurement in the NaI detector. This containment procedure was used by the COMET, 2017, project which was continued in this study.

Preservation of samples was achieved using 100% ethanol. Animal samples preserved in 70% to 100% ethanol showed the ability to preserve samples, however Preservation in dilute ethanol, 80% and lower, after 6 months DNA was almost completely destroyed (Fukatsu, 1999). Preservation in ethanol is cost effective and can be kept at room temperature during transport, this is better for samples taken from the field (Staube and Juen, 2013).

Sorting and Measurement of Samples.

Previous to the present study, the earthworm samples had been roughly screened for radiocaesium content according to site, but not systematically measured to enable an assessment of the variability in content between individuals. For this study, all samples were first recounted using the NaI detector to compare with previous measurements, and then bulk samples that were above detection limit were separated into individual worms and recounted

Originally samples collected from the FDNPP 100 km Area were contained within 53 vials and the samples collected from the ChNPP Exclusion Zone were contained within 23 vials. As a result of the further subdivision of samples, this number grew to 110 vials containing samples from the FDNPP 100 km Area and 37 vials from the ChNPP Excluision Zone.

In total this results in 154 measurements from the FDNPP 100 km Area samples and 91 measurements from ChNPP Exclusion Zone.

Total Sample Weight

Equipment and Method

All earthworm samples were weighed individually on the Mettler Toledo AX204 weighing scales. This brand of scales includes a draft shield which was necessary as some of the samples were very light. The scales were set to 0 (tare) after regular intervals of about 5-10 measurements in order to maintain the best accuracy. Between the samples and the weighing scales a small plastic hexagonal bowl was used to prevent the sample from touching the scale itself, and set the tare to include this bowl in the nett zero.

Samples from vials containing multiple worms, were weighed individually and the total number of weights of all worms in the vial was noted. In addition to providing information on the number and weight of individual earthworms, this separation allowed calculation of the individual Bq/g for the worms having sufficient levels of radiocaesium and assisted in the selection of samples for dissection. Labelled pictures were also taken throughout this process to aid in later identification.

The individual earthworms were removed from the ethanol and placed on a paper towel to remove the liquid that may remain in the surface of the sample, as this could contribute to added weight. Samples were left on the paper towel for approximately a minute, including dabbing the sample until there was no apparent liquid on the surface. They were then transferred to a bowl on the scales where a measurement was recorded after proximately 10 seconds to allow the scale measurement to settle. The samples continued to reduce in weight at first rapidly however slowing down after a few seconds as the acetone evaporated, therefore it was important to decide when to take the recording as it was impractical to wait for complete evaporation. By keeping a similar wait time before recording, in addition to using the paper towel, as much of the excess liquid was accounted for as was possible and practical.

Measuring Cs-134 and Cs-137

NaI Measurement

The model of NaI detector used was a PerkinElmer 2480 Automatic Gamma Counter using the software WIZARD 2. The NaI detector was used for its higher counting efficiency with ɣ-radiation. Using a cylindrical NaI-crystal, optically connected to a photo-multiplier tube (PMT), scintillations (light) produced in the crystal is absorbed by the PMT and the signal is amplified, converted to a current signal, then to a voltage signal, and finally to a digital signal. The number of scintillations is proportional to the energy of the incoming photon; therefore, the energy distribution of the signals can be measured and the intensity plot against the energy enables a ɣ-spectrum can be produced. The energy is the divided into channels and the counts for each channel is collected and displayed. This provides information on the total counts detected in the allotted window, the counts per minute, (CPM) and Error % of Cs-134 and Cs-137 in each sample. Samples ran for a count time of 1500 seconds each, this was split into three runs. The first included both samples from the ChNPP Exclusion Zone and FDNPP 100 km Area with four blanks for separation, and the two subsequent runs were only samples from the FDNPP 100 km Area

The standards used in these runs were: “IAEA 300”, “IAEA 373”, “Low Cs-137”, and “Cs-134+137”. These standards of mixed Cs-137 and Cs-134 were included to enable the calculation of the spill over between the two counting windows for the samples.

Dissection

Selection of Samples for Dissection

The samples selected for dissection were based on the initial Cs-137 and Cs-134 measurements taken of the whole earthworm body. Samples from both locations were selected for dissection based on the Cs-137 measurement exceeding the detection level. With the expectation that the majority of the radiocaesium would be within the gut (see Literature review) these were selected as samples that would most likely produce a result discernible from background radiation when dissected into separate smaller parts of the whole.

It was also necessary to consider the size of the earthworm for the ease of the dissection process. This could be evaluated from the weight and also visually. Over time as experience of the dissection procedure was gained smaller samples were possible to dissect. The smallest earthworm to be dissected was sample 23 at 0.041g, this sample was chosen because of the high Bq/g measurement compared to other samples. Due to the small scale some skin was still attached to the gut, however, since it was more important to avoid contamination of the skin, this was not considered to be a large source of error.

In addition to this samples from the ChNPP Exclusion Zone were selected to represent the ecological groups: Epigeic and Endogeic. Through evaluating both the Cs-137 and Cs-134 measurements and the representation of the ecological groups, 6 Epigeic and 3 Endogeic earthworms were selected. This is slightly bias towards more representation of samples from the Epigeic group however this was due to more samples from this group being available from the initial COMET, 2017, sample collection. This separation was not possible for the samples from Fukushima because the samples had not been identified for their ecological groups. For these samples the highest measurement of Cs-137 Bq/g was used as the indicator to which samples were selected for dissection. For the sample from Fukushima in most cases although Cs-134 was above background radiation it was not by an amount that influences the decision for dissection selection.

Dissection Procedure

The dissection procedure follows a very similar procedure as Berman, 1985, however was altered for the effects of the long preservation of the earthworm samples since collection.

The materials used in the dissection process included: Stainless steel 2A tweezers, Metal pointing tool, OrtoMedic Rudolf RU 2240-11 Stainless steel scissors, 5ml pipette, thin metal pins, Eppendorf tubes: safe lock 2.0ml, Zeiss Stemi DV4 Miscroscope, Petri dish with set plaster of Paris, 70% ethanol and Mili-Q water.

The plaster of Paris in the petri dish was saturated with 70% ethanol until 1mm of liquid above the plaster of Paris was able to sit and not be absorbed. Continuously throughout the dissection the dish was routinely washed with the 70% ethanol to maintain saturation.

The sample was taken from the scintillation vial and placed on the petri dish where the worm was pinned at the head an anus as close to the ends as was practical as to not pierce the gut however keep the sample secure. If possible, the sample was pinned straight however in their preservation the samples were very stiff and sometimes could not be straightened, maintaining a curve which required slight adaption to the dissection procedure. The sample was also kept moist throughout the dissection with 70% ethanol using the 5ml pipet.

The pinned sample in the petri-dish was placed under the Zeiss Stemi DV4 Miscroscope which was adjusted for focus. Using the metal pointing tool and the OrtoMedic Rudolf RU 2240-11 Stainless steel scissors a small incision was made below the clitellum that enabled the scissors to be inserted between the skin and the gut and continue to cut along the body. The pointing tool was used to stabilise the sample and hold open the edges of the incision to help to ensure that the gut had not been pierced. When the incision was long enough to allow the edges to be pulled away by the pointing tool the connective tissue was revealed and could also be cut using the scissors or scraping using the pointing tool. Connective tissue was around the entirety of the circumference of the body therefore when necessary the gut had to be lifted with either the pointing tool or the Stainless steel 2A tweezers to allow the separation from the underside. The skin that had been separated from the gut was pinned to the plaster of Paris using the long metal pins set at approximately a 45° angle that left space as to not obstruct visibility or movement of dissection tools.

Once the skin had been separated from the gut from the clitellum to the anus and pinned to the side the gut had to be detached from the skin. A cut was made as close to the anus opening as possible from the inside of the body cavity. It was decided that it was a cleaner division to include a very small amount of gut material, while avoiding the inclusion of any gut contents, at the end of the skin sample.

The incision was then continued from the location of the first incision to the mouth of the earthworm sample. The texture of the clitellum is denser and tougher, potentially as a result of the preservation. The clitellum was cut down to the intestines and separated to be included with the skin. Other organs, such as the reproductive organs, crop, gizzard etc, were either included in the gut sample due to the inability to separate them without risk of tearing the gut wall., or were separated and disposed of if they were able to be separated. At the opening of the mouth a similar procedure as with the anus was followed, unlike the anus there was usually not much gut contents around the mouth and therefore there was little risk of losing gut contents from the sample. The incision was cut to potentially include a small amount gut material at the pharynx and oesophagus with the skin sample. Due to the lack of gut contents in this area it should be inconsequential to the measurements.

