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The Forest Trees of Chernobyl

A Master's Thesis submitted for the degree of
"Master of Science"

supervised by
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Affidavit

I, **MYLENE GRACE V. VELASQUEZ**, hereby declare

1. that I am the sole author of the present Master's Thesis, "THE FOREST TREES OF CHERNOBYL", 81 pages, bound, and that I have not used any source or tool other than those referenced or any other illicit aid or tool, and
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ABSTRACT

The thesis reviewed and assessed studies on forest trees inside the Chernobyl Exclusion Zone. Eleven studies discussing radiation impact on various levels were evaluated and analysed. Consequences were found in terms of population, cellular and subcellular, genetic and intergenerational impacts.

The noticeable recovery of biota in the vicinity of the ChNPP raised the issue of redevelopment and resettlement in areas affected by the fallout. A contrasting opinion offered by scientists stated that visible signs of repair by the biota were not enough indications of recuperation. Forest trees surrounding the V.I. Lenin reactor as a component of the environment that survived the worst of the fallout is a good starting point for discussion but it is not taken as a representative of the whole ecosystem.

Results of the study signified that forest trees which did not absorbed the lethal threshold dose employed different mechanisms including morphological and physiological means to limit the damage. The low intensity of mutation found in specimens exposed to either high acute or chronic or both types of radiation suggested that defense mechanisms that do not excessively rearrange DNA were preferred over other repair systems. The strong relationship between mutability and sterility is interpreted as protecting strongly damaged genetic material from being passed on to future generations.

Assessment of other environmental components is further recommended.

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LIST OF ABBREVIATIONS

AFLP – Amplified Fragment-Length Polymorphism
ATP – Adenosine Triphosphate
Cat – Catalase
CEC – Cation Exchange Capacity
CERRIE – Committee Examining Radiation Risks of Internal Emitters
CEZ – Chenobyl Exclusion Zone
ChNPP – Chernobyl Nuclear Power Plant
DNA – Dioxyribonucleic acid
cDNA – compementary DNA
rDNA – recombinant DNA
DNMT – DNA Methyltransferase
DSB – Double Strand Breaks
DSBR – Double Strand Break Repair
IAEA – International Atomic Energy Agency
GPx – Glutathione Peroxidase
LET – Linear Energy Transfer
MFA – Microfibril Angles
NER – Nucleotide Excision Repair
NPP – Nuclear Power Plant
PAL – Phenylalanine ammonialyase
ROS – Reactive Oxygen Species
SSB – Single Strand Breaks
SSRs – Simple Sequence Repeats
UDS – Unscheduled DNA Synthesis

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1. Introduction

This thesis is a review and assessment of selected studies and publications on forest trees in the immediate vicinity of the Chernobyl Nuclear Power Plant (ChNPP) with the intention of consolidating data to provide more clarity regarding the effects of extremely high radiation due to fall-out from a nuclear power plant on trees.

Two polarised development policy views concerning areas heavily affected by the ChNPP accident fall-out had emerged when it comes to the effect of the explosion decades after the event. One of the views advocated the resettlement and redevelopment within the areas outside the exclusion zone but which were evacuated in the immediate aftermath of the ChNPP (WNN, 2010) while the other view cautioned against any action due to known and still unknown environmental impacts (Mousseau et al., 2005). Ramifications of radiation damage must be accounted for before any economic consideration can be contemplated. The full resolution of these concerns is beyond the scope of this thesis. It does, however, aim to pin a starting point from which the discussion of this issue can commence.

Forest trees inside the 30-km exclusion zone were chosen as suitable for several reasons. First, the 30-km exclusion zone experienced the worst case-scenario. Any negative effects observed inside the exclusion zone is assumed to weaken or diminish outside the said area. Second, the forest cover in the vicinity of the ChNPP received the brunt of the initial impact during the explosion which tested the ability of the trees to survive under extreme radioactive conditions brought about by nuclear power plant accidents. Third, the forest trees covered a wide range of radio sensitivity or radio resistance which is expected to provide insights on the adaptability of plants. Fourth, forest trees are perennials as opposed to many crops that are often the subject of induced mutation. Prior to 1986, knowledge regarding the effect of radiation on plants were largely from short-lived crops which were conducted under controlled environment and with the particular purpose of improving genetic material. In contrast, forests in the vicinity of ChNPP made the observance of long-term effects of exposure to extreme radiation possible. These trees may possibly provide better perspectives on plant mechanisms and functions that respond to long-term extremely high radioactive environmental stress. The perennial life of trees also signifies that it will be possible to compare the mechanism at work of a parent tree with its progenies. Lastly, the trees have economic value in contrast to herbaceous forest plant species.

1.1 | Objectives

The overarching aim of this thesis was to review and consolidate data regarding the effect of ChNPP explosion on forest trees through the review and assessment of selected studies and publications.

It aimed specifically to:

1. Review and assess selected studies on forest trees which had been subjected to the effect of the ChNPP explosion on various levels.
2. Analyse the implications of the results of the aforementioned studies to the survival of forest trees.
3. Identify gaps in the study of forest trees within the vicinity of Chernobyl.
4. Recommend approaches for potential avenues to extend the body of knowledge.

1.2 | Methodology

This thesis explored and investigated the consequences of exposing plants to a situation characterised by extreme radiation. It relied primarily on collecting and examining existing data from studies done within the environs of Chernobyl in the aftermath of the Nuclear Power Plant explosion.

A thorough search for studies, publications and other relevant materials through the internet and libraries was conducted at the start of this thesis. A set of criteria, detailed below, was established for filtering materials for inclusion. The process allowed the staggering amount of materials present on library, Internet searches, databases and online journals related to Chernobyl and nuclear topics to be whittled down into a manageable number. From numerous studies, the final number of studies found to have satisfied the set of criteria were eleven. Numbers from 1-11, based on the chronological order of their publication year except for the case of study number (SN) 4, were assigned to the studies for identification. SN4 deviated from the numbering because it was a follow-up to the study preceding it. SN8, SN9, and SN10 were also related studies but published consecutively so the need to deviate from numbering chronologically by year published was not necessary. These eleven studies were reviewed and analysed concerning their premise, year and duration in the conduct of study, results, consequences of said results and other factors. Data relating to the survival and recovery of forest trees were extrapolated, then compared and analysed to existing knowledge from reference materials. Gaps in existing body of literature regarding the study of forest trees in Chernobyl were then identified and potential avenues for impending studies were suggested.

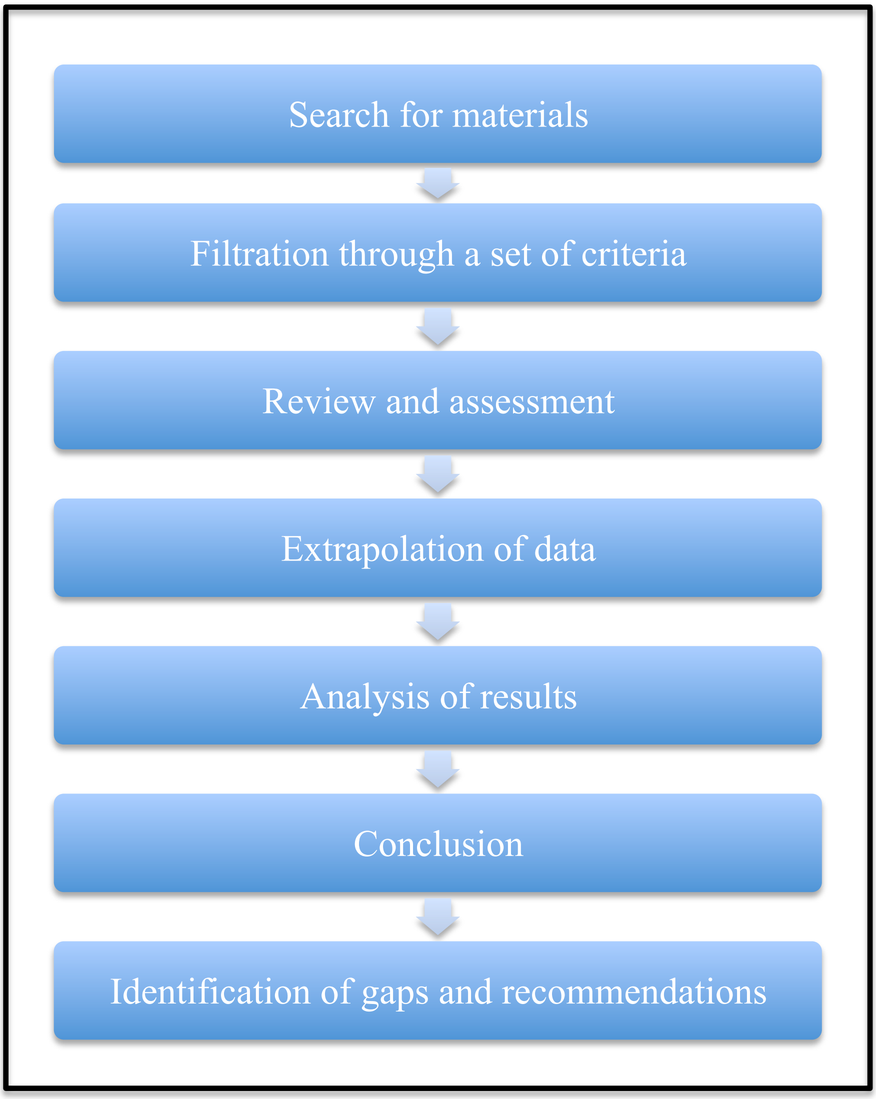

Figure 1.1 | **Methodology**

Criteria

1. Forest species must be the subjects or one of the subjects of investigation.
2. The said forest specie should be located within the 30-km Chernobyl Exclusion Zone (CEZ).

3. The time of conduction must be wholly or partly after the ChNPP explosion.
4. The core methodology must not rely on reviewing, assessing, analysing, compiling and summarising data from other studies.
5. The core methodology must not rely on analysing satellite images.
6. Access to the study or a translated copy into English must be open.

1.3 | Scope and Limitations

This thesis reviewed and assessed studies focused on forest trees surviving in a very particular setting. The overarching aim was to provide a starting point on the progression of recuperation of forest trees within the CEZ in the context of redevelopment or resettlement. Data extrapolated was limited to studies which were readily accessible and written or was already translated to English.

2. The ChNPP Explosion and the List of Studies on Forest Trees

The majority of forest plant species are divided into soft woods and hard woods. In general, hard woods and soft woods are differentiated through the density of their material although the actual dividing line between the two is the seed structure. Wherein hard woods, classified as angiosperms, have seeds that are covered, soft woods or gymnosperms let their seeds fall to the ground with no covering. Hard woods were established in existing literature to be more radioresistant than soft woods like Pinus sylvestris, which was the specimen under scrutiny in most of the studies listed. Evidence of external recuperation in damaged forest plants existed as trees which sustained extensive damage but were not exposed to the threshold of lethal dosage rebounded back after a few years.

Official publications from the UN in the 10^{th} and 20^{th} year of the ChNPP recorded recovery of coniferous species in most areas except the Red Forest Zone. It had been an accepted fact a decade after the explosion that the most sensitive life stage for all organisms, including plants, are the earlier ones like embryo and juvenile which are periods of active growth and development phases (IAEA, 1996).

Initial evaluation of the studies revealed the prominence of Scots pine trees as the most studied forest tree specie due to the variety of observed consequences after the accident but a few comparison studies involving hard woods with other forest species like herbaceous ones were also found. In cases like the latter, only data concerning the forest specie is considered for inclusion.

Eleven studies were found to comply with the established set of criteria. These studies were assessed according to their objective, years of conduction, specimen collection as well as the results with regards to survival, fitness and adaptability of forest trees. Table 2.1 is the final list of studies reviewed and assessed for this thesis.