The Eppendorf safe lock tubes, prefilled with 70% ethanol. Firstly, the now fully separated gut was carefully lifted from the skin using the pointing tool and Stainless steel 2A tweezers. In some places connective tissue may have been missed and the scissors were utilised to separate them. Each gut was placed in an individual Eppendorf tube. Since gut contents may have spilled out of small accidental cuts in the intestine lining or at either end of the incisions made at the mouth or anus. Using the suction of the 5ml pipette was utilised to extract the loose gut contents along with the 70% ethanol that the sample was saturated with continuously during the procedure.

The skin smaple was transferred to the second Eppendorf tube using the 5ml pipette. The pins were removed and the skin was lifted from the plate using the Stainless steel 2A tweezers, washed with more 70% ethanol in order to remove any gut contents that may have been missed, and transferred to the tubes.

The petri dish containing the set plaster of Paris was then washed with Mili-Q water to remove any material from the previous dissection and could be used for the next dissection procedure.

Measuring Gut Contents and Skin

NaI Measurement

The samples from the two sample locations were measured in two separate runs to keep the background to a minimum during the measurement of the lower activity of the samples from the ChNPP Exclusion Zone. Both sets of measurements were recorded on the same PerkinElmer 2480 Automatic Gamma Counter as the previous measurement taken before dissection.

The samples from the ChNPP Exclusion Zone included 11 blanks and three standards, the standards included were “137 Cs low 3 test”, “IAEA 373, 6.812g” and “IAEA 300, 10.14g”. Each sample was counted for 7199.98 seconds and only measured for Cs-137. Blank samples used throughout all measurements in this study are scintillation sample rack spaces empty of any contents.

In addition to this the samples from the FDNPP 100 km Area included eight blanks, as well as the same standards “IAEA 373, 6.812g” and “IAEA 300, 10.14g”, as were included in the run for the samples from the ChNPP Exclusion Zone. Additionally, for the samples from the

FDNPP 100 km Area three more standards were used, these standards of mixed Cs-137 and Cs-134 (explained in the previous NaI detector section). Each sample was counted for between 3600 and 3600.02 seconds however this set of samples was measured for both Cs-137 and Cs-134.

Individual Skin and Gut Weight

Similar to the method of weighing the whole sample before dissection, the same process was followed including the same Mettler Toledo AX204 weighing scales, the small plastic hexagonal bowl (with the tare taken to include this) and skin samples were dabbed on a paper towel before weighing and measured for a similar time each.

The skin of the dissected earthworms was weighed rather than the gut due to the ease of handling the entire structure whereas the gut was prone to breakage and potential loss of contents. In most cases the skin was able to be kept in one structure, although some samples had undergone separation in a previous study where the head section from the rest of the body resulting in a two skin sections for one sample, such as sample 20 from the ChNPP Exclusion Zone. Samples like this had been combined again as if there was no separation and the two parts of the skin was weighed together. Once the weight of the skin had been recorded the weight of the gut and the contents of the gut was calculated from the total weight taken before dissection. In some dissected samples there would have been a small amount of gut contents material lost through handling and dissection mistakes where the gut may have been pierced, although this loss was negligible and if possible, this matter was recovered.

Ge Measurement

The skins of the samples from the ChNPP Exclusion Zone were collected, excluding the skin of sample 20, to be measured together in the Ge Detector for further analysis in a detector with a higher sensitivity, to distinguish if a measurement exceeding background could be recorded collectively. Ge-detectors use semiconductors, in this case Ge, to get a higher resolution of a photo peak at the Full width at Half Maximum (FWHM), the width at 50% of the maximum intensity. Ge-detectors can potentially measure FWHM values of less than 2keV however, in comparison to the NaI-detector the Ge-detector has a lower efficiency.

The samples remained inside the 2.0ml safe-lock Eppendorf tubes inside the 70% ethanol, however were placed vertically in a cylindrical geometry.

Data Analysis

Calculations from NaI Detector and Ge Detector Measurements

The initial results from the NaI detector were converted from CPM to Bq/g using the calculated efficiency for the individual counts, the average background count, and the sample weights. The Germanium (Ge) Detector measurements used internal calibrations for energy and efficiency, and background, producing an output measurement as Bq which can be used directly. The reported counting errors were converted to Bq for both measurements.

Background Calculations, Critical Limit L_c and Detection Limit L_d

Blank samples were used to determine the background radiation level, then to take this away from the radioactivity measurement so that it can be confirmed that the remaining measurement is from the source that can be distinguished as separate from background radiation. Blank samples are used to ensure that samples are neither dismissed due to: assuming that the measured radioactivity from the source was not present when in fact it was, or the reverse where samples are included when they should be dismissed, assuming that there was a recorded measurement above background when there was not (Currie, 1968).

In this case from radionuclides in the soils of the ChNPP Exclusion Zone and the FDNPP 100km Area. Averages of the background (blanks) were calculated: mean, maximum value and standard deviation.

Results

Initial NaI Detector Measurements

Result comparison to the previous study

Some measurements of Cs-137 and Cs-134 had been recorded for the previous COMET project, 2017. The first step of the current study was to re-measured all samples to compare the results with the previous study, and to identify any indiscretions between measurements. Indiscretions between the old and the new measurements could indicate that the samples had been miss-labelled, swapped or missing, as well as differences in the measurements instrument. The time difference (over one year) between the new and the old measurements could have been the difference between the detection above background radiation levels for Cs-134, resulting in some previously detectable samples in the previous study being discounted in this study, due to the lower half-life of Cs-137 (2.06 year vs. 30 years for Cs-137) and the time passed from the initial deposition at both sites. Therefore Cs-137 measurements were more reliable for comparison.

Comparison of the Cs-137 results of the FDNPP 100 km Area samples between both studies followed similar patterns. For example, samples 35, 36, 37 and 38 (all collected from the same site) were the highest measurements in the previous study were now most of the highest measurements in this study.

Samples where dividing the composite samples into individual earthworms and remeasuring showed differences in the activity in individual earthworms and that in some cases most of the activity could be attributed to just one earthworm of the collective sample. This observation aided in the selection of which samples to dissect.

Samples from the ChNPP Exclusion Zone were harder to correlate with results from previous measurements from the COMET, 2017, project. The sites with the highest quoted DPM and Bq/g did not match the sample sites giving the highest DPM for the current study. This is due to a different a NaI detector, counting set up and efficiency used in the COMET, 2017, project, therefore, a comparison is difficult to make.

Samples from the ChNPP Exclusion Zone

The mean blank measurements for Cs-134 for the samples from the ChNPP Exclusion zone was a CPM of 15.51 with a standard deviation of 1.06, resulting in a nett L_c of 17.30

and net L_d of 19.00. None of the earthworm samples gace Cs-134 levels over the limit of detection, with the highest recorded CPM being 17.08. This was not suprising since it is unlikely that one would be able to detect any Cs-134 remaining from the ChNPP Accident in 1986. The t½ of Cs-134 is 2.0648 years, after 10 t½ around 0.1% of the original activity remains (NCRP, 2007), therefore in the 33 years since the ChNPP Accident almost 16 (15.98) half-lives have passed. Due to this Cs-134 will be indistinguishable from background radiation, as was found in these measurements.

In contrast, Cs-137 with a t ½ of 30.17 years, therefore only 1 half-life and 8.58% of the second half-life has been achieved since the ChNPP Accident. Nethertheless, many of the earthworm samples were however still below the L_c. The mean background for Cs-137 for the samples from the ChNPP Exclusion Zone was a CPM of 0.89 with a standard deviation of 0.92. This gave a nett L_c CPM of 2.45 and nett L_d was a CPM of 3.92. Most samples that did not exceed the L_d, and in some cases the L_c were from sample sited described as a "control site" in Chernobyl City and Glinka, although three samples from Chernobyl city exceeded both the L_c and L_d.

In some cases, for example those collected in Zamoshnya where several earthworm samples were collected in the same sample location (GPS) and identified as the same ecological group, there was an extreme variation of Cs-137 radioactivity. A sample of large Epigeic earthworms collected in Zamoshnya ranged from a CPM of 3.50 and 36.07, resulting in a difference of not meeting the L_d and exceeding the L_d by 8.42 times.

The earthworms collected in the ChNPP Exclusion Zone were of varying weights, ranging from 0.007 g to 1.48 g. some samples however were a collection of multiple earthworms measured in the NaI detector as one sample with individual earthworm weights added together as mentioned in the method section: Ordering Sample, Number of vials and number of samples. In total 17 samples, some containing multiple earthworms and some with only an individual, exceeded L_d. Using the weight of the sample the Bq/g was calculated, making the measurement of radioactivity of a sample relative to another by mass. Of the samples that exceeded L_d for Cs-137, this ranged from 0.05 Bq/g to 3.71 Bq/g.

Epigeic vs Endogeic Ecological Groups

Measurements exceeding at least 1 Bq/g were 64.70% of all samples, 11 of 17 samples that had exceeded the L_d. Of these samples 90.90% were of the Epigeic ecological group, whereas only 1 sample exceeded 1 Bq/g of the Endogeic ecological group, and this was at the highest activity site, Gluboky Lake. This earthworm contained the highest measurement (Cs-137) at 18.9 Bq/g. Unfortunately, no Epigeic samples were found at this sample site, Tables 5 and 6. The opposite is observed at Zamoshnya, no samples from the Endogeic group were collected, whereas the Epigeic earthworms from this sample site had the highest maximum Cs-137 of this ecological group of 10.3 Bq/g and the highest mean of 2.6 Bq/g. Despite Chernobyl city being regarded as a control site it is the only sample site which both the Epigeic and Endogeic samples exceeded the L_d, with the higher Cs-137 Bq/g mean and maximum from Epigeic earthworms, this comparison of sample sites and ecological groups is displayed in Figure 10.

Table 5, Mean, minimum value, maximum value, range and standard deviation for Bq/g per site for earthworms identified as from the 8Epigeic ecological group. No Epigeic earthworms were identified at the sample sites Glyboky lake and the Road Near Ukraine – Belarus Border. All samples found at Glinka did not exceed L_d. When “N/A” is used this represents that samples were not collected at this location.

Chernobyl NPP Exclusion Zone Epigeic Earthworm Samples

Site Name	**Mean Cs-137 Bq/g**	**Min Cs-137 Bq/g**	**Max Cs-137 Bq/g**	**Range Cs 137 Bq/g**	**SD Cs-137 Bq/g**
Glinka	$<L_d$	$<L_d$	$<L_d$	$<L_d$	$<L_d$
Zamoshnya	1.946	$<L_d$	9.410	9.667	2.509
Glyboky lake	N/A	N/A	N/A	N/A	N/A
Road Near Ukraine – Belarus Border	$<L_d$	$<L_d$	$<L_d$	$<L_d$	$<L_d$
Chernobyl City	0.249	0.143	0.354	0.211	0.149

Table 6, mean, minimum value, maximum value, range and standard deviation for Bq/g per site for earthworms identified as from the Epigeic ecological group. No Epdogeic earthworms were identified at the sample site Zampshnya. All samples found at Glinka did not exceed L_d. When "N/A" is used this represents that samples were not collected at this location.

Chernobyl NPP Exclusion Zone Endogeic Earthworm Samples

Site Name	**Mean Cs-137 Bq/g**	**Min Cs-137 Bq/g**	**Max Cs-137 Bq/g**	**Range Cs 137 Bq/g**	**SD Cs-137 Bq/g**
Glinka	$<L_d$	$<L_d$	$<L_d$	$<L_d$	$<L_d$
Zamoshnya	N/A	N/A	N/A	N/A	N/A
Glyboky lake	9.293	$<L_d$	18.586	18.586	13.142
Road Near Ukraine – Belarus Border	0.054	$<L_d$	0.112	0.112	0.056
Chernobyl City	0.039	$<L_d$	0.077	0.077	0.055

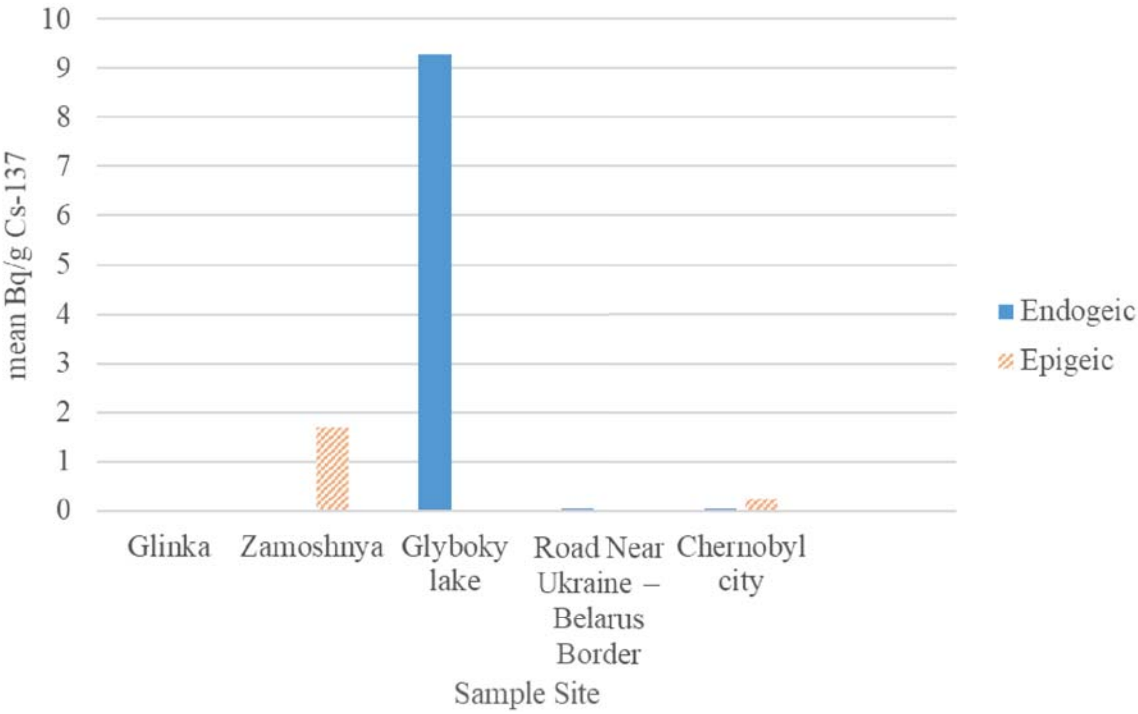


Figure 10, Comparison of the mean Bq/g Cs-137 for earthworms identified as from the Epigic and Endogeic ecological groups from samples sites in the ChNPP Exclusion Zone.

Although, comparison of activities at the Chernobyl City sample site suggested that the epigeic earthworms had a higher uptake of radiocaesium than the Endogeic earthworms, the difference for both groups was examamined acros all sites. The statistical software "Minitab 18" was used to test the significance of the difference between Cs-137 Bq/g of the earthworms from the ChNPP Exclision Zone identified as from the Epigeic and Endogeic ecological groups. For these tests samples with did not meet the L_d in the original measurments from the NaI detector were excluded. The alternative hypothesis H_1 for this outcome of the tests is that there is enough of a difference between the different earthworm ecological groups to be statisically significant, whereas the null hypothesis (H_0) for this test would be that there is no difference between the two groups.

To apply the correct statistical test the variables were tested for normility (normal distribution). This produced the probability plots displayed in Figures 11 and 12 for each variable. The P values were P=0.012 for Epigeic earthworms and P=0.007 for Endogeic earthworms. The H_0 for the normality test is that the samples come from normally distributed populations therefore H_0 accepted if $P>0.05$ and reject H_0 if $P\leq 0.05$. Therefore, both the normality tests for Epigeic and Endogeic had a P-value ≤0.05 indicating that the data is normally distributed.

In addition to this a test for 2 variances was applied using the F-test (Table 7) as the two samples come from normally distributed data. This tests if the variances are equal or not. If the variances are equal the ratio should contain 1, however as seen in Table 8 the ratios for the Epigeic and Endogeic earthworms does not contain one therefore it is concluded that the variances are not equal. Further evidence for this is that the P-value is below 0.05, at P=0.001, which implies that the data is different and not equal.

The conclusion of these tests is that the data is normally distributed however not equal variances, therefore a 2-sample t test cannot be applied and instead a Mann-Whitney test was used. A Man-Whitney test is a non-parametric test that tests the difference between 2 independent populations using the medians of the data rather than the mean.

The Mann-Whitney test produced a P-value of P=0.157, as can be seen in Table 9 As this P-value is >0.05 this means that the strength of evidence against the H_0 is insufficient and the H_1 is rejected. The H_0, that there is no statistically significant evidence supporting that the 2 earthworm ecological groups, Epigeic and Endogeic, are different from one another.