Table 2.1 | **List of Assessed and Reviewed Studies**

Study #	Year Published	Authors	Title
1	1994	N.P. Arkhipov, N.D. Kuchma, S. Askbrant, P.S. Pasternak, and V.V. Musica	Acute and long-term effects of irradiation on pine (Pinus silvestris) stands post-Chernobyl
2	1998	A.P. Møller	Developmental Instability and Radaition from Chernobyl
3	2002	M. Tulik	Cambial history of Scots pine trees (Pinus Sylvestris) prior and after the Chernobyl accident as encoded in the xylem
4	2004	M. Tulik and A. Rusin	Microfibril angle in wood of Scots pine trees (Pinus Sylvestris) after irradiation from the Chernobyl nuclear reactor accident
5	2003	O. Kuvalchuk, P. Burke, A. Arkhipov, N. Kuchma, S.J. James, I. Kovalchuk, and I. Pogribny	Genome hypermethylation in Pinus silvestris of Chernobyl – a mechanism for radiation adaptation?
6	2005	L. Zelena, B. Sorochinsky, S. von Arnold, L. van Zyl, and D.H. Clapham	Indications of limited altered gene expression in Pinus sylvestris trees from the Chernobyl region
7	2007	I. I. Boubriak, D. M. Grodzinsky, V. P. Polischuk, V. D. Naumenko, N. P. Gushcha, A. N. Micheev, S. J. Mccready and D. J. Osborne	Adaptation and Impairment of DNA Repair Function in Pollen of Betula verrucosa and Seeds of Oenothera biennis from differently Radionuclide-contaminated Sites of Chernobyl
8	2011	O. Kuchma and R. Finkeldey	Evidence for selection in response to radiation exposure: Pinus sylvestris in the Chernobyl exclusion zone
9	2011	O. Kuchma, B. Vornam and R. Finkeldey	Mutation rates in Scots pine (Pinus sylvestris L.) from the Chernobyl exclusion zone evaluated with amplified fragment-length polymorphisms (AFLPs) and microsatellite markers
10	2011	B. Vornam, A. Arkhipov, and R. Finkeldey	Nucleotide diversity and gene expression of Catalase and Glutathione peroxidase in irradiated Scots pine (Pinus sylvestris L.) from the Chernobyl exclusion zone
11	2013	T.A. Mousseau, S.M. Welch, I. Chizhevsky, O. Bondarenko, G. Milinevsky, D. J. Tedeschi, A. Bonisoli-Alquati and A.P. Møller	Tree rings reveal extent of exposure to ionising radiation in Scots pine Pinus sylvestris

2.1 | The Zone of Focus

Studies conducted in the Chernobyl Exclusion Zone (CEZ) were chosen due to the extent of radiation impact on forest trees recorded in the region therefore it was assumed that any negative effects observed outside the CEZ was magnified inside it.

The exclusion zone was located in Ukranian Polessye physiographic province central part which originally referred to a 30-km radius institutionally-controlled sealed-off site established immediately after the accident as a precaution (Faybishenko, 2004). The boundaries were, however, adjusted as more detailed monitoring of the contaminants revealed the need to increase or decrease these borders. Due to several factors which affected radionuclide distribution and activity concentration, the effect of the ChNPP accident in various locations varied in intensity from those which were outside this area. Faybishenko citing Shestopalov (2004) stated that the CEZ within Ukraine borders only excluding the Kiev reservoir had a total area of 2,044 km^2 composed of 50% forest, 30% arable land and the remaining 20% to be a mixture of urban areas, forest, marchlands, and waterbodies. Sokolov et al. (1993) estimated that around 70% of the forest which covered the vicinity of accident site, 63% were coniferous trees (Pinus sylvestris and Picea abies) and the remaining 37% were hardwood species made up mainly of birch (Betula pendula), aspen (Populus tremula), alder (Alnus glutinosa), and oak (Quercus robur). The canopy of these forests intercepted the cloud of radioactive material which exposed them more to radioactive material due to the filter effect (Tikhomirov and Shcheglov, 1994). It served as the primary mechanism for preventing the full impact of the contamination to reach the public in the immediate aftermath of the accident.

Contamination within the 30-km exclusion zone were uneven. Former caesium-137 contamination in hot spots reached around 22 370 000 kBq/m^2 or 10 000 Ci/km^2 while strontium-90 were up to 222 185 000 kBq/m^2 or 5000 Ci/km^2. Plutonium ones were up to 555 kBq/m^2 (15 Ci/km^2) (IAEA, 1996). Fission products which had accumulated in the reactor core was given off in a thermal and steam explosion. This was different from Hiroshima and Nagasaki bombs which were nuclear explosions that caused thermal and blast effects with a great deal of direct radiation to intentionally devastate large urban areas but little production of fallout radionuclides (IAEA, 1996).

Table 2.2 | **Comparison of Atomic Bomb and ChNPP Hot Particles**		
Characteristics	Atomic nuclear bomb	ChNPP Accident*
^{60}Co and other activation products	Present	Absent
^{25}Sb and ^{144}Ce presence	Absent	Present
^{137}Cs presence	Present	Larger fraction
^{154}Eu/^{155}Eu ratio	Present	10-fold larger
U and Np radiation	Present	Lower
Pu content	Present	Lower
Data extrapolated from Faybishenko (2004) *ChNPP values of lower and higher pertain to comparison with atomic nuclear bomb fallouts		

Prevailing weather conditions affected the deposition of the radionuclides. Non-uniformity was due to the shifting direction of the winds and sporadic rainfall necessitating more detailed measurements (IAEA, 1996). Smith and Beresford (2005) cleared the difference of the implications between wet deposition and dry deposition. The efficiency of wet deposition washout by rain, snow, fog or ground-level cloud culminated into hot spots 150-250 km to the northwest of Chernobyl and areas of comparatively high deposition through Europe. Dry deposition, on the other hand, happened through the direct adsorption onto the earth's surface of gaseous elements, the impaction of airborne particles with the surface and the fallout of heavier particles near the source. In the forest, the speed of dry fallout of small particles was significant due to the more turbulent atmosphere and efficiency of absorption by tree phytomass (Tikhomirov and Scheglov, 1993). Volatile radionuclides such as ^{134,137}Cs, ^{132}Te, ^{131,133}I and $^{103, 106}$Ru were released from the core by evaporation and attached to small duct particles (aerosols) in the atmosphere through a large part of the radio iodine remained in gaseous form. Variation in radionuclide precipitation processes affected the difference in the ^{90}Sr and ^{137}Cs circulation patterns (Faybishenko, 2004).

2.2 | Radioactive Material from ChNPP

The reactor emitted a total mass of 6-8 tons of radioactive particles which settled in the ground or close to the ground surface in Byelorussia and Ukraine over a period of 8-10 days (Sandalls et al, 1993). The initial estimate of the Soviets of released radioactive material was 4 EBq (1 ExaBecquerel = 10^{18} Becquerels) but this included only to European part of the Soviet Union deposited materials (Anspaugh et al., 1988). It was later corrected to be about 14 EBq5 which included 1.8 EBq of ^{131}I, 0.085 EBq of

^{137}Cs, 0.01 EBq of ^{90}Sr and 0.003 EBq of various plutonium radioisotopes (Kinly, 2005). Condensable materials were around 2 x 10^{18} Bq (Sandalls et al., 1993).

Measurements of radionuclides in the environment prior to the Chernobyl explosion were different because previous experience in fallout radionuclide monitoring was derived from nuclear testing of which the prominence of short-lived radionuclides was reduced by radioactive decay during delayed deposition. Chernobyl, on the other hand, came from a single surface location making the deposition 100 times greater with iodine and other short-lived radionuclides prominent in the first day and weeks after the accident (IAEA, 1996).

The IAEA (1996) reported that the estimated total activity of all the radioactive material released were around 12 x 10^{18} Bq including those due to noble gases. During the review of the impacts a decade after the fall-out, documentation showed that lethal radiation doses were reached in some radiosensitive local ecosystems which affected certain plant species within 10 km of the reactor site after the first few weeks of the explosion (IAEA, 1996). Yet dose rates were reduced by a factor of 100 by autumn of the same year and the natural environments begun to recover at around 1989. Jaworowski (2010) notes in his study that the total emission in Chernobyl was still 200 times less when compared to all of the 543 nuclear warheads exploded in the atmosphere since 1945. Atmospheric testing of nuclear weapons was the greatest global release of radionuclides from a man-made source while the Chernobyl NPP incident was attributed with the greatest local impact of man-made releases (IAEA, 1996). Table 2.3 summarised the radionuclides released during the ChNPP fallout. As the ^{137}Cs decay into more stabilised form, radioactive contamination from Pu isotopes is expected to be more evident.

The ^{90}Sr, ^{106}Ru, ^{134}Cs, ^{137}Cs, ^{144}Ce, and ^{147}Pm radionuclides were some of the sources of the total β-activity of hot particles while actinide elements ^{238}Pu, ^{239}Pu, ^{240}Pu, and ^{242}Cm isotopes were chief contributors to the total a-activity of hot particles (Faybishenko, 2004). The large-scale and non-uniform contamination was affected by two important but very different controlling processes which played a part in the formation of the radioactive material emitted from the reactor. It had been established that radiation elicits different responses from various organisms but the non-uniformity of the release in the case of Chernobyl shaped the biota at different levels of biological organisation from molecular and cellular to the whole ecosystems (Geras'kin et al., 2008). Sandalls et al. (1993) in their review of radionuclides released from Chernobyl identified the two processes and its resulting emissions. The first one resulted into mono-elemental or bi-elemental particles so-called due to the fact that gamma-ray spectroscopy can detect only one or two radionuclides but in truth contains radioactive

Table 2.3 | Radionuclides Released during the ChNPP Fall-out

Half -Life	Element	Classification	Activity Released
20.8 h	Iodine-133	Volatile	910
2.35 d	Neptunium-239	Refractory	400
2.75 d	Molybdenum-99	Refractory	>72
3.26 d	Tellurium-132	Volatile	~1150
5.25 d	Xenon-133	Inert gas	6500
8.04 d	Iodine -131	Volatile	~1760
12.7 d	Barium 140	Intermediate Volatility	240
13.1 d	Cesium-136	Volatile	36
32.5 d	Cerium-141	Refractory	84
33.6 d	Tellurium-129m	Volatile	240
39.3 d	Ruthenium – 103	Intermediate Volatility	>168
50.5 d	Strontium-89	Intermediate Volatility	~115
64.0 d	Zirconium-95	Refractory	84
284 d	Cerium-144	Refractory	~50
368 d	Ruthenium-106	Intermediate Volatility	>73
2.06 a	Caesium-134	Volatile	~47[a]
10.72 a	Krypton-85	Inert gas	33
14.4 a	Plutonium 241	Refractory	~2.6
18.1 a	Curium-242	Refractory	~0.4
29.12 a	Strontium-90	Intermediate Volatility	~10
30.0 a	Caesium-137	Volatile	~85
87.74 a	Plutonium-238	Refractory	0.015
24 065 a	Plutonium-239	Refractory	0.013
376 000 a	Plutonium-240	Refractory	0.00004

Data derived from IAEA Chernobyl Forum Expert Group ‘Environment’ (2006)
[a]Based on $^{134}Cs/^{137}Cs$ ratio of 0.55 as of 26 April 1986

elements from the second transition series. The result of the second process when examined under gamma-ray spectroscopy revealed fragments of uranium oxide fuel containing the range of fission products although depleted in more volatile elements. Fuel particles with 10µm formed around 90% of the released ^{90}Sr, $^{141,144}Ce$ and ^{241}Am. The different characteristics of both particles contributed to their variation in distribution and deposition but it is the fuel particles played prominent role inside the CEZ as large fuel particles consisting of depleted cesium isotopes were primarily deposited within a 5-km zone of the reactor (Faybishenko, 2004).

2.3 | CEZ Forests Radionuclide Cycle

The release of the massive amounts of radionuclides was the most concerning consequence of the accident. Humans were prioritised for protection measures but impact in the environment should have been considered as well. Forest ecosystems were vulnerable, in particular, because of the filter effect and the continuing cycle of the radioactive materials in its components.

Tikhomirov and Shcheglov (1994) posited that deactivation and root absorption were the two processes that determined the achievement of equilibrium in the radionuclide distribution dynamics. Deactivation dominated in the initial period as contamination was observed to decrease in the structural parts of the tree. This was later counter-balanced by the escalation of radionuclide in the above-ground phytomass which resulted in some sort of quasi-steady state as the annual soil radionuclide transfer exceeds its return through fall of foliage only by the phytomass annual accretion content.

The above-ground parts of forest stands initially intercepted 70-80% of the total radioactive materials deposited of which pine stands interception factor was 2-3 times greater than deciduous ones (Ipatyev et al., 1999) although other estimates widened this interception percentage range to 60-90% as evidenced by model field experiments (Tikhomirov and Shcheglov, 1994). This strong contamination from the canopy could have generated secondary transfers to neighbouring territories in the initial post-accident period by spreading radioactive aerosols in the event of strong winds (Tikhomirov and Shcheglov, 1994).