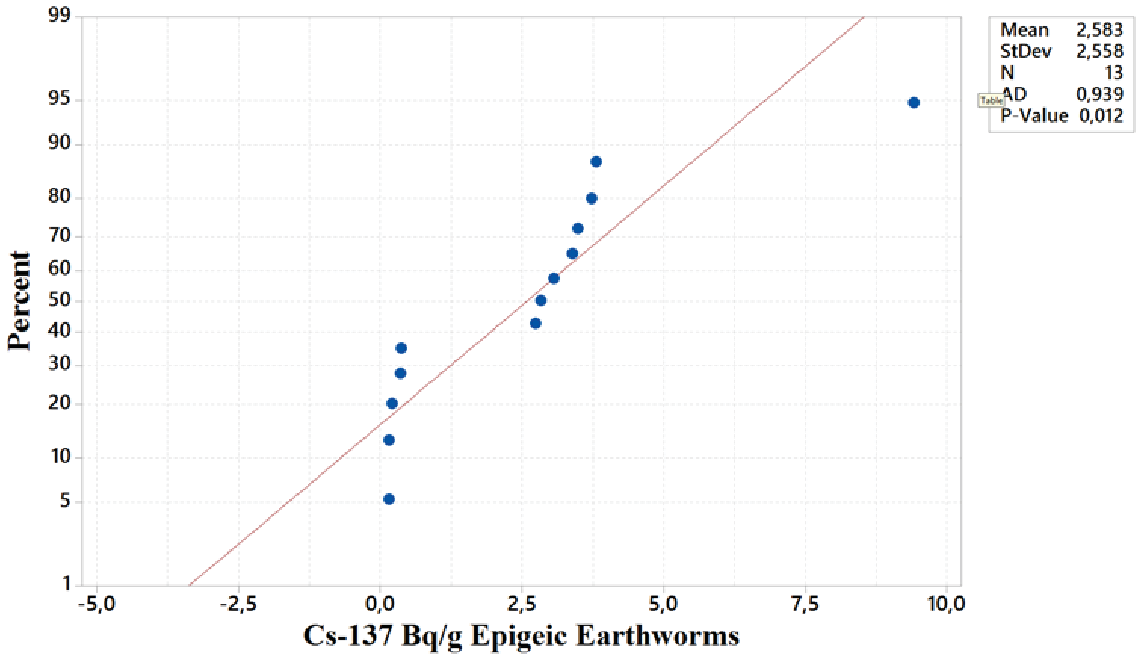


Figure 11, Probability Plot of Cs-137 Bq/g for Epigeic earthworms testing the normality of the distribution of the data. P=0.012.

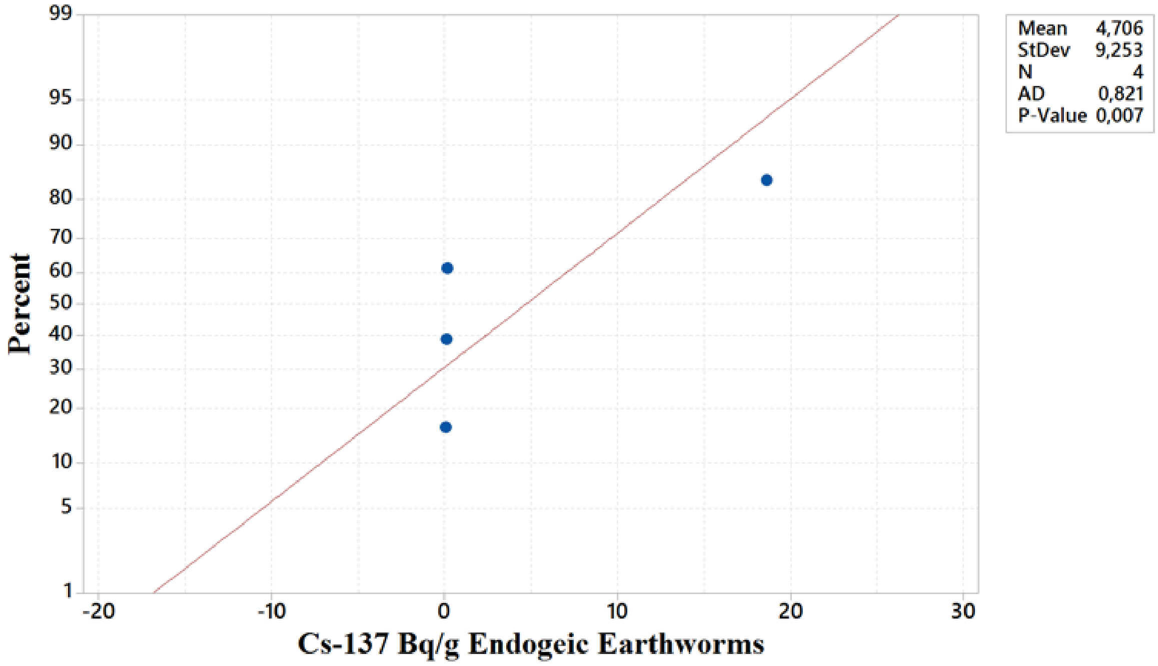


Figure 12, Probability Plot of Cs-137 Bq/g for Endogeic earthworms testing the normality of the distribution of the data. P=0.007.

Table 7, F- test comparing for the Cs-137 Bq/g for Epigeic earthworms and the Cs-137 Bq/g for the Endogeic earthworms. P=0.001.

Test

Null hypothesis	$H_0: \sigma_1 / \sigma_2 = 1$
Alternative hypothesis	$H_1: \sigma_1 / \sigma_2 \neq 1$
Significance level	$\alpha = 0,05$

Method	Test Statistic	DF1	DF2	P-Value
F	0,08	12	3	0,001

Table 8, Descriptive statistics for the Cs-137 Bq/g for Epigeic earthworms and the Cs-137 Bq/g for the Endogeic earthworms for the 2-variences test.

Descriptive Statistics

Variable	N	StDev	Variance	95% CI for σ
Cs-137 Bq/g Epigeic	13	2,558	6,543	(1,319; 5,842)
Cs-137 Bq/g Endogeic	4	9,253	85,622	(2,613; 64,256)

Table 9, Non-parametric Mann-Whitney test for the Cs-137 Bq/g of Epigeic earthworm samples and the Cs-137 Bq/g Endogeic earthworm samples from the ChNPP Exclusion Zone.

Method

η_1: median of Cs-137 Bq/g Epigeic
η_2: median of Cs-137 Bq/g Endogeic
Difference: $\eta_1 - \eta_2$

Descriptive Statistics

Sample	N	Median
Cs-137 Bq/g Epigeic	13	2,82526
Cs-137 Bq/g Endogeic	4	0,09446

Estimation for Difference

Difference	CI for Difference	Achieved Confidence
0,296365	(-15,2085; 3,60095)	95,25%

Test

Null hypothesis	$H_0: \eta_1 - \eta_2 = 0$
Alternative hypothesis	$H_1: \eta_1 - \eta_2 \neq 0$

W-Value	P-Value
130,00	0,157

Samples from the FDNPP 100 km Area

The blank sample counts for Cs-134 for the samples from the FDNPP 100 km Area depended on the run that the sample was included in, since different counting windows were used for the measurements. In addition, it is not possible to separate Cs-137 from Cs-134 due to the similar energies, so correlations need to be made for the Cs-134 levels. Unlike the samples from the ChNPP Exclusion Zone the samples from the FDNPP 100 km Area were split into 3 runs with the mean Cs-134 CPM of the blank samples of 15.31, 40.08 and 40.06, with standard deviations of 0.60, 1.40 and 1.28 respectively. Therefore, the CPM L_c was 16.33, 42.45 and 42.22 and the L_d was 17.28, 44.69 and 44.27.

As mentioned in the previous section the t½ of Cs-134 is 2.0648 years, from the day of the FDNPP Accident to the time of this study ~39.43% of the 10x t½ has been reached. This, together with the high background count and interference with Cs-137 mean that the Cs-134 levels have a higher error than Cs-137. However, with the t ½ of Cs-137 of 30.17 years therefore only 26.99% has passed for one t ½ for this radionuclide from this source. So, the main focus for the rest of the study was placed on the Cs-137 results.

For all samples, the ratio of the mean Cs-134/Cs-137 was 0.0565 and for the maximum value 0.087. Correcting this back to the ratio at the time of release would give a Cs:134/Cs-137 ratio of 0.68 and 1.06 for the mean and maximum, respectively. This is reasonable agreement with the expected 1:1 ratio in the initial release, especially for the maximum value which is the number that is least compromised by the number of measurements below the detection limit.

. The mean Cs-137 CPM of the blank samples of 1.08, 0.42 and 0.67, with standard deviations of 0.73, 0.51 and 0.6 respectively. Therefore, the CPM L_c was 2.31, 1.28 and 1.68 and the L_d was 3.48, 2.10 and 2.64. All but two of the Cs-137 measurements from active sites (not control sites) did not exceed the L_d. Of the control sites all samples from "Mt. Sasamori, Abukuma River tribute" did not exceed the L_d, however only one sample from the sample site "No Name" and 62.5% of samples from the sample site "Observatory Site did not exceed the L_d .

For direct comparison it is better to use Bq/g due to the different weight of each sample, comprising of either individual earthworms or groups of earthworms. The mean Bq/g of the Cs-137 of earthworms found at each sample site can be seen in Figure 13.