The migration of the main part of radionuclides from the canopy to the forest litter was dependent mainly on the physiological status of woody plants indicating that the radioactive materials travel to the forest litter through biogenic transfers such as epidermal leaf, bud scales and bark scales shedding (Tikhomirov and Shcheglov, 1994). These biogenic processes are influenced by seasons as active growth, which occurs mainly during spring, accelerates the deactivation of trees.

Table 2.4 | **Percentage of ^{137}Cs in Different Kinds of Soils**

Type of Soil	Average	Leaf Litter + 5 cm	5-10 cm	10-20 cm
Automorphic	37	92.5	3.2	1.6
Semiterrestial	15	85.6	9.6	4.8
Hydroterrestial	5.8	67.6	19.9	12.5

* All values in percentage
Data from Ipatyev et al. (1999)

As the radionuclides moved from the forest canopy to the forest litter, the soil characteristics became more essential as vertical migration factored in the root absorption. Most radionuclides have moved to the leaf-litter and remained accumulated in the upper 3-5 cm soil layer within approximately 7 years (Kalchencko and Fedotov, 2000). The topsoils in the exclusion zone consisted mostly of podsols and peaty podsols with relative homogeneity (Faybishenko, 2004). Ipatyev (1999) used automorphic (akin to sandy-podzolic soil), semiterrestial and hydroterrestial to characterise the soil. Automorphic soil is akin to sandy-podzolic soil. Table 2.4 compared the migration of ^{137}Cs in different kinds of soils.

Table 2.5 | **Total percentage of ^{137}Cs Distribution in Forest Automorphic Soils**

Soil Layer (cm)	**1987**	**1988**	**1889**	**1990**	**1991**
0-3	95.0	89.0	85.3	80.8	76.6
3-4	2.3	5.6	6.7	8.7	10.6
4-5	1.2	2.4	3.5	4.6	5.6
5-6	0.6	1.3	1.9	2.5	3.0
6-7	0.3	0.7	1.0	1.3	1.6
7-8	0.2	0.4	0.6	0.7	0.9
8-9	0.1	0.2	0.3	0.4	0.5
9-10	0.06	0.1	0.2	0.3	0.3

All values expressed in percentage
Adapted from Tikhomirov et al. (1993)

The migration of ^{137}Cs in podzolic-soil is further elaborated in the Table 2.5 adapted from Tikhomirov and Shcheglov (1994).

The impairments due to internal exposure as the migration of ^{137}Cs transferred from the crowns to the leaf litter and soil in the forest floor became significant in assessing the complications of the ChNPP accident and the type of soil signified. Ipatyev et al. (1999) traced the transfer of the radionuclides in Chernobyl forest systems. The uppermost 1 cm layer of automorphic soils demonstrated even ^{137}Cs deposition which decreased with depth while superficial layers of semiterrestial and hydromorphic soil deposition percentage was lower in the forest litter. ^{90}Sr showed similarities with ^{137}Cs where the concentration decreased with respect to depth but the it was shown to be more mobile. Caesium binds strongly to clay minerals making it relatively immobile in soils. Chemical properties of the elements influence their migration due to the cation exchange capacity (CEC) (Smith and Beresford, 2005). Strontium uptake of plants is approximately equal with calcium-strontium-ratio in soils while the ^{137}Cs uptake is similar to potassium. Active accumulation by plants of more potassium when it is scarce results into more strongly accumulated caesium due to the similarity of their soil fixation properties (Smith and Beresford, 2005).

Levels of beta and gamma radiation contributions to the total radiation exposure became comparable due to the migration of ^{137}Cs into the soil although this balance was affected by the bioaccumulation of ^{137}Cs in organisms and the behaviour of said organism. Deposition of ^{137}Cs ranged from 96.6 to 99.2% in mineral soil layers of forest floors (Ipatyev, 1999).

3. The ChNPP Explosion and Conduct of the Studies

Radiation had been established since the late 1920s to have mutagenic properties and used in inducing physiological and morphological changes in crops to improve variety. Experimental use of radiation, however, were applied with limits and control so as to not cause excessive damage. Under the Chernobyl scenario, limits and control were impossible. The level of radionuclide contamination within the mentioned area is interspersed irregularly due to different deposition processes influenced by the weather and the combination of different radioactive products. Nevertheless, the existing body of knowledge on the effects of exposure and induced mutation is considered as a prospective frame of reference for the observed effects in Chernobyl.

The results of the fallout were catastrophic but it also provided an opportunity for scientist to study the effects of irradiation outside laboratory settings as elevated levels of different radionuclides cycled among different parts of the ecosystem. The distribution of radionuclides and activity concentration tend to be dictated or influenced by several factors such as the course of event, release scenario, distance from the source, dispersion processes and others (Salbu and Oughton, 1995).

The massive amount of ionising radiation released in Chernobyl caused immense damage to all biological material in its vicinity. This was particularly observed in the stand of forest trees near the reactor which turned red and died gradually months after the explosion. This area had been named the Red Forest zone due to the change in the color of the foliage. Comparison of the data from 1986 after the immediate aftermath of the accident and information ten, twenty and thirty years after, however, suggested that the surrounding plant biota had recovered from the initial damages it has sustained. Conifers that did not revive in the Red Forest zone were replaced by other species which were able to thrive despite the levels of radiation. This review and assessment examined deeper the mechanisms of the recovery and adaptation of forest trees.

Table 3.1 summarised pertinent information of the studies done on forest trees surrounding the ChNPP reactor.

Table 3.1 | **Summary of Study Review and Assessment**

Study #	Forest Species	Tree Parts	Data Collection	Year	Methods/ Procedure	Level of Study	Results	Implications
1	Pinus sylvestris	Foliage and other observable parts	Sequence	1986-1991	Observation	Population, External form	Absorbed irradiation is directly correlated to stand viability, mortality rate, development of tree canopies and reproduction anomalies.	Trees which absorbed less radiation have bigger chances for recovery and survival
2	Robinia pseudoacacia, Sorbus aucuparia	Symmetry of leaf arrangement	Single	1996	Observation	Population, External form	^{137}Cs has a strong positive relationship to developmental instability	Developmental instability results in symmetry fluctuation and morphological deviants which can lessen longevity and survival prospects
3	Pinus sylvestris	Meristematic tissue cambium in the trunk/branch	Single	1997	Laboratory analysis of serial photographs	Morphology, Life Processes	Frequency of cambial cell events were three times higher after the ChNPP accident	The higher frequency of said events possibly eliminates mutated or genetically damaged cells
4	Pinus sylvestris	Microfibril Angles	Single	1992, 2002	Electron microscopy scanning and analysis	Morphology, Life Processes	The S_2 and S_3 layer of the tracheids cell wall changed after the ChNPP accident	Not stated
5	Pinus sylvestris	DNA	Single/ Intergenerational	1992, 2002	DNA Extraction and cytosine extension assay	Intergenerational, Methylation	Hypermethylation occurred in genomic DNA of exposed pine; level of hypermethylation depends on the dose of radiation absorbed	Hypermethylation impacts tree survival by prevention of hereditary material reshuffling and genome instability
6	Pinus sylvestris	Needles, DNA	Single	2010	Array analysis, cytological analysis	Genetic expression, External form	Comparison of genes from normal and dwarf needles indicated 12 significantly up- or down regulated expression of which 5 were linked to development or stress	The altered gene expression is possibly related to the presence of dwarf needles but was relatively too small to indicate further obstacles in a considerable recovery

Table 3.1 | **Summary of Study Review and Assessment (Continuation)**

Study #	Forest Specie	Tree Parts	Data Collection	Year	Methods/ Procedure	Level of Study	Results	Implications
7	Betula verrucosa	Pollen, DNA	Sequence	1987-1997	DNA isolation, Southern blot hybridisation, nucleosome analysis	Intergenera-tional, Dark repair systems	Pollen at g/b-emitter sites have recovered the ability to perform UDS which had been impaired and non-functioning in 1987. Pollen from sites with a- and g/b emitters has reduced UDS capacity and failures in repairing lesions in rDNA repeats.	Exposure to chronic radiation may affect DNA repair mechanisms by making it more efficient except in the case of exposure to alpha emitters
8	Pinus sylvestris	Needles, DNA	Single	n.d*	DNA isolation and AFLP genotyping	Genetic, Selective response	Selective response was demonstrated by 15 of the 222 loci or 6% of the tested material instability	Evolutionary adaptations can help long-living plants to adjust under extreme environmental change
9	Pinus sylvestris	Needles, DNA	Single	n.d*.	DNA isolation, microsatellite and AFLP genotyping	Genetic, mutation	AFLP markers indicated three-fold higher statistically significant number of mutations than microsatellite markers	Not specified
10	Pinus sylvestris	Needles, DNA	Single	n.d*	Identification of genomic Cat, GPx and a-Tubulin sequences by PCR	Genetic expression,	Cat was up-regulated while GPx was down-regulated when compared to control. GPx nucleotide diversity was higher than Cat in analyzed samples	Not specified
11	Pinus sylvestris	Tree rings	Sequence	1980-2008	Standard dendrochrono-logy methods, Digital imaging	Morphology, Tree growth	Background radiation level elevation is associated with increase variance in the size of growth increments and reduced growth increments	Affects quantity and quality of wood and the process of decomposition

Data extrapolated from the enumerated studies on Table 5.1
*Not specified but determined to be afer the ChNPP accident

3.1 | Objectives, Methods and Levels of Study

The objectives of the study determined the method and level examination applied. All 11 studies had different objectives and methods although SN3 and SN4 and SN8, SN9 and SN10 were connected through their sources of specimen.

Examinations became more sophisticated through the years due to technological breakthroughs. Experimental designs kept up with the progression thus yielding more information on the processes that the trees undergo under extreme situation. Four levels of studies identified were population, cellular and subcellular, genetic and intergenerational assessments.

Both population, and cellular and subcellular levels relied on observed changes on the external, structural or functional form of the trees but population level was deduced to have direct consequences on the survival and continuation of tree lineage and other organisms dependent in any capacity on the subject under study. SN1 and SN2 were all done at the level of population, which meant that the outer appearance of the species under investigation indicated the health of the trees.

SN1 systematically observed and recorded physiological and morphological forms of Pinus sylvestris at population level by indicating mortality or recovery and return to normality of trees through sequential monitoring of forest plots. These plots were categorised depending on the absorbed dose of radiation to determine the effects of contamination levels on stands of Scots pine trees. SN2, on the other hand, also used methodical observation by studying deciduous trees and plant species with symmetrical structure with varying distance from the Reactor IV to establish the correlation between deviance from symmetrical formation of physiological structures and ^{137}Cs.

Studies at the level of cellular and subcellular addressed phenotypic indications of exposure to radiation and established correlations or links between the detected effects and possible causes but it did not inquire how and why those outcomes came to be. SN3, SN4 and SN11 were all done at this level.

SN3 and SN4, and SN11 all studied tree rings but where SN3 and SN4 delved into cellular and subcellular processes in the years prior and ensuing the ChNPP incident through inferences from examination through serial photography and electron microscopy, SN11 examined tree growth and development in relation to external environment.

The third level of examination assessed life processes that specimens undergo by subjecting DNA to various procedures uncovering how the species reacted to environmental stresses and how do these changes signify for an organism. The enormous amount of DNA, however, of one organism and the variation that existed between these individual DNAs posed difficulty in establishing norms and correlations between the observed visible outcomes and the aspect of DNA processes under scrutiny. This was specially true in the case of Pinus sylvestris because it is not a model organism.

The fourth level of study overlapped with both population and genetic levels but separated due to the implication of their results to the next generation of trees. SN5 and SN7 were both classified to be under this study.

3.2 | Forest Species and Tree Parts

Of the eleven studies, nine were overwhelmingly focused on a type of coniferous tree called Pinus sylvestris more commonly known as Scots pine trees. Picea abies, another coniferous specie, was also reported to had been affected heavily to the point of near extinction in the vicinity of the damaged reactor but no studies were readily accessible. The other two studies were focused on hardwoods, SN2 on Robinia pseudoacacia and Sorbus aucuparia, commonly known as black locust and rowan trees, respectively, while SN7 chose Betula verrucosa, a type of birch tree. SN2 chose species based on the physiological arrangement of the leaves of trees. The study relied on the premise that maintenance of symmetrical structures was indicative of developmental stability. Tree parts utilised for the studies of Pinus sylvestris are needles and tree rings. The studies on deciduous trees, SN2 and SN7, looked at symmetrical formation and pollen repair mechanisms, respectively.

The type of forest specie came into close scrutiny because it determines the uptake of ^{137}Cs (Tikhomirov and Shcheglov, 1994). The ^{137}Cs distribution was differentiated among leaves/needles, timber, bark and branches so the part of the tree

Table 3.2 | **Summary of Contamination Level and Specimen of the Studies**

SN	Contamination	Specimen
1	**Ci/km^2** Control: 5-8 Hotspots: 800 Near the reactor: 200-400	250 Pinus sylvestris trees in the vicinity of the ChNPP classified according to exposure dose in percentage : Acute – 100% High – 65% Medium – 15% Low – 5% No injury - >1%
2	1.10 -4.66 **Ci/km^2**	Species from 9 sites in a gradient starting from security zone to Kiev Black locust trees: total of 1300 leaves from 260 trees Rowan: 26000 from 260 trees
3	**kBq/m^2** Initial levels: 18.5×10^5 Specimen collection: 3.7×10^5	Two Pinus sylvestris trees located 5 km from the ChNPP and 100m from each other. Aged 30 and 42 year old. Sample was from stems 1.3 m from the ground
4	**kBq/m^2** Initial levels: 18.5×10^5 Specimen collection: 3.7×10^5	Three Pinus sylvestris trees located 5 km from the ChNPP and 100m from each other. Aged 16, 30 and 42 year old. Sample was from stems 1.3 m from the ground
5	**Ci/km^2** **1st Exp:** Control: 0.01 Burial site: >4000 **2nd Exp: Ci/km^2** Control: 0.01 Contaminated: ~600	Experiment 1: Control: Seeds from unexposed 40 yr-old trees planted in clean soil Exposed 1.1: Seeds from Red Forest trees planted in clean soil Exposed 1.2: Seeds from unexposed trees planted in burial sites Experiment 2: Control: 10 y.o. trees from seeds of 40 y.o. trees planted in clean soil Exposed 2.1: 10 y.o. trees with 12 Gy dose, acute as seeds and chronic Exposed 2.2: 10 y.o. trees with 5 Gy dose, acute as seeds and chronic
6	**Initial levels:** 3-5 Gy **Specimen collection:**1-2 mR/h	Dwarfed and normal needles from five 7-12 year old Pinus sylvestris located about 10 km from ChNPP collected during the summer and winter of 2000
7	**Bq/km^2** **Initial levels:** Control: $5{\cdot}2 \times 10^{10}$ Level II: $4{\cdot}9 \times 10^{13}$ Level III:$1{\cdot}3 \times 10^{14}$ **Final year:** Control: 3.3×10^9 Level II: 1.1×10^{12} Level III: $1{\cdot}8 \times 10^{13}$	Pollen from Betula verrucosa collected every year since 1987 on 30-km CEZ
8	**Control:** 0.005-0.008 mR/h Accumulated: 3×10^{-6} Gy/year **Red Forest:** 0.9 - 4.4 mR/h Accumulated: 9.8 Gy/year	**P1:** 50-year old Pinus sylvestris located at heavily radiated sites; exposed to acute high radiation at 30-year old followed by chronic radiation **P2:** 20-year old trees planted on heavily contaminated plots after the ChNPP accident; exposed to chronic radiation and exhibits strong damage **P3:** 20-year old trees planted on heavily contaminated plots after the ChNPP accident; exposed to chronic radiation and shows no damage or only minor ones **P4:** Pinus sylvestris planted the same year and from the same year as the P2/P3 in locations with low radiation but comparable climactic and edaphic conditions. Served as control for P2/P3 **P5:** 50-year old Pinus sylvestris not exposed to irradaiton. Served as control for P1
9	**Demes: mR/h** P1: 0.6–1.1 P2: 0.9–4.4 P3: 0.9–4.4 P4: 0.005–0.008 P5: 0.005–0.008	
10	**Demes: Gy/yr** P1: 0.035-0.11 P2: 0.070-0.21 P4:0.0004-0.0007 P5: 0.0004-0.0007	
11	Various levels on 14 sites **confirmed by the Ministry of Emergencies**	112 Tree cores collected from eight Pinsu sylvestris each in 14 different sites

Data extrapolated from the list of studies in Table 2.1

being examined may also signify although none of the studies considered this aspect. None of the studies also documented the type of soil where the trees were growing but whether this omission had any significant impact on the studies is indeterminable.

There was an evident imbalance on existing number of studies between deciduous and coniferous trees believed to be due to the extent of recorded damage on coniferous trees. The relative higher radiosensitivity of coniferous trees meant that the release of radioactive materials had higher population impacts due to expected consequences like reduction of lifespan, population rate, fertility rate and extinction, damage to genetic material and, possibly, migration of mutation.

Table 3.3 | **Percentage Distribution of Radionuclides in Different Tree Parts**

	1986	**1987**	**1988**	**1989**	**1990**	**1991**
Automorphic Soil						
Leaves, needles	6.7	0.87	0.92	0.51	0.22	0.09
Timber	1.72	1.48	0.77	0.71	0.41	0.35
Bark	4.9	2.95	2.9	3.5	3.0	2.17
Branches	4.3	2.85	1.4	1.2	1.04	0.4
Total overground phytomass	17.62	8.15	6.0	5.9	4.7	3.3
Hydromorphic Soil						
Leaves and needles	6.0	0.48	0.50	0.35	0.69	1.11
Timber	1.7	1.3	0.84	0.64	1.7	2.6
Bark	4.9	3.9	2.9	3.4	3.8	5.4
Branches	4.3	2.0	0.9	0.7	1.4	1.75
Total overground phytomass	16.9	7.7	5.1	5.1	7.6	10.9

*All values expressed in percentage.
**Majority of the radionuclides remained in the soil
Adapted from Tikhomirov and Shcheglov (1994)

Table 3.3 compared the ^{137}Cs distribution between different plant parts found in automorphic and hydromorphic soils. If the extent of damage was directly related to the amount of absorbed radiation, and the amount of radiation absorbed varied by tree type, plant part and soil type, then information on the type of tree, the part and the soil type should had been included in every study and not just the background radiation. Only SN10 addressed this difference by tabulating the absorbed dose of the needles tested for gene expression rather than just giving background radiation levels.

3.3 | Time and Frequency of Specimen Collection

An important aspect in the review and analysis of these studies is the year when the specimen for the experiment had been collected due to the continuous variation in the intensity of the contamination level as the short-lived radionuclides decayed into their half-lives and the medium and long-term radionuclides cycled into different sections of the ecosystems and eventually stabilised.

Most of the trees included in the studies both experienced acute and chronic exposure but the time when the specimen was observed or collected for further investigation would determine which of these influenced the results more. None of the studies examined solely the effects of acute exposure since determination of effects from chronic irradiation would be difficult due to constant exposure after the CHNPP explosion. A number of studies, however, initiated observation or collection of specimen a few months after the accident. SN1 started from 1986 and sequentially observed their established plots from thereon but was limited by the depth of the study. It would have been interesting to see if there was a change in the repair mechanisms employed by the affected trees through the years. Defence mechanisms to chronic radiation had been determined to be different from acute ones in other plant species (Kovalchuk, 2000). SN7 collected pollen from birch trees in 1987 until 1997.

Time and frequency of specimen collection were factors in circumstances like the ChNPP accident where the strength of the stimuli became weaker with the passage of time and with lengthening distance from the immediate vicinity. Sequential data-gathering would had been preferred as a data-collection procedure as the constantly changing level of contamination was expected to elicit differing mechanisms for plant survival. Only SN1 and SN7 gathered information or specimen through the years but SN3, SN4 and SN11 focused on tree rings which can shed light on cell processes in the trunk of the tree retrospectively. On the other hand, SN5 focused on comparing the parent tree with its progenies making a yearly track less necessary. The other five studies, however, would have benefitted from a sequential specimen collection for a better understanding of the interaction between of tree and the environment. Sequential collection of specimen would have differentiated the specimen source reaction to the declining level of radiation. SN2 tried to compensate to this limitation by distance but this in itself is limited by the variation of biological between each individual organism and DNA material even between the same species of organisms.

The passage of time played a part in describing the level of radiocontamination in the aftermath setting apart what researchers have classified as acute and chronic radiation to demarcate levels of radioactivity from the occurrence of the explosion until the present. This classification also had connotations in the external, contact and internal components of radiation contamination (Kal'chenko and Fedotov, 2000; Smith and Beresford, 2005). External contamination were caused by radionuclides which passed vaporous-gaseous aerosols clouds and accumulated on the soil surface while internal ones came from incorporated radionuclides. Contamination upon contact, meanwhile, was caused by radionuclides which accumulated on the surface of a given tree. For the purpose of delineating the acute and chronic exposures of biota around the Chernobyl NPP, Hinton et al. (2007) discussed three distinct time periods which have been arbitrarily categorised.

The first 20-30 days were recognised as the acute exposure due to the quantities of short-lived radionuclides including ^{99}Mo, ^{132}Te/I, ^{133}Xe, ^{131}I, and ^{140}Ba/La impacting biota due to its large accumulation and deposition into plant and ground surfaces. Gamma irradiation from deposited radionuclides was the main source of exposure rates but beta-radiation also played a factor for biological targets and surface tissues. External radiation from aerosol precipitation on trees which polluted various wood-stand components accounted for approximately 80% of the dose received by trees (Kalchencko and Fedotov, 2000). Absorbed dose was one to two orders of magnitude higher than that of incorporated radionuclides.

Anspaugh et al. (1988) depicted this period as initially a large release of radioactive material including fuel fragments which later dwindled to a lower level only to rise up again after several days when heat from the decay of residual fission products caused the temperature within the remaining core to intensify and the fission products to begin distilling out of the reactor. Exposure measured in the immediate vicinity of the reactor reached 20 Gy/d from the day of the accident (Hinton et al, 2007). This was, however, offset by the observance of researchers in the heterogeneity of dose rates which can decline rapidly over short distances as seen in Pripyat where it declined by three orders of magnitude over three km.

Of the studies, SN1 described the radionuclide composition as made up of Caesium 137, Strontium-90 and Pu isotopes derived mainly from fuel particles. Fuel particles started disintegrating a year or two after the accident meant that their effects can be observed later as opposed to other forms of elements that spewed from the reactor. It was not cleared whether the delayed effect of fuel particles made an impact in the study.

Delineation of the effects of the aforementioned 20-30 days or acute radiation from chronic ones was complicated due to the continuous stream of radioactivity from the background but several pronounced effects on biota had been identified including but not limited to the mortality of more sensitive species depending on the absorbed dosage. Of the studies, only SN1 was initiated the same year as the explosion but it only focused on physiological and morphological changes. Since it collected data sequentially, it also covered the second and third temporal demarcations as well. Other studies like SN3 and SN4, and SN11 also tried to examine tree processes during this time but through tree rings posing limitations to some degree. On the other hand, SN7 collected pollen from Betulla verrucosa which were presumed to had been exposed to the initial fall-out. The rest of the studies were initiated during chronic exposure period past the second temporal demarcation and well after the third.

The second temporal demarcation (Hinton et al., 2007) of radiation exposure saw the decay of the short-lived radionuclides while the longer-lived radionuclides cycled from one component of the environment to another through physical, chemical and biological processes. These processes included rain-induced transfer from plant surfaces onto soil and bioaccumulation through plant tissues.

Within three months of the accident, total radiation dose accumulated by plants and animals were approximately 80% which consisted of 90% beta radiation (Hinton et al. citing UNSCEAR, 2007). The decay of the short-lived radioisotopes resulted to the decline of the soil surface dose rates by this time to 10% of the initial values but damaging total doses still accumulated. External radiation was surmised for 2-4 year as the main pathway for contamination (Smith and Beresford citing Tikhomirov and Scheglov, 2005).

One year post-accident marked the third and continuing phase of the chronic radiation exposure where it was estimated the only approximately 1-2% of the initial values remain which is derived mainly from ^{137}Cs (Hinton et al., 2007; Smith and Beresford, 2005). Levels of beta and gamma radiation contributions to the total radiation exposure became comparable due to the migration of ^{137}Cs into the soil although this balance was susceptible to the bioaccumulation in organisms of ^{137}Cs and the behaviour of organism.

The study by Kozubov and Taskaev (cited by Zelena et al., 2005) on the dynamics of radiation damage and repair processes in coniferous stands provided a similar alternative of classification based on contamination by dividing affected the zones into four based on the growth response of the Pinus Sylvestris and dosimetric data. Areas were classified as the lethal damage zone estimated to be over 600 ha. (hectares) where the radiation level reached over 100 Gy, the zone of sublethal damage where the absorbed dose reached 50 Gy and covered 3800 ha. of pine forest, the 3-5 Gy zone and the fourth zone which included the absorbed does was 0.5-2 Gy and included major part of the pine of the forest within the 30 km exclusion zone.