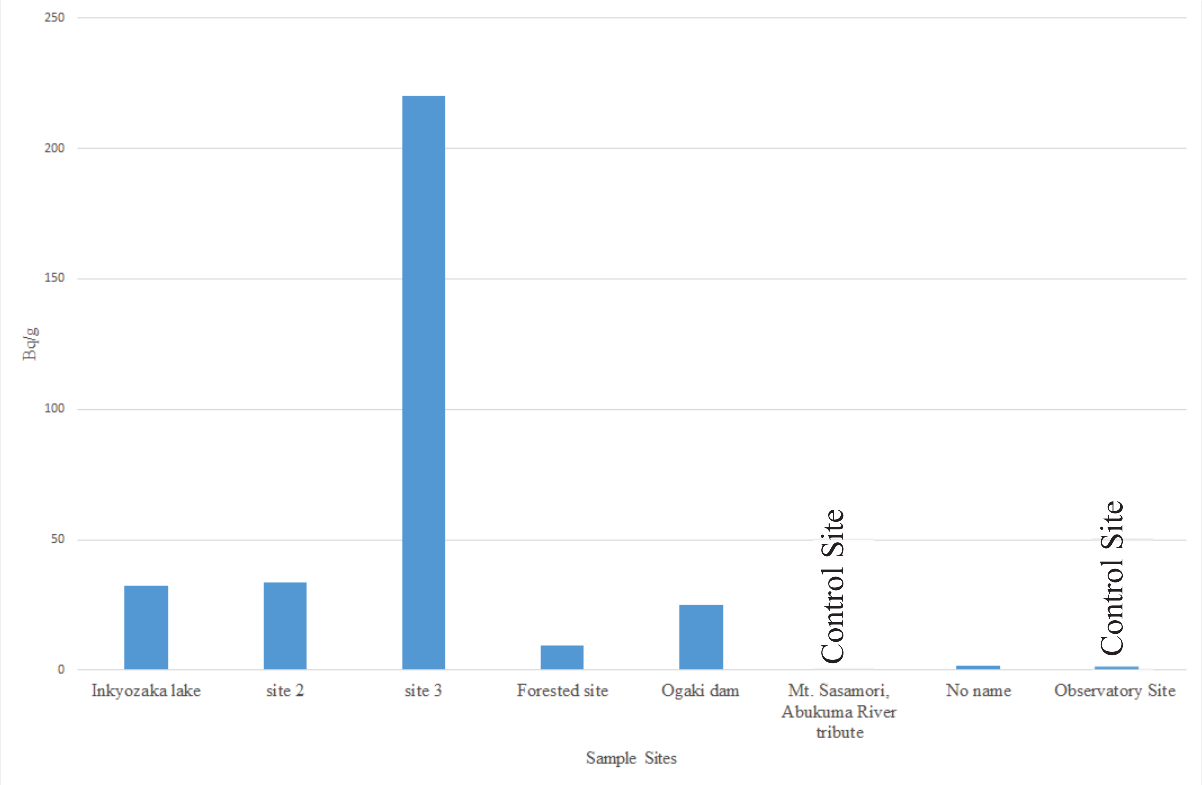


Figure 13, Comparison of Cs-137 Bq/g of sample sites for samples from the FDNPP 100 km Area.

Post Dissection

Using the measurements from the NaI detector samples from the FDNPP 100 km Area were selected due to the high activity and representation of sample sites. Samples from the ChNPP Exclusion were selected for their higher activity however selection was also influenced by the representation of sample sites and the descriptive information of the ecological groups. This selection process for samples from the ChNPP resulted in some samples with very low activity being selected for dissection. One worm from vials with multiple worms was selected, usually the heaviest to aid in the dissection process. The weight of individual as a whole was recorded individually from the total weight of all earthworms in that samples and a picture was taken for identification. This enabled more accurate

Descriptive statistics created using Minitab 18, for the skin and gut of each sample, combined to make mean minimum, maximum and median of the Cs-137 Bq/g for all skin samples and all gut samples, displayed in Table 10. For samples from the FDNPP all samples, both skin and gut, exceeded the L_d, however for the ChNPP samples due to the different selection methodology applied to this set of samples, sometimes the L_d was not met, seen in the minimum of both Skin and Gut samples in Table 11.

Table 10, Descriptive statistics, created using Minitab 18, of the earthworm samples collected in the FDNPP 100 km Area that had been dissected into separate skin and gut samples, from the Cs-137 results produced and calculated from the NaI detector. All measurements from all parts of the dissected samples exceeded the L_d, for this data set the L_d= 0.1458 Bq/g.

Statistics

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Cs-137 Bq/g SKIN	10	0	13,09	4,06	12,85	2,19	3,58	7,56	25,20	38,81
Cs-137 Bq/g GUT	10	0	472	105	332	23	155	527	762	968

Table 11, Descriptive statistics of the earthworm samples collected in the ChNPP Exclusion Zone that had been dissected into separate skin and gut samples, from the Cs-137 results produced and calculated from the NaI detector. When "0" this represents that the measurement (Bq/g) did not exceed the Ld, for this data set the Ld= 0.174 Bq/g.

Statistics

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median	Q3	Maximum
Cs-137 Bq/g SKIN	9	0	0,118	0,118	0,354	0,000	0,000	0,000	0,000	1,061
Cs-137 Bq/g GUT	9	0	12,54	6,07	18,22	0,00	0,00	8,35	22,77	50,34

The graph in Figure 14 shows the comparison of the Cs-137 Bq/g of the skin and gut of each sample of those collected from the FDNPP 100 km Area. The Cs-137 Bq/g of the skin is very variable compared to the corresponding gut sample. For sample 25.6 the percentage Cs-137 Bq of the skin, of the total Cs-137 Bq, is 6.85%, calculated from the data from Table 9. For Cs-137 Bq/g, as the skin is always lighter, the skin is 12.48% of the total Cs-137 Bq/g of the earthworm sample, using the data in Figure 14. In contrast sample 38 shows a much larger disparity between the skin and gut samples. The percentage Cs-137 Bq of the skin, of the total Cs-137 Bq, is just 0.26 and from the data displayed in Figure 14 the skin is just 0.61% of the total Cs-137 Bq/g.

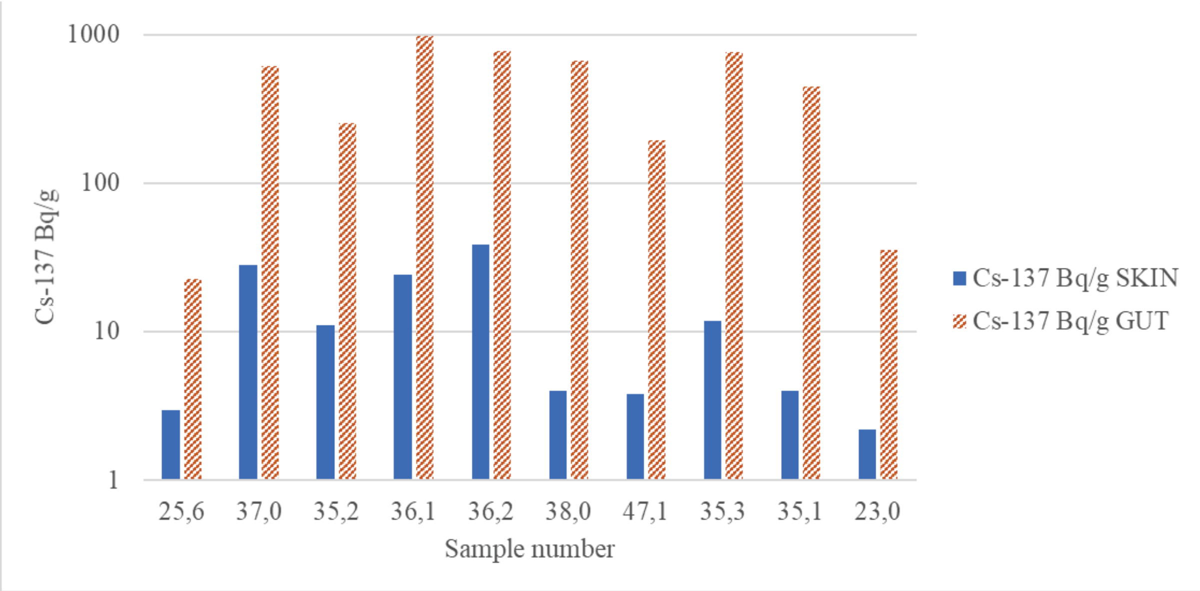


Figure 14 Graph to display the difference in the Cs-137 Bq/g in the skin and the gut of samples collected in the FDNPP 100 km Area. Note: Logarithmic scale.