4. The ChNPP Explosion and Population Impacts

Population impacts were effects that are felt by numbers. It had the tendency to affect not just individual organisms but the survival of the species in a specific location. It was indicated by extinction, reduced population size, reduced lifespan and decreased fertility rates among others depending on the dose and type absorbed.

Plants, on the other hand, employed different mechanisms to combat these effects. This was evident by the recuperation of pines tree stands which were not exposed to the lethal threshold dosage.

4.1 | Ionising Radiation

Radioactive material releases vast amount of energy as its nucleus tries to stabilise itself from a higher energy state to a lower one at a constant decay rate (λ) independent of its surroundings. Ionisation takes place when this energy dislodges orbital electrons from the atoms it encounters releasing approximately 33 eV per event which is enough to break strong chemical bonds (Hinton, 2014). Ionisation energy is transmitted by X rays, gamma rays, beta particles (high-speed electrons), alpha particles (the nucleus of the helium atom), neutrons, protons, and other heavy ions such as the nuclei of argon, nitrogen, carbon, and other elements which are classified by physical parameters into high/low-linear-energy transfer (High-LET and Low-LET) to describe their released energy average per unit length of track. High-LET are particles like neutrons and alpha-particles which cause dense ionisation that are more destructive to biological material due to the transfer of their energy to a small region of the cell. Low-LET radiation, meanwhile, produces ionisation sparsely along their track such that it is homogenous within a cell (Radiation Effects Research Foundation, 2007).

Direct and indirect effects occur as consequences of exposure to ionising radiation when orbital electrons are released. The direct effect occurs when significant damage to biological cells results from the process or from the produced charged particles by breaking one or both of the sugar phosphate backbones, the base pair of the DNA or the hydrogen bond of the base pairs (Teachnuclear.ca, 2016). The indirect effects causes biological damage when the ionisation process produce and leave fragments of atoms, called free radicals, which are highly unstable chemically due to unpaired or odd number of orbital electron. The most common free radical formed by the ionisation of water is OH• which is dependent on the water content of a body. The G-value is the amount of free radicals produced per 100eV of energy absorbed by a medium. It is estimated that around 50 000 free radicals of OH• can theoretically be produced when a 5 MeV alpha particle dissipates all of its energy in cellular water (Hinton, 2014). Both the direct and indirect effects can cause alterations in the DNA like single-strand (SSBs) and double-strand breaks (DSBs). Other damages are hydrolytic deprivation, apyrimidic sites and oxidative damage to bases and phosphodiester backbone of the DNA (Gill et al., 2014).

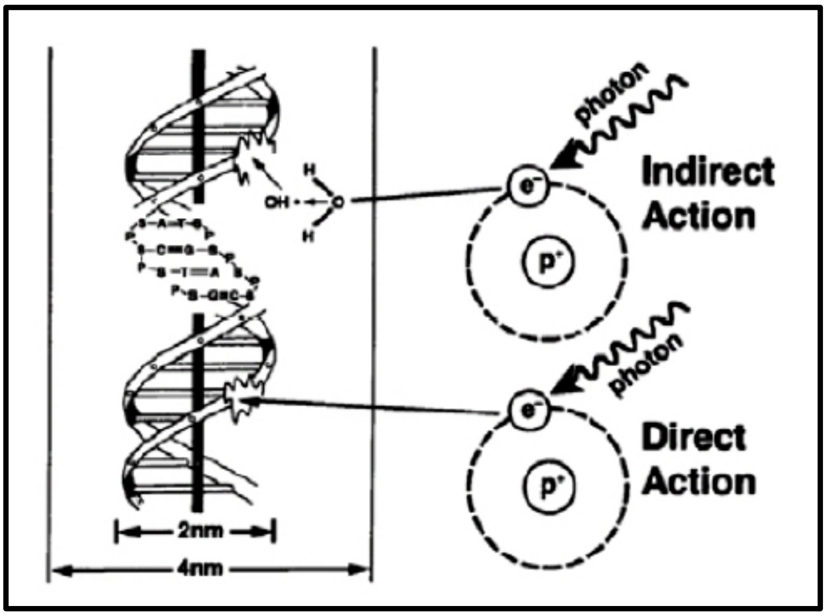

Figure 4.1 | **Direct and Indirect Action of Ionising Radiation**
(Hinton, 2014)

Other reactive oxygen specials produced aside from OH• are O_2•, and H_2O_2 (Griffiths et al, 2000). These are the most biologically relevant reaction products which can damage bases and cause different adducts and degradation products. Thymine glycol and 8oxodG are among the most prevalent DNA damage products formed after the attack by oxygen radicals which results to mutation. The breakage due to ionising radiation of N-glycosydic bond leads to the formation of AP sites, and stand breaks that have lethal effects (Griffiths et al, 2000).

Radiation affects organisms through external or internal exposures. Internal exposure is the incorporation into the body of radionuclides thereby having an internal effect through radiating body tissues internally. According to the report on internal emitters by CERRIE (2004), internal emitters are characterised by short-range charged particle emissions which may be the dominant or sole contributors to radiation dose and risk depending on the emissions of the radionuclide concerned and its location within tissues and cells as opposed to external exposures which can be limited by the ability of the radiation to permeate the body which gives rise to radiation doses to body tissues. This distinction is crucial when the types of radiation are being discussed because alpha particles are densely ionising while gamma rays are more sparsely ionising. Low energy electrons also lead to greater ionisation densities bringing about greater damage per unit dose.

The document by CERRIE (2004) goes on further in differentiating internal and external exposure through the challenges of measurements. External exposures, in general, are directly measurable. Radionuclides which have been incorporated, on the other hand, differ substantially in their physiological behaviour which makes it necessary to rely on estimates from biokinetic models of radionuclide behaviours like uptake, transport and distribution of an element within the body for chemically similar group of elements.

Whereas internal and external exposure outcomes are influenced by the location and nature of radionuclides able to penetrate the organism, acute and chronic considerations depend on the dosage and duration of the exposure. Acute radiation exposure is associated with high-dose rates over a short period of time in relation to the manifestation of obvious effects. On the other hand, exposure to chronic radiation is susceptibility to sufficiently low-dose rate decay emission that transpires continuously over a significant portion of an organism's life or throughout some particular life-stage but does not produce effects that are akin to the acute exposure. (Hinton et al, 2007).

Since the autumn of 1986, the relatively high doses of acute radiation in Chernobyl had stabilised to chronic exposure with low dose rates that persists up to the present (Geraskin et al., 2002)

4.2 | Damage and Recovery of Coniferous stands

The ChNPP fallout heavily affected Scots pine trees. The first study assessed how radionuclide exposure possibly impacted biodiversity by affecting the population of Pine trees.

Table 4.1 | **Conditions of Pine Stands from 1986 to 1991**

Degree of injury	**1986**	**1897**	**1988**	**1989**	**1990**	**1991**
No injury < 0.1	Normal growth	Normal status	Normal	Normal	Normal	Normal
Low 0.1 - 1.0	Depression of growth	Occasional morphoses	Normal	Normal	Normal	Normal
Medium 1.0 - 10	Strongly depressed growth, morphoses, occasional death of trees	Partial forest restoration, morphoses, absence of flowering	Rehabilitati on of timber output, morphoses	Normal	Normal	Normal
High 10 - 60	Interrupted timber output, browning of needles, death of individual stands	Rehabilita-tion of individual sites	Rehabilita-tion timber output, numerous foliar morhoses	Growth of foliage, undergrowt h of grass	Growth of foliage	Growth of under-growth grass
Acute >60	Total forest destruction	Needles fall-off, splintering of bark	Bark fall-off, creation of foliage and undergrowt h of grass	Collapsing of stems, creation of a new plant community	Collapsing of stems, creation of a new plant community	Creation of a new plant commu-nity

Adapted from Arkhipov et al. (1994)

Arkhipov et al. published a study in 1994 focused on forest stands within the first 10-km of forest area nearest the ChNPP from 1987-1991. The specific interest of this investigation depended in the observance of the consequences of the said filter effect phenomena which skewed the distribution of the particles and caused death of trees on the outer borders (Fedotov et al, 1989). It is noteworthy of its systematic investigation of the effects of radiation on pine stands from 1986 to 1992. The findings are summarised in Table 4.1.

The 250 trees under observation in this study were affected by short-lived radionuclides at the time of the accident even if the conduct of the study itself started some time after when these elements have turned to more stabilised forms. The nuclear fall-out caused damages that triggered various repair mechanisms. These mechanisms restored and tried to maintain genomic stability even as it tried to eliminate deleterious mutations leading to apparent recovery of pine stands.

The researchers determined death of pine stands aged 20 years-old located in highly injured areas reached up to 35% while this mortality was not detected in low injured stands. Pine stands located in areas of no injury displayed no visible injuries but anomalies in the reproductive system were observed. Percentage of regeneration by the end of 1987 for medium and low areas were 5-7 and 20-30%, respectively while no regeneration were observed in stand areas of highly injured trees affected by acute radiation. By 1988, mortality in latter were reported to be between 37 and 100%. In 1987, the reestablishment of stands could have been possibly adversely by pathogenic insects which have settled in the highly injured stands but was prevented in spreading to the adjacent stands by air-chemical treatment which prevented insect settlement but did not completely eliminate them. The population of insects by 1992 was equal to normal background levels which was not deemed to be a threat to forest stand viability. Natural reproduction of birch, asp and other species have replaced pine stands in areas where injury and mortality was high.

This study provided a database on the effects of radiation in Pinus sylvestris through systematic observation and categorisation of damage in connection to the level of absorbed radiation and the length of time it took for those damages to be repaired. Since morphological changes are rooted in cellular changes, it can be assumed that there was damage at the DNA molecular level and one or several of the known plant repair mechanisms have been engaged in order to maintain the stability of the genome. In the context of this study, the almost uniform indications of instability proved to be interesting in its implications. The morphoses mentioned in this study had been further elucidated by Kal’chenko and Fedotov (2000) in which trees that absorbed certain dosage exhibited similar forms of phenodeviants. For example, the broom-like shoots, gigantic/dwarfed needles and shoots and low number of seeds in cones have been observed in trees categorised as having absorbed 5-15 Gy.

Changes in the phenotype do not only affect the individual organism but other populations of organisms relying on them one way or the other. Background research materials often report established threshold only for lethality as indicated by the level of radiosensitivity or resistance of an organisms. These results, however, indicated that there are various thresholds for a plant organism when it comes to the effects of radiation. Threshold levels existed for certain kinds of effects and damages because biological material reacts differently to radiation depending on various factors. The case in point was the mentioned example above wherein broom-like shoots, gigantic/dwarfed needles and shoots, and low number of seeds in cones were manifested in Pinus sylvestris after absorbing 5-15Gy. These thresholds vary from organism to organism wherein a certain dosage may cause one plant to flourish while it causes mortality in another. The threshold of lethality signified internal damage caused at the level of the absorbed radiation could not be repaired anymore which, in turn, propels other species to flourish or die. Population impacts pertains to influences of the ChNPP event to the species being examined as a whole but it has ramifications on biodiversity because the reduction in the numbers can give rise to the prominence of other species as in the case of Red Forest zone.

Recuperating abilities of the pine stands were observed as early the second half of 1986 where the buds of brachyoblasts from 1985 sprouted into new growth. By 1987, the phytomass reestablishment of pine trees was found to be dependent on the number of live buds. Highly affected stands were found by the authors to have less than 5% of the phytomass of the control group while trees in the medium and low affected areas exhibited the absence of any central leader in clusters of growing sprouts (Arkhipov et al. citing Pasterknak et al., 1994). Their report stated that only the most highly injured have not recovered their crowns completely by 1991 and that the viability of medium-injured stands were almost completely re-established.

The production of seeds again, in terms of population impacts, signified that the population size in the future would have the capacity to regenerate decreasing the probabilities of total extinction. It was reported by Kal'chenko and Fedotov (2000), however that the quality of these seeds upon further examination were not up to par in terms of amount and size. This, in itself, is predicted to have complications on future populations of the Pinus sylvestris. Monitoring of the progenies from trees which turned sterile would have been advisable.

The unique growth and development of trees must also be taken into consideration in interpreting the events in Chernobyl. Plants possess an indeterminate growth pattern contrary to faunal growth. Meristems or undifferentiated cells are retained in certain areas precluding a predefined body plan and retaining the ability to divide rapidly all throughout the life of the plant (Franklin and Mercker, n.d.). These undifferentiated cells eventually form specific tissues and cell types with defined functions and characteristics after rapid division. In areas where the absorbed radiation was not enough to cause lethality, this meant that the parts which were badly damaged could be replaced by new growth as long as there are brachyoblasts which are capable of promoting growth. Occurrence of somatic mutations with deleterious effects can be replaced can be replaced by new growth in the affected branches. This characteristic is not often observed during induced mutation experiments as crops like barley or rice have a life span of less then a year which do not allow for the possibility of a reverse mutation or recuperation from damages by replacement of new plant parts.

4.3 | Symmetry Fluctuation in Deciduous Trees

Moller conducted the study in 1996 and published in 1998. It examined the developmental stability of plants through fluctuating asymmetry and frequency of phenodeviants as sensitive indicators of current environmental conditions than traditional measures like growth, fecundity and survival due to its tendency to give indications of instability before the environmental condition actually results in a reduction of fitness. It was conducted a decade after the accident which meant that the elements influencing contamination were Krypton-85, Curium-242, Strontium-90, Caesium-137, Plutonium-241, Plutonium-238, Plutonium-239, and Plutonium-240 if the depiction of CEZ radionuclide cycle and percentage on soil contamination are accurate. Of the elements mentioned, Caesium-137 was the most likely cause for contamination while the possible impact of the Plutonium series which might have exerted their influence in the first ten kilometres from the site of the accident (Sokolov et al., 1992) were not given consideration. The study was done on a westward gradient from the vicinity of Chernobyl to Kiev and areas further away. The researcher chose a south-westward direction due to the calculated path of the plumes when the accident first occurred. No information regarding soil components was given but the consistency of the effects observed by the author seems to negate any variance that soil components might have affected.

The article was clear in terms of objective and specific depiction of methodology both in processing of data and gathering measurement of specimen even taking into consideration the calculated direction of the fallout in 1986. Data targeted by the study is concerned with the structural changes in the asymmetry of the specimens, observation and measurement was adequate as methods of data collection. It would have been interesting to see this study conducted within a period of several years rather than in just a single year since one of the mentioned concerns was the effect of asymmetry and aberrance to fitness with several studies proving the association of symmetrical phenotypes with survival prospects and longevity. A comparison study would have revealed whether there is a year-to-year significant variation in the asymmetry and if the instability had begun to recuperate with the declining levels of radiation. An intergenerational comparison would also be important in determining if this instability is inherited by testing if it would be passed down to their progenies. Changes in phenotypes are normally passed down when germline is affected (Scofield, 2016) or the pattern of methylation is passed down. However, since plant cells do not differentiate somatic and germline cells from the beginning as animals do, any somatic mutations have the capacity to be passed down to the offspring if it happens in the undifferentiated cells.

The study found out that the developmental instability observed in all species tended to be statistically significantly larger by a factor of three or four in areas which are near the security zone as opposed to specimens collected from areas with no radionuclide contamination and located far from the exclusion zone. The species studied by Moller followed the similar patterns marked by high levels of developmental instability near the security zone and decreasing levels near Kiev and further away from the Chernobyl area. Due to the highly consistent pattern of co-variation showing similarities in deviant morphology, the author concluded that there is a common cause instigating the developmental instability in the plant species. This common cause was attributed to caesium-137 as a strong positive relationship emerged for all three species with a fivefold increase in radiation being associated with a fourfold increase in developmental instability confirming radiation as being associated with variation in developmental instability of plants in different sites. In other forest species like Scots pines, changes in structure of the trees that did not die recuperated after several years. Since this study was done a decade after the accident, it would have seemed likely that recuperation should have started or occurred prior to the time of conduct.

Moller (1992) concluded that one of the specific consequences of Caesium-137 in biological material is a fluctuation in symmetry. The study did not offer any specific cause of disruption of the feedback mechanism which affects symmetry. Freeman et al. (citing various papers, 2005) attributed these disruptions to the feedback between the left and right sides of bilaterally symmetrical structures is said to cause the asymmetrical fluctuations demonstrating the failure of a genotype to consistently produce the same phenotype due to genetic or environmental influences which disturbs side-to-side communication and potential compensatory allocation making the preservation of structural symmetry or recovery from stress difficult .

Difficulties in the nuances of phenotypic definition comes into play in interpreting this study because phenotypes covers all observable traits including those that can be examined under the microscope. The consistent fluctuations of asymmetry in all three species suggested that the vertical migration of radionuclides have reached depths deep enough to be absorbed by the roots of rowan and the black locust trees although it must be noted that the latter have shallow, aggressive root systems. Methylation patterns or mutation could have caused said disruption between the left and the right feedback mechanisms, the former by silencing the expression of some genes to protect DNA structural integrity and the latter by changing genetic structure. If mutation caused a change in the genotype, then it could be inferred that the genes coding for phenotypic symmetry is one of the so-called hot spots characterised by low-level biochemical reactions (Loewe, 2008) because the reaction is manifested in a number of species. Since mutations are often deleterious rather than advantageous and often excised by plant repair systems, the persistence of asymmetry, therefore, could imply that it might serve some purpose in the environmental conditions existing in Chernobyl or it could be a pleiotropic effect wherein a gene mutation plays a crucial role to the survival of the plant but it also affects a seemingly unrelated phenotypic trait. In pleiotropic cases, the change in symmetry is a by-product or linked to a change in another phenotypic trait. It is also possible that the observed indications of instability have been caused by the establishment of new methylation patterns of the plants wherein the attachment of methyl groups to cytosine residues as a defense mechanism have silenced the some of the genes responsible for coding for symmetry.

Moller asserted in the study that the developmental instability manifested through asymmetry and irregular phenotypes in plant structures were currently unknown but could possibly have repercussions in fitness components because selection acts against developmentally notable phenotypes. Background research reveals, however, that this might not seem to be always the case as attested by toadflax (Linaria vulgaris) studied by Linnaeus 250 years ago where the wild-type actually formed asymmetric bilateral flowers while it is the mutant which forms symmetrically radial flowers due to the changes in methylation patterns (Sano, 2010). This is not to say that radially asymmetrical plant features are more fit than symmetrical ones but there is a need to establish if the symmetry does play an important role in fitness of plant organisms especially in terms of pollinating success in a wide variety of plants.

5. The ChNPP Explosion and Cellular Impacts

SN11 examined tree rings as indicative of the external environment. SN3 and SN4 studies were written and conducted by Tulik (1997), and Tulik and Rusin (2004), respectively, confirming the changes in the cambial events and orientation of MFA in the aftermath of the ChNPP accident. Tracheids, together with the trachea, xylem fibers and xylem parenchyma, make up the xylem of the tree (Kantharaj, n.d.). The xylem, together with the phloem, makes up the primary components of the vascular tissue which are conducting tissues. The xylem is responsible for the transport of water.

The main pathway of contamination was external or internal rather than contact contamination and the elements with short half-lives have stabilised during the collection for specimen for examination but the years of tree growth under investigation was the three-year period immediately after the ChNPP accident where contact and external contamination were giving way to external and internal pathways. The trees examined were 5 kilometres south from the site of the explosion making it very probable that alpha emitters were present in the absorbed radiation.

5.1 | Tree Growth and External Conditions

Mousseau et al. (2013) undertook further investigations regarding the growth of Pinus sylvestris to associate elevated ionising radiation background levels with standardised growth rates of Pinus sylvestris trees. It confirmed that the strongest influence of radioactive of materials on the trunk and branches of the trees was felt 2-3 years after the accident. This is in agreement with the findings of SN3 and SN4. Compared to SN3 and SN4 which examined retrospectively cellular processes and tree processes, it only studied tree growth in relation to external environment. Eight tree cores each from 14 different sites were collected but seven of the collected specimens disintegrated leaving a total of 105 samples which yielded 3,758 tree rings. The increment cores obtained were magnified under a 20x dissecting microscope after standard dendrochology procedures of mounting and polishing.

Tree growth rate change was visible in pine logs from the CEZ with significant growth variation from 1986-2008. This variance was more evident when compared to growth during 1914-1985 when there was little change in growth rates. Growth was severely depressed with annual increments of 0.10 mm from 1987-1989 recurred then from 1992, 1996, 2003 and 2006. Average annual increments under environmental conditions before the ChNPP were measured to be 2.93mm. The researchers commented that the radiation effect depended on the size of the trees with smaller trees demonstrating disproportionate reduced growth when contrasted with larger trees.

Growth trends are very good indications of tree health. The severe depression of growth, especially in locations where competition for resources may be tight, is indicative of disturbances in plant processes which affects the health of an organism. For example, small trees, when surrounded by taller trees, have less capacity to catch sunlight which is vital to maintaining and producing plant parts. The confirmation of the relationship between environmental radiation levels and depression of tree growth increments was not unexpected. The strongest depression of growth happened 2-3 years after the accident. The researchers hypothesized that the extent of the damage was due to weather and drought exacerbating the effects of radiation contamination. Analysis of the radionuclide cycle and the findings of SN3 and SN4 led to the conclusion that the severely depressed growth was possibly due to the cellular damage in the trunk caused by the disintegration of fuel particles containing alpha particles. The primary pathway of contamination was also changing from external to internal pathway since the radionuclides in the soil were migrating deeper.

5.2 | Cambial Events

SN3 was a study by Tulik (200) examining the development of cells from undifferentiated cells in the cambium using 30- and 42-year-old Pinus sylvestris located 5 kilometers from the ChNPP and 100 meters from each other. The tree rings of interest were three years prior and the years succeeding the incident. The researcher of the study hypothesised that cambial events increased following the Chernobyl accident. Cambial events consists of oblique anticlinal division, intrusive growth and cell elimination. Cambium is composed of 2 types of meristematic cells called fusiform initials, which are vertically-oriented xylem and phloem, and ray initials, which are isodiametric and radially oriented xylem and phloem ray parenchyma. Periclinal division is a process of cell differentiation leading to the production of xylem or phloem cells while anticlinal divisions improve secondary growth through the multiplication of the cambial cells thereby impacting the quantity of radial file cambial derivatives (Cell Types, n.d.).

Cambium is the only tissue wherein arrangement, numbers, and dimensions of xylem and phloem cells are suggestive of their development in the past.

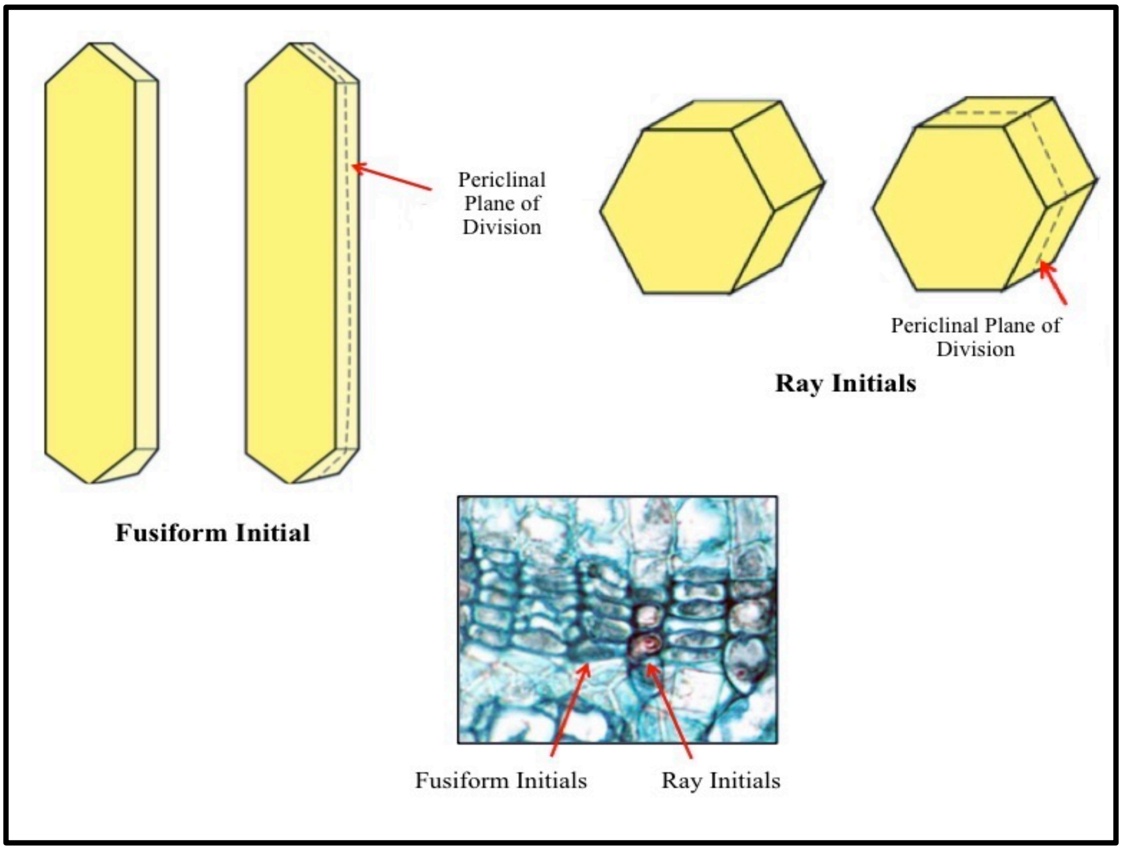

Figure 5.1 | **Two Types of Cambial Initials**
(Cell Types, n.d.)