Table 12 also shows the percentage of the Cs-137 Bq of the skin is of the gut samples. Commonly the skin samples are not above 10% of the gut samples, save for sample 23, however this is due to the skin sample not exceeding the L_d, therefore the gut sample was compared against the L_d value which was 0.12. In no cases did the skin exceed the gut of the sample for either Cs-137 Bq, or Cs-137 Bq/g.

Table 12, Measurements taken from the NaI detector of the Skin and Gut of each samples that was selected for dissection and calculated for Bq/g and the % of Cs-137 Bq the skin is of the gut for samples collected from the FDNPP 100 km Area. (including some blanks due to a formatting issue from transfer from excel). Unfortunately, due to time constraints measurements for Cs-134 Bq/g were unable to be calculated due to missing the efficiency for this radionuclide (%).

Description	Sample No.	Total Weight	Individual Weight (g)	Cs-134 Bq/g	Cs-137 Bq/g	L_d	Bq Cs137	%
BLANK								
GUT	25.6	0.144	0.095	//	22.6886234		2.16	
SKIN	25.6		0.049	//	3.234422603		0.16	7.352941
GUT	37	0.686	0.501	//	609.4538938		305.34	
SKIN	37		0.185	//	28.02741392		5.19	1.69815
GUT	35.2	0.228	0.115	//	253.3920156		29.14	
SKIN	35.2		0.113	//	11.22934651		1.27	4.354539
BLANK								
GUT	36.1	0.315	0.205	//	967.8038805		198.40	
SKIN	36.1		0.110	//	24.40974159		2.69	1.353364
GUT	36.2	0.289	0.195	//	775.6069425		151.24	
SKIN	36.2		0.094	//	38.95270417		3.66	2.420969
GUT	38	0.735	0.514	//	663.2868635		340.93	
SKIN	38		0.221	//	4.066846182		0.90	0.263624
BLANK								
GUT	47.1	0.300	0.181	//	194.4096081		35.19	
SKIN	47.1		0.119	//	3.900946882		0.46	0.131923
GUT	35.3	0.291	0.173	//	757.674622		131.08	
SKIN	35.3		0.118	//	11.89733458		1.40	1.071033
BLANK								
GUT	35.1	0.263	0.113	//	443.9166079		50.16	
SKIN	35.1		0.150	//	4.055896387		0.61	0.913662
GUT	23	0.041	0.014	//	36.22553316		0.51	
SKIN	23		0.027	//	2.688782852	$<L_d$		<23.66129

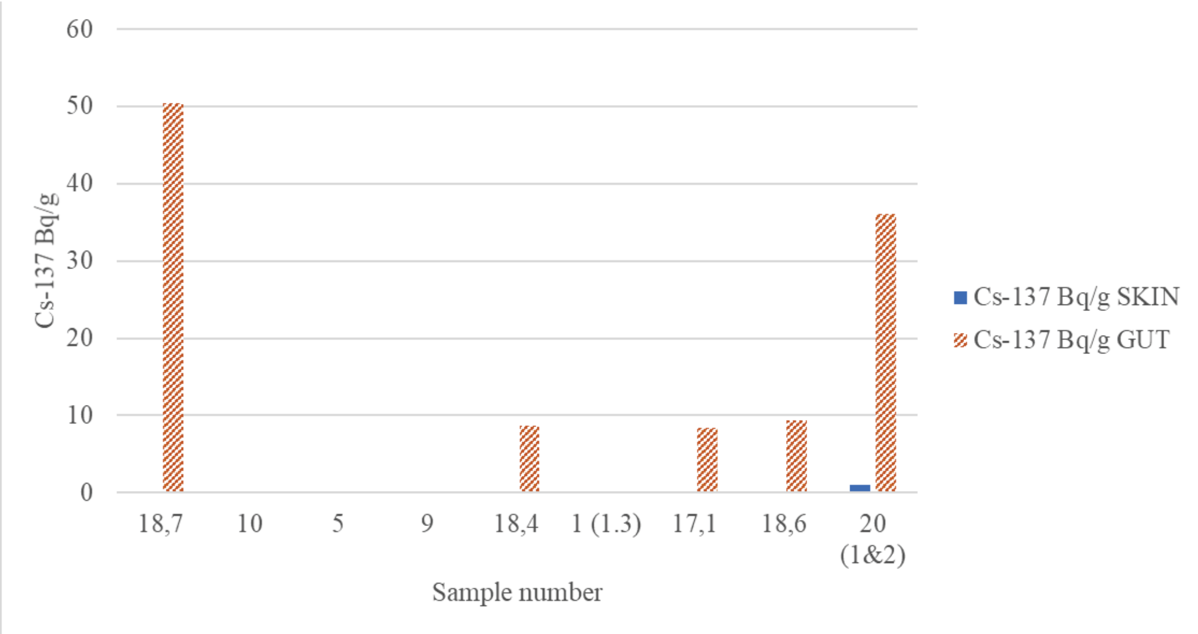


Figure 15, *Comparison of Cs-137 Bq/g in the skin and gut of earthworm samples collected from the ChNPP Exclusion Area.*

Samples from the ChNPP resulted in a lot of samples not exceeding the L_d, more commonly for skin samples however, likely due to the different parameters use for the selection of the dissected samples, some gut samples also did not exceed the L_d. A comparison of the dissected samples in Figure 15 shows that only sample 20 (1&2) exceeded even 1 Bq/g.

Table 13 shows that only samples 17.1 and 20 (1&2) had skin samples that exceeded the L_d, Sample 20 (1&2) is an Endogeic earthworm species and sample 17.1 is an Epigeic earthworm species.

To test if the skin samples from this location had any detectable activity the skin (only) samples were measured in the Ge-detector collectively, however excluding the skin sample of 20 (1&2). This produced a result of 0.232 ± 5.7% Bq (with background radiation already taken away)

Table 13, Measurements taken from the NaI detector of the Skin and Gut of each samples that was selected for dissection and calculated for Bq/g and the % of Cs-137 Bq the skin is of the gut for samples collected from the ChNPP 100 km Area. (including some blanks due to a formatting issue from transfer from excel).

Description	Sample No.	total weight (g)	individual weight (g)	Cs-137 Bq/g	L_d	Bq Cs137	%
GUT	18.7	0.12	0.025	56.94916944		1.423729236	
SKIN	18.7		0.095	0.338866032	$<L_d$		10.53571
GUT	10	2.5	2.038	0.101909757		0.207692084	
SKIN	10		0.462	-0.157342488	$<L_d$		72.2223
GUT	5	0.09	0.039	-0.213017522	$<L_d$		
SKIN	5		0.051	-0.162895752	$<L_d$		
GUT	9	0.79	0.555	0.117879291		0.065423006	
SKIN	9		0.235	-0.136988396	$<L_d$		72.2223
GUT	18.4	0.12	0.049	11.99527751		0.587768598	
SKIN	18.4		0.071	0	$<L_d$		25.52025
GUT (control site)	1 (1.3)	0.36	0.125	-0.373845751	$<L_d$		
SKIN (control site)	1 (1.3)		0.235	-0.101636552	$<L_d$		
BLANK							
GUT	17.1	0.46	0.212	9.13061426		1.935690223	
SKIN	17.1		0.248	0.443858083		0.110076805	5.686695
GUT	18.6	0.12	0.038	13.77326452		0.523384052	
SKIN	18.6		0.082	0.08864906	$<L_d$		28.65964
BLANK					$<L_d$		
GUT	20 (1&2)	0.46	0.227	36.84018398		8.362721763	
SKIN	20 (1&2)		0.233	1.769393935		0.412268787	4.92984

Discussion

Comparison of Earthworms Ecological Groups

As previously discussed, earthworm species can be divided into the ecological groups Epigeic, Anecic and Endogeic, based on their behaviours and habitats which was first proposed in 1971 and 1977 by Bouché (Edwards and Bohlen, 1996). For this study only the earthworm samples collected from the ChNPP Exclusion zone were identified by ecological group and only Epigeic and Endogeic earthworm species were identified, leaving no representation of Anecic earthworms. The differences between the Epigeic and Endogeic earthworms are the source of food. Epigeic earthworms live and feed from the leaf litter whereas Endogeic earthworms live in deeper mineral soil layers and feed on a mixture of on decomposing roots and soil organic matter (Edwards and Bohlen, 1996; Lavelle and Spain, 2005). As the earthworm samples were all collected from sample sites located within the ChNPP Exclusion Zone comparing the two ecological groups is of interest to observe the different effects that the fallout from the 1986 ChNPP Accident may have had.