Results of the study revealed that the three processes of cambial events increased in the aftermath of the accident when compared to the prior years. The frequency of fusiform anticlinal divisions reached three times higher two years after the accident for the 30 year-old and two times higher three years after the accident for the 42 year-old when compared to the mean growth years before the accident. Intrusive growth for both the 30 and 42 year-olds intensified the rate prior the accident two-fold. The highest rate of intrusive growth in the 30 year-old occurred two years after the ChNPP accident then continued to decline but not to the levels of the years before the accident. Cell elimination frequency for the 30 year old peaked 2-3 years after the accident then slowly declined but did not reached any of the levels prior the accident. The 42 year-old cell elimination frequency rate peaked on the fifth year after the accident where a three-fold increase was observed. Wood sample from both trees exhibited discernible increase of tracheid length. This increase in frequency of cell elimination was interpreted by the researcher as a way of disposing mutated cells while the lengthening of the tracheid was seen as a sharpening of cambial cells selection wherein the longest of the fully formed fusiform initials is a factor of tracheid proper length maintenance (Tulik, 2002).

The analysis of this study required knowledge of cambium which causes secondary growth in trees through the production of secondary vascular tissue resulting into radial growth in stem and root tissue. For the purposes of this study, only the cambium in shoots is discussed. Cambium is composed of two types of meristematic cells called fusiform initials, which are vertically-oriented xylem and phloem, and ray initials, which are isodiametric and progresses into radially-oriented xylem and phloem ray parenchyma after division. Ray initials function as vegetative storage tissue (Cell Types, n.d.)

Hejnowicz (1961) in his study of cambial events in non-storied cambium of trees reported that secondary growth or increase in girth of trees with non-storied cambium like conifers is decided by intrusive growth of fusiform cambial cells which is defined as the active cell tip advancement. The tips pushes neighbouring cells apart by forcing their way through radial walls. The role of anticlinal divisions at this point is to increase fusiform initials and also to counteract the average-length growth. The increase of fusiform initials, on the other hand is counteracted by elimination of some initial cells which occurs as more dense cambial cells in the radial row force themselves sidewards in-between tangential cell walls resulting into breaks in the row where the loss of a cambium initial takes place. Shorter initials had been found to have the tendency to be eliminated and this process of elimination invigorates in the neighbouring cells stronger intrusive growth (Hejnowicz citing various authors, 1961).

The results of this study regarding the increase in frequency of anticlinal division was noteworthy given that SN11 reported that the years after the ChNPP accident marked by severe growth depression coincided with the years when anticlinal divisions were reported to be highest. This was two years after the ChNPP explosion. These years should have shown strong growth patterns. It is hypothesized that this increase in anticlinal division was offset by the increase in the frequency of cell elimination. Cell elimination is a normal process in the cambium even under ordinary circumstances. Under CEZ conditions, the conjecture of the SN3 researchers that fusiform elimination removed mutated or damaged cells wherein it became a mechanism for selection in the light of the study by Hejnowicz (citing Bannan, 1961) was given credence but it did not consider this cambial event as a factor in the lengthened tracheids. The excessive elimination encouraged intrusive growth resulting into lengthened tracheid cells. Elimination of cells accelerates the rate of intrusive growth and anticlinal divisions in the cambium but the presence of mutagenic environmental stimuli caused damaged cells and genetic material to be eliminated in excess. It is possible that the increased frequency rate of all cambial events examined and observed begun from the increased rate of cell elimination. SN4 sheds more light in the tree ring processes.

5.3 | Microfibril Angles

The researchers observed that the differences were affected by the age of the specimens with changes being more evident in the younger ones. Tree rings of three trees aged 16, 30 and 42 year-olds, at the time of the accident aged 5, 19 and 31 year olds respectively, were analysed where the steepening of the microfibril angles which normally occur as reported by Walker and Butterfield, and Donaldson was not observed (cited by Tulik and Rusin, 2004). On the contrary, the researchers reported that the MFA of the 16-year-old tree at the time of the study, and 5-year-old during the time of the accident, reversed from the expected angles which was from 20° to over 30° to the long axis instead of becoming steeper. They qualified the data findings by stating that while the steepening is the known normal occurrence in species which have been studied like Picea abies (Norway Spruce) and other conifers, the angles of the microfibrils of Pinus sylvestris have not been really been examined before although pines other than Pinus sylvestris have also demonstrated this phenomena.

To properly review and assess SN4, background research was done on microfibril angles. A study by Lichteneger et al. (1999) observed that microfibril angles under normal circumstances were higher in early wood which is produced in spring than in latewood which is produced in the summer or fall. The general conclusion derived by Lichtenegger et al. (1999) stated that MFAs, despite considerable differences in the detailed course with annual rings, decreased from pith to bark and confirmed that Pinus sylvestris MFA was supposed to steepen as the tree gets older. The observed results of SN4, together with the results of an earlier study by Tulik (2001) which found that the cell length after the ChNPP accident lengthened instead of shortened as it was expected to happen under stress conditions, attributed to the delay of the transition of cambium development wherein the juvenile wood is produced to the mature wood is deposited.

The possibility that the utilisation of enzymes needed for the process to occur as one of the possible causes is considered in analysing this study. The results of the study by Zelena et al. (2005) in studying gene expression levels noted that phenylalanine ammonialyase or PAL, which was determined to be a key enzyme in the secondary metabolism of higher plants in catalyzing L-phenylalanine into trans-cinnamate through non-oxidative conversion but is also essential for ozone tolerance, defense against pathogens and metabolism of exogenous compounds, is up regulated. The secondary metabolism is essential because trans-cinnamate is the initial substrate of phenylpropanoid in wood formation (Zelena et al, 2005). The role of PAL in the formation of tracheids had not been explicitly stated. The connection lies tenuously at best in both being parts of secondary metabolism in wood formation, yet, it cannot be ignored. In a study by Rubery and Northcote (1968), PAL is given a role in the synthesis of lignin during xylem differentiation. It does not mention tracheids specifically but instead the process of xylem formation from the cambium which are lateral meristematic cells leaving its role in the aberrant formation of S_2 layer in the MFA open to speculation and prone to conjecture.

Analysis at this point suggests that the increase in PAL was not cause of the delay in the transition of cambium development although the change in the quantity can be interpreted another way. The additional production of the enzyme implies that the plants need more quantities of it because it is being utilised as well in its other capacity as a defense against exogenous compound to combat the effects of ionisation which may result in the unavailability of the enzyme even when its production is increased for its function as a catalyst resulting in the said delay of cambium transition development.

Comparison of the S_3 layer of tracheids cell wall before and after the ChNPP disaster by the researchers indicated the abnormality in the morphology of the MFA where the arrangement of cellulose microfibrils was altered. The normal development of

70° to the long axis was found to have deep fissures and was grouped in bars oriented transversally or obliquely to the long axis of the cell.

As mentioned in the study currently under assessment, the S_2 layer plays an important role with regards to mechanical support as it is the thickest layer with it comprising 75-85% of the tracheid's cell wall. The steepening of the microfibril angels from the pith to the bark is connected to the mechanical optimisation for a particular given specie. In the study by Lichtenegger et al., (1999) the difference in the MFA of softwoods and hardwoods, had been related to loading condition in relation to lateral forces like the wind, vertical compressive forces due to gravitation and bending up to a critical angle wherein the typical decrease of MFA from juvenile to mature wood would therefore result from typical loading patterns during the development of the tree.

The study confirmed that the observed changes in the tracheid of the Pinus sylvestris occurred in the aftermath of the ChNPP accident. Longer cells have higher chances of surviving the process of cell elimination but the unusual length of the tracheids in the specimens collected also had disadvantages. The unusual orientation of the MFA was expected to have an impact on the survivability of the organism to withstand outside forces. It is, however, prudent to consider that the tracheids are part of the water transport system of the trees. The results of SN11, SN3 and SN4 were observed a few years after the ChNPP accident. This might be due to delayed reaction or a new environmental stimuli. In the examination of the radionuclide cycle, the disintegration of the fuel particles which often have alpha particles occurred 1-2 years after the incident. The failure of the MFA to steepen was due to the utilisation of vital PAL enzymes in their role of pathogenic metabolism resulting into late deposition of wood was considered but it was also possible that the failure of the MFA to steepen plant mechanism to prevent the damaging radionuclides from the soil to reach more sensitive plant parts such as undifferentiated cells or the buds.

6. The ChNPP Explosion and Genetic Impacts

Mutations result in the aftermath of environmental insult and repair processes of genetic material. These are changes in the genetic sequence that occur at different levels with varying consequences that causes diversity among organisms. Large mutational effects can be caused by a single mutation but, more often than not, result from accumulation of mutations with small affects impacting the evolutionary change. Mutational effects are dependent on their context or location and can be beneficial, harmful or neutral. Generally, the greater the number of base pairs affected by a mutation is accompanied by a larger effect and a bigger probability of being deleterious (Loewe, 2008).

6.1 | DNA Lesions

Plants endure numerous biotic and abiotic conditions that affect their survival by wreaking havoc on their structural, cellular or genomic systems. Of these, the genomic integrity is paramount to the survival of the plant. The genome encompasses the Deoxyribonucleic Acid (DNA) which carries genes made up of the four bases adenine, guanine, thymine and cytosine arranged in specific sequences that encodes the information of inheritable traits and characteristics (Tabatabaei, 2009). It has been noted by Gill et al (2015) that despite the stability of plant genome, nuclear DNA is still an inherently unstable molecule that can be damaged by important DNA-damaging agents like ionising radiation, chemical mutagens, cross linking agents, alkylating agents, fungal as well as fungal and bacterial stress factors. Table 6.1 adapted from Lodish et al. (citing Kornberg and Baker, 2000) enumerated various lesions that require repair.

DNA damages in the form of altered bases and sugars, formation of DNA-protein and DNA-DNA crosslinks and single- (SSBs) and double-strand breakages (DSBs) had been proven to result from radiation (Hinton, 2014; Kovalchuk et al., 2000). A dose of 1 to 2 Gy is enough to cause DNA lesions of around 1000 base damages, 1000 single strand breaks (SSBs), and some 40 double strand breaks (Hinton citing IAEA, 2014). Of these effects, extreme cytolethal forms of DNA damage are represented by DSBs due to the absence of an intact template which can be used for repair as both strands of the double helix are broken (Lodish et al, 2000) posing a threat to the preservation of genetic information (Orlowski et al. 2011).

Table 6.1 | **DNA Lesions that Require Repair**	
DNA Lesion	**Cause**
3' deoxyribose Fragments	Disruption of deoxyribose structure by free radicals leading to strand breaks
Altered Base	Ionising Radiation Alkylating Agents
Bulge due to deletion or insertion of a nucleotide	Intercalating agents that cause addition or loss of a nucleotide during recombination or replication
Cross-linked strands	Covalent linkage of two strands by bifunctional alkylating agents
Incorrect Base	Mutations affecting 3'→ 5' exonuclease proofreading of incorrectly incorporated bases
Linked pyramidines	Cyclotubyl dimers resulting from UV radiation
Missing base	Removal of purines by acid; Removal of altered bases
Single or Double Strand Breaks	Breakage of phosphodiester bonds by ionising radiation or chemical agents
Adapted from Lodish et al., (2000)	

Since DSBs can be caused by radiation, it is correlated with radiosensitivity and becomes central to the probability of cell survival. Kuchma and Finkeldey (citing Holst and Nagel, 2011) stated that the responses radiation exposure elicits from plants are comparable to other stress factors due to the uniformity of metabolic pathways reactions to stress. Despite broad similarities of cellular responses from different organisms to radiation, however, these do not preclude the differences in radiation sensitivities (Hinton, 2014). Plants cover a wide range in the scale of radiosensitivities varying by three or four orders of magnitude when comes to the range of lethality. Viruses are, on the other hand, among the most radioresistant while mammals are among the most sensitive. (Hinton citing Whicker and Schultz, 2014). Different factors of cell features able to affect resistance and sensitivity of plants have been identified in Table 4.2 adapted by Hinton et al. (2007) from Sparrow, and Whicker and Schultz. In 1992, Syomov et al. asserted that the properties of a plant's DNA repair system contributes to the radioresistance or radiosensitivity of an organism.

DNA lesions pose threats to the stability of the genome. In the event of disturbance, repair and maintenance of DNA to stabilise genomic integrity through various pathways must be initiated by the effective detection of DNA damage followed by the removal of damaged nucleotides to be replaced with undamaged nucleotides by DNA synthesis in order to lower the risk of having permanent genetic modifications (Gill et al., 2014). This makes DNA repair systems as one of the most essential features influencing natural plant radioresistance (Syomov et al, 1992) in the face of varying insults.

Table 6.2 | **Nuclear Characteristics and Factors Influencing Sensitivity of Plants to Radiation**

Factors	Sensitivity	Resistant
Nucleus	Large with high DNA content	Small with low DNA content
Heterochromatin	Abundant	Little
Chromosomes	Large	Small
	Acrocentric	Metacentric
Centromere	Normal	Polycentric or Diffuse
Cells	Uni-nucleated	Multi-nucleated
Chromosome Number	Low	High
	Diploid or haploid	High polyploidy
Reproduction	Sexual	Asexual
Intermitotic time	Long	Short
Dormancy Period	Long	Short or none
Meiosis	Slow	Fast
(Hinton citng Sparrow; Whicker and Schulz, 2007)		

These damages occur at the molecular level but can be manifested at the physiological one as the result of the interplay between the abiotic stress and the struggle of the internal plant systems to preserve genome integrity. At the cyto/genetic level, repair or persistence of damages and mutation are relevant considerations.

6.2 | Selection Response

SN8 was the first of the studies which utilised specimens from the same five diverse tree stands of Pinus sylvestris (Scots pine) also used in SN9 and SN10. The demes, at the time of the study, were 50 year old trees (labelled in the study as deme P1), 20 year old stands with differing outward appearance even when exposed to similar levels of contamination (deme P2 and P3) and two stands which served as control (deme P4 and P5).

Deme P1 were 50-year-old trees exposed to the original myriad of elements spouted by the ChNPP accident and thereafter. Acute and chronic radiation exposure were expected to have affected the trees in this group and pathways of exposure would have been through contact and external in the immediate aftermath of the accident which gave way to internal and external ones as time passed. Demes P2 and P3 were planted around 1991 in similar conditions, exposed to chronic radiation and were classified according to their outward appearance. Deme P2 were trees with strong damage while P3 showed slight or no damages at all despite being exposed to the same level of contamination. The authors did not expound how variation in biological material was accounted for as a possible reason for variation. These deme of trees were influenced by external and internal pathways of contamination and were also exposed to radiation during their seedling stage as opposed to the trees in P1 which were already around 25 years old during the ChNPP accident.

This study by Kuchma and Finkelday (2011) examined selection response as an adaptive mechanism of plants in Chernobyl. Selection response is the differentiation of adaptive genes with main functions and close associated genomic regions as a feedback to environmental stimuli. Adaptive differentiation is proven through comparison between populations of large allele frequency differences at the loci being investigated. It relies on the assumption that phenotypic traits which adapt to the environment are associated with DNA regions are which functionally-active. The authors used amplified fragment length polymorphism (AFLP) instead of the candidate gene approach since the Pinus sylvestris was not a model plant and prior information about genetic sequences and functions are inadequate. It was expected that AFLP variation comparison between loci which had adapted would yield higher differentiation than loci of trees from the control plot. Specimens for experimentation were chosen from demes P2 and P3 which were 20 year old Pinus sylvestris while material from deme P4 served as control.

The results of the study yielded statistically insignificant results where variation of genetic material of trees from P2, P3 and P4 was very low. Since P2 and P3 trees were cultivated under similar circumstances and in plots near each other, the researchers expected that the opposing development of physiology of trees from these stands would be a result of the difference in genetic material. The researchers found strong evidence that genetic structure from a number of markers changed as a reaction to high radiation but, due to the result of the study, they conjectured that these polymorphisms controlled adaptive responses rather than selective genes.

The genetic differentiation was a possible explanation for the difference between P2 and P3 trees in an environment with the level of radiation existing on the exclusion zone. The mechanisms of selective response, however, relies on genetic modification which contradicts the preservation of genetic structure because most of evolutionary change by which selection acts is based on the accumulation of numerous mutations with small influences instead of being point mutations. Survival on environments with high mutagenic factors would depend on maintaining the structural integrity of the genes. Since these trees had been planted on high radiation-contaminated soil, other defense mechanisms minimising DNA rearrangement were favored. This gave credence to the hypermethylation theory by Kovalchuk et al. (2003).

6.3 | AFLP and Microsatellite Mutations

This was a study by Kuchma et al. (2011) comparing results of Amplified Fragment Length Polymorphism (AFLP) against microsatellite markers in identifying mutations. A microsatellite can be a DNA motif ranging in length from two to five nucleotides repeated typically from 5-50 times (Wikipedia, 2016c) but genomic satellites which were used by the researchers were simple sequence repeats (SSRs) of one- to six-base nucleotides. It is differentiated from other genetic modifications by the gain or loss of an entire repeating unit instead of affecting just a single nucleotide or random sequences of nucleotides. It often happens at noncoding sites which are spliced out during translation but can occur in coding sites or exons as well and when it does, the physical and chemical properties of proteins can be affected through producing gradual and predictable changes in protein action. An example of dinucleotide microsatellite is TATATA while the trinucleotide microsatellite is GTCGTCGTCGTC (Wikipedia, 2016c). On the other hand, AFLP is the detection of deviation from the absence or presence of polymorphisms which is the association of several phenotypic forms with alleles of one gene or homologs of one chromosome occurring in a population (Griffiths et al., 1999). It was hypothesised by the researchers that mutation rates at SSRs would be high as mutational events per locus under normal circumstances are high although this hypothesis was cautioned by the functional importance of SSR motifs during adaptation. They reasoned that change of SSR at loci involved in adaptation would preempted by selection.

Microsatellites genotyping identified four mutations in demes P1, P2 and P3 and none in control plot, P4 and P5. The mutations represented deletions found in repeat motifs of three different microsatellite loci recognised as LOP5, PtTX3107 and PtTX2146. The researchers did not elucidate what are the exact ramifications of modifications at these points but microsatellites were hypothesised to play a role in translation, transcription, recombination, DNA replication, chromatin organisation, and the cell cycle (Kuchma et al., 2011). The single instance of mutation in the deme P1 is a loss of twenty nucleotides at LOP5 in one of the trees. The other three were located from trees in deme P2 and P3. The detection of mutation using microsatellite loci markers showed no statistically significant difference between the irradiated and the control groups even if no mutations were found in the control

The researchers detected twelve events in the control plot P4 while P2 and P3 yielded in 34 and 32 events, respectively, through the utilisation of the AFLP method. Whole fragments deletions and insertions were recorded as well as single nucleotide deletions and insertions. Mutations at different AFLP loci were, in some cases, found to exist in the same tree but in different branches. Yet, as with the results of the microsatellite markers, no significance was found between the comparisons in the number of mutations between the irradiated damaged P2 and irradiated healthy P3 although the difference was highly significant when compared to the control. The AFLP method calculated a significant difference of 3.8 for P2 and 3.5 for P3 higher mutation rate when compared to the control group.

The higher number of mutational events in demes P2 and P3 when compared to deme P1 could signify that the effect of radiation during seedling stage have a more prominent and lasting effect than the effects of acute radiation when it is received by 25-year old trees. On the other hand, it is entirely possible that trees which had been exposed first to acute radiation characterised by high levels of contamination developed a form of immunity to radionuclides by having more efficient repair systems able to cope with the lower levels of contamination because it had been able to cope with more numerous damages in the past.

The numbers of mutational events were significantly lower than expected. The authors suggested that these might be due to various factors that influences mutation rates in at microsatellites or more efficient plant repair systems but it is also likely that epigenetic mechanisms or reverse mutation may have played a part on maintaining the fidelity of the genome. Since no radionuclide analysis information was included in the study, the presence of high-LET radionuclides which would have affected the efficiency development of plant repair system cannot be truly be certain.

Comparison of microsatellite mutation events between demes P2 and P3 located in the Red Forest was not found to be statistically significant. This is revealing as both P2 and P3 were growing under the same contamination levels and the trees were near each other enough for weather and other environmental elements not to factor in the difference of their appearance. Yet, deme P2 displayed large amounts of damage while P3 had only slight damages or none at all. From the differences in their appearance, it was expected that there would be a significant difference in the frequency of mutation events from these two different stands even if the microsatellites occur at the non-coding sites since it was posited that noncoding sites may be responsible for the regulation of gene expression. The comparison of deme P2 and deme P3 in this study is indicative of the unexplored nuances of plant responses to high radiation. To recap, both are stands of trees planted after the ChNPP nuclear accident and were 20 years old at the time of the study. The researchers took care to factor out extraneous factors as much as they can in cultivating these trees including the level of contamination that they are exposed to by planting trees from one deme close to another. However, one set of trees exhibited high damage while the other one had minimal or no damage at all. This difference in form and structure is attributed to the differing response of trees to radioactive materials. Examining the number of events in both microsatellite and fragment-length polymorphisms mutation showed the number of differences was not too large. P2 had two while P3 had one mutational microsatellite events. In the study AFLP study, P2 had 34 while P3 had 32. This effectively demonstrates that morphologic phenotypic deviance does not rely strongly on the frequency of mutation revealed by micro satellite markers or AFLP. Even the influence of the mutation loci on the significant outward difference between the two groups were analysed but even that seems to be under question as an examination of the mutational events reveals that these occurred at different loci. While induced mutation relies on the effect of mutation hotspots wherein propensity of mutations to happen at these areas are higher because of low-level biochemical reactions (Loewe, 2008) bring about the desired morpho-physiological changes, the mutational events in the DNA of trees proved that radiation wreaks in DNA were randomly affected rather than specific point areas of the chromosome or DNA in each tree. The consistency in the manifestation of instability in Pinus sylvestries after the ChNPP accident in this study seems to rely on other internal processes of the plants such as hypermethylation or ATP energy distribution rather than a direct consequence of mutation. Such mechanisms at the moment are, however, still for further investigation.

6.4 | Gene Expression

Two studies, SN6 and SN10, focused on differential regulation of gene expression. The up-/down regulation of gene expression is postulated to have an effect in the formation of deviant physiology.

Table 6.3 | **Comparison of Needle Dimensions**		
	Length	**Weight**
Dwarf	19 ± 3 mm	14 ± 2 mg
Normal	60 ± 4mm	80 ± 3 mg
Data extrapolated from Zelena et al. (2005)		

The study by Zelena et al. (2005) was conducted in 2000 from trees which were 7-12 years old. This meant that the specimens had not been exposed to acute radiation but had been subjected to chronic radiation at the rate of 1-2 mR/h at the time of the study. The researchers described the location as sublethal zone at about 10 km from the ChNPP site did not clarify whether the presence of alpha particles in the soil had been tested. No information on soil components had been given either.

The researchers performed several procedures with the length and cytology of normal and dwarfed pine needles. Seedlings grown from seeds collected from Red Forest trees exhibited phenotypical deviances. Table 6.3 compared normal with dwarf needles attributes. The study also analysed the content of radioactive caesium-137 in normal and dwarf needles of pine trees to discover the reason for the coexistence of morphologically normal and dwarf needles on the same branch of trees. It was initially thought that the difference might be due to the differences in local adhesion of radioactive or toxic particle but the results of their analysis were statistically insignificant. A cytological analysis of the meristematic tissues at the base of the needles was then carried out. The presence of mitotic abnormalities was found to be highly significant at $P<0.001$ with the percentage for normal needles at 23% while it was 35% for dwarf needles. Mitotic abnormalities are indicated by c-mitosis and presence of chromosome or chromatid bridges or fragments at telophase. In control groups with absorbed radiation of 0.007Gy/year, this figure was 0.5±0.1%.

Analysis