The difference between the Cs-137 Bq/g for each sample (excluding samples that did not meet the L_d) split into their ecological groups was tested for the statistical significance using the statistical software "Minitab 18". The data was tested for their distribution and equal variances, however, despite the distribution of both ecological groups being a normal distribution they did not test positively for equal variances and therefore a non-parametric Mann-Whitney test was used. The Mann-Whitney test showed that there was not enough evidence to support any statistically significant differences between the data. This suggests that despite the difference in feeding and burrowing behaviour and depth of habitat that the different ecological groups follow, does not affect the level of Cs-137 radioactivity of the earthworm

In some cases, samples of earthworms collected from one of the ecological groups was not found at a site where the other ecological group was reprisented. Such an example occurred at the sample site "Glyboky Lake" were only Endogeic earthworm samples were collected, whereas at the sample site "Zamoshyna" only Epigeic earthworms were sampled whereas Endogeic earthworms were not. The samples that contained examples of both ecological groups were the sample sites described as "control sites" by the COMET, 2017, project, "Glinka" and "Chernobyl City". Of the samples collected from "Glinka" no samples of either ecological group exceeded the L_d and therefore cannot be differentiated from background radiation, of samples collected from the sample site "Chernobyl City", however, there were samples representing examples from both ecological groups that did exceed L_d, although not all. The mean Cs-137 Bq/g of earthworm samples identified as from the Epigeic ecological group from the sample site "Chernobyl City" was 0.25 Bq/g, and for sample collected from the same sample site however were identified as from the Endogeic ecological group the mean Cs-137 Bq/g was 0.039 Bq/g. This shows that when samples from both represented ecological groups are represented in the same sample site Epigeic earthworm samples are more radioactive (Cs-137) than Endogeic earthworms.

The Data collected from this sample site would have been useful to test for the statistical significance of the difference using the Minitab 18 statistical software. However, there is a lack of samples to test for parameters such as the test for normality using a probability plot as there was only one sample (vial) of an Endogeic earthworm and two samples (vials) of Epigeic earthworms. These samples all contained multiple earthworms within one vial, therefore in further analysis it would be possible to separate these samples and, provided that the activity is not just confined to one earthworm of the collective and multiple earthworm samples exceed the L_d, this test could be conducted. However, due to time constraints this was not possible in this study.

The observations of "Chernobyl City" is only the case for this sample site, when all sample sites are collected together the total mean of all Epigeic earthworms from all sample sites was 2.58 Bq/g and the total mean of all Endogeic earthworms from all sample sites was 4.71 Bq/g. However, the later measurement is skewed by the presence of the high activity of the sample site.

Endogeic earthworm from the samples site "Glyboky Lake" had the highest soil activity, nearly 50 times that of Zamoshnya, (COMET, 2017). The highest Bq/g of Epigeic earthworm samples was collected from the sample site "Zamoshnya", at 9.4 Bq/g.

Taking the whole sample set, of the 17 samples that exceeded the L_d 64.70% of these samples exceeded 1 Bq/g, of which 10 of these were of the Epigeic earthworm group and only one was of the Endogeic earthworm group, which, as stated above was from the site with the highest activity. In total however there are more than double the number of examples of Epigeic samples in vials, 25 samples, than there are examples of Endogeic samples, nine samples, including samples that did not exceed the L_d. Only four of these 9 Endogeic samples, or 44.44%, exceeded the L_d, whereas only 13 of the 25 Epigeic earthworm samples, or 52%, exceeded the L_d. Reviewing this raw data supports the hypothesis that there is a difference between the ecological groups, that there is a higher transfer of radiocaesium to Epigeic earthworms than to Endogeic earthworms.

The discrepancy of ecological groups not commonly being represented in the same sample site is confirmed preliminary observations in the COMET, 2017, project, as seen in Table 1 (COMET, 2017). Where the highest quantity of *Eiseniella tetrahedra*, 13 samples from "Control 1", the highest number of any other earthworm samples at this sample site was 6, *Eisenis fetida* which is also an Epigeic species. Another example was *Apporrectodea caliginosa*, which the highest quantity of this species, 32, was collected from the sample site "Control 2". The only other species collected at this sample site was *Aporrectodea rosea*, of which there was only one example collected. *Aporrectodea rosea* and *Apporrectodea caliginosa* are both of the Endogeic ecological group. The only example where there is a large quantity, although still almost half, of two different species identifiedis from the sample site "Medium 2", with 37 samples of *Octolasion lacteum* and 20 samples of *Apporrectodea caliginosa*. These samples are both identified as being from the Endogeic Ecological group, which is consistently the case in all examples. Either there are no significant numbers of any other species at the sample site, or when there is a significant quantity of another species they were identified as being from the same ecological group which reflects in the samples of this study. This would suggest preferences of particular sample sites being preferable to just one of the ecological groups, or potentially evidence of a competitive relationship between the ecological groups of earthworm ecological groups. This factor could explain why it was difficult to compare ecological groups from the same sample sites as commonly there would not be many, if at all, representative samples of both of ecological groups at same sample site.

Several studies have shown the movement of radionuclides through the soil over time, many of these studies use the ChNPP Accident and the FDNPP Accident to observe the factors effecting the rate at which the fallout distributes through the vertical soil profile over time (Schimmack et al, 1989; Korobova et al, 2014; Shcheglova et al, 2014; Teramage et al, 2014; Matsuda et al, 2015; Takahashi et al, 2015; Teramage et al, 2016; Mishra et al, 2018; Takahashi et al, 2018). Previous studies show that the distribution of radionuclides in the soil profile is a patchy distribution and not easily predicted. Cs-137 has been shown to collect in areas with high organic matter, a major food source for earthworm species, and become bound to clay minerals hindering the bioavailability of the Cs-137 (Fuller et al, 2015;

Hasegawa et al, 2013). In the time directly following the deposition of radionuclides following a nuclear accident all of the deposition would be collected in the leaf litter layer and most interaction would be by Epigeic (and Anecic) earthworm species. In contrast Endogeic earthworm species my not have as much initial exposure due to this lack of interaction with the surface leaf litter, and instead consuming mineral soil and decomposing leaf litter within the deep soil layers. The time that has passed in the ChNPP Exclusion Zone could be enough time that the migration of radionuclides has reached areas inhabited by Endogeic earthworm species (depending of factors that may affect the rate of the migration of radionuclides in the soil profile). This factor may be why we see no statistical significance, in the analysis using Minitab 18, between the earthworm ecological groups as both ecological groups now have pathways of exposure. If the ecological groups of the earthworm samples from the FDNPP 100 km Area were identified, due to the smaller amount of time passing since the FDNPP accident, a different result may have been observed.

Previous studies have suggested because Epigeic earthworms have a higher rate of reproduction and exhibit a rapid growth rate, these features allow for the adaption to dramatic changes in their environment (Ray (ed.), 2018). The reason why there are more examples of Epigeic samples rather than Endogeic samples could be due to there just being a larger number of this species because of their better adaption to the contaminated environment. Despite neither species showing to be statistically any more radioactive than the other group, it could be that Epigeic earthworms are thriving in populace at these sample sites whereas Endogeic earthworm populations are suffering in comparison. This however is countered by the preliminary COMET, 2017, project study as the highest quantity of earthworm samples at all sites was *Octolasion lacteum,* an Endogeic earthworm species (table 1). Provided the same methodology of sampling for the samples collected for the COMET, 2017, project (the samples used in this study) as was used in the preliminary study the sampling technique did not show a bias towards the sampling of one ecological group over the other.

Dissected Samples, Transfer from Gut Contents to Skin

Samples selected for dissection were based on a few factors depending on their collection location but mostly on the basis of the highest Cs-137 Bq/g with a representation of multiple sample sites. At the ChNPP samples were also selected to represent the earthworm ecological groups: Epigeic and Endogeic. This resulted in some higher activity samples not being selected for dissection in favour of samples with a lower activity in order to better represent the present ecological groups or representation of more variety of sample sites. In addition to this a sample from a control site was selected for dissection from the ChNPP Exclusion Zone samples to confirm the sample would show very low activity, not likely to exceed the L_d, which was confirmed.

By separating the skin and the gut, lining and contents, it is possible to see if the activity measured by the NaI Detector in the initial runs discussed in the precious section was due entirely from the soil that remained in the gut or if there was transfer to the skin.

The results of the dissected samples from the FDNPP showed that the activity of the gut contents did not dictate the activity of the skin, which could be shown as a percentage, ranging from 6.85% of the total Cs-137 Bq of sample, or 12.5% of the total Cs-137 Bq/g. in contrast the samples with the highest disparity between the results was just 0.26% of the total Cs-137 Bq, or is just 0.61% of the total Cs-137 Bq/g. The sample with the highest percentage was from the sample site Inkyozaka lake, which was the sample site closest to the location of the FDNPP, as seen in Figure 8, however the sample with the lowest percentage was from a sample site with notably high radioactivity at 40 µSv. Unfortunately, this site did not have a GPS location. This sample site was the location of the highest measured Cs-137 and Cs-134 activity in earthworms for both the COMET, 2017, project and this study. Therefore, the samples with both the highest and the lowest percentage of the total in the skin were from sample sites with a notable amount of radioactivity in the soil, confirming a variation of transfer even at the same site.

The skin sample with the highest percentage of the total Cs-137 Bq/g and Cs-137 Bq was still only 7.35% of the gut sample, therefore no skin samples, excluding samples where at least one component (skin or gut) did not exceed L_d, had more than 10% that of the gut sample. Most of the activity seen in the initial NaI detector measurements can be attributed to the gut, which was similarly found in the autoradiography results of Fujiwara et al, 2015, and Tanaka et al, 2018.

Similar results were found in the samples from the ChNPP, although most result did not meet the L_d most likely due to the sample selection method. The samples that did show detectable measurements from both the skin and the gut were both of Epigeic and Endogeic species, although the higher result was of the Endogeic ecological group. Due to a lack of examples exceeding the L_d, however, it is not possible to draw conclusions about ecological groups from this data. Where samples did exceed the L_d a similar result as the samples from the FDNPP was observed, no skin sample was above 10% of the gut sample, and only one skin sample exceeded 1 Bq/g. in comparison 50% of the samples from the FDNPP 100 km Area exceeded 1 Bq/g, this is due to the higher activity of Cs-137 in that location as the FDNPP accident was more recent.

Skin samples from the ChNPP Exclusion Zone that did not exceed the L_d, and the inclusion of one very low activity skin sample, we grouped together to be recorded on the Ge-detector to see if there was any activity above background radiation. The results of the Ge-detector found that these samples collectively showed a result of 0.232 ± 5.7% Bq above background radiation, showing that there was a small amount of transfer detectable from using a more sensitive tool. This is 4.9% of the collective Cs-137 Bq of gut samples that exceeded the L_d (and excluding sample 20 (1&2), which was also excluded from the collective skin sample).

However, 5 of the 9 of the dissected samples from the ChNPP Exclusion Zone had only the gut measurements exceeding the L_d and not the skin samples. This could potentially be due to the time since the ChNPP accident and therefore the Cs-137 has already passed one t½ making the Cs-137 in the earthworm samples more difficult to detect, as evident in the initial measurements of the earthworm sample as 52.78% of samples from this location did not exceed the L_d. A factor that affects this could also be the availability of the Cs-137 in the soils of the ChNPP Exclusion Zone. Potentially the Cs-137 could be bound to clay which would be consumed by the earthworm however would just be passed through and could not be transferred by the earthworm to the tissue as the Cs-137 is no longer bioavailable (Fuller et al, 2015; Hasegawa et al, 2013).

Despite the skin samples showing lower Cs-137 Bq/g compared to the gut samples, there is evidence of a small amount of transfer to the skin of the earthworm. This is supported by the study by Sheppard et al 1997 into the depuration rate in tissues, which found that Cs-134 still remained within the tissues of *Lumbricus terrestris (an Anecic earthworm species)* for 80 days, with 20% of the Cs-134 still remaining. Although this study concentrated on Cs-134 it shows that the uptake into the skin is long term, as least in comparison to an earthworm lifespan of up to 4 years. This contrasts with study by Fujiwara et al, 2015 determined after just one day of culture in soil not contaminated by radiocaesium the activity in the earthworm, *Eisenia fetida (an Epigeic earthworm)* was not detectable above the L_s. This potentially highlights the impact of the difference between using different earthworm species, identified as different ecological groups.

Conclusion

Samples collected from the ChNPP Exclusion Zone and the FDNPP 100 km Area by the COMET, 2017, project, underwent several measurements to understand the differences between the ecological groups: Epigeic and Endogeic, and the uptake to the tissue of the earthworm from the gut contents.

Earthworm samples from the ChNPP Exclusion Zone were identified for their ecological group. The Bq/g of the samples from each group was tested for the statistical significance using a Mann-Whitney non-parametric test. The results showed that there was no statistically significant evidence to support that there was a difference between the Bq/g of each group.

Previous studies of the distribution of radionuclides in the soil profile may hint to different exposures for the ecological groups over time due to the difference in habitats. Epigeic earthworms would experience more exposure in the initial deposition onto the leaf litter layer following a nuclear accident however over time as the radionuclides migrate down through the soil profile to the mineral soil layers. In the time since the ChNPP accident this effect may be being observed.

The result of the statistical analysis, however, is contested due to the difference in the activity of the sample sites. In most cases the Bq/g of Endogeic earthworms was less than that of Epigeic earthworms but the highest Bq/g of the earthworm samples, from the sample site "Glyboky Lake" which has an activity nearly 50 time that of another sample site Zamoshnya, skewed the mean of the Epigeic earthworm samples. By reviewing the raw data, the hypothesis that there is a difference between the ecological groups, is supported and would appear that there is a higher transfer of radiocaesium to Epigeic earthworms than to Endogeic earthworms. It is also noted that there is a higher number of Epigeic earthworm samples rather than Endogeic earthworm samples. This could be due to factors allowing Epigeic earthworms to adapt to the contaminated environment better than Epigeic earthworms, such as a higher reproduction rate and rapid growth rate.

The earthworm samples were dissected and tested for the distribution of the Cs-137 throughout the body. The skin and the gut, with gut contents, were separated and measured individually to investigate the transfer of Cs-137 from the gut contents to the tissue (skin). It was found that most of the activity measured was contributed to the gut contents and the skin was less than 10% of the activity of the gut sample. This result is in agreement with previous studies, that most of the activity is from the gut contents, however, there was still evidence that a small percentage of the radionuclides is transferred to the tissues of the earthworm. The samples from the ChNPP Exclusion Zone were collectively measured in the Ge-detector and found that samples, that did not exceed the L_d when measured in the NaI detector, still had a small amount of activity, 4.9% of the collective Cs-137 Bq of gut samples, excluding those that did not exceeded the L_d. Samples from the ChNPP Exclusion Zone commonly did not exceed the L_d, which could be attributed to a lot of the Cs-137 in the ChNPP Exclusion being bound to clay minerals and therefore not bioavailable.

Limitations of the Study

Samples collected from this study observed a discrepancy of representation of ecological groups at different sample sites. This same discrepancy was observed in the preliminary sampling in the COMET, 2017, project. Where a sample of one species of the represented ecological group was collected from a sample site, any sample of the other represented ecological group is commonly under represented or not present. In cases where there are two species present at a noTable quantity at the same sample site it is usual that they are of the same ecological group. What cannot be observed in the study but could be observed in the initial sampling is the specific species. This study only uses the ecological group, not specific species, however this same limitation is observed. It is unlikely to be a sampling error as it is likely that the same sampling technique was used at every sample site (although this is not actually specified in the methodology in the COMET, 2017, project) then there would be an under representation of an entire group at all sample sites, not a variable representation. It is also possible that there is competition between the two ecological groups in the same area, which there is potentially evidence for in the initial sampling that there is usually a dominance of a single species in a sampling site. However, it appears that this is not a theory represented in the literature and is an area that could benefit from further study.

This underrepresentation of ecological groups at sample sites potentially created issues with the data analysis. Where there was data of earthworms of an ecological group at a sample site, the other ecological group at the sample site had no representation and therefore could not be directly compared. At a sample site which had both ecological groups represented ("Chernobyl City") on ecological group had a mean Cs-137 Bq/g that was higher for one ecological group than the other, although this sample site only had one sample identified as from the Endogeic ecological group and two samples as from the Epigeic ecological group that were above the L_d. In future this samples could be split into multiple samples and each individual measured, potentially creating more samples to work with. Or additionally if identification in the field is possible then to collect for a longer time at an individual site until example of both groups have been sampled, however reviewing the preliminary study by COMET, 2017, commonly only one of the ecological groups are found at one site.

In this study no Anecic earthworms were identified. It could potentially be an oversight to not include this ecological group in a study comparing ecological groups. This could however just be that no Aneic earthworms inhabit the ChNPP Exclusion Zone, or at least the sample sites selected. Studying Epigeic and Endogeic earthworms at least covers the earthworms living predominantly in the leaf litter and top layers of soil and the earthworms living in deeper layers of the mineral soils, whereas Anecic earthworms' traverse in between the habitats of these two groups.

It would have been a great benefit to the study to be able to identify the earthworms that were collected in the FDNPP 100 km Area. This limitation was due to a time constraint as the earthworm samples would not have been identified by the time of the submission. Samples from this area mostly exceeded the L_d for Cs-137, whereas a lot of earthworm samples from the ChNPP did not, due to the more recent event of the FDNPP accident. If these were identified then the comparison may have had stronger data to work with as the data from the ChNPP had a very few Endogeic earthworm examples.

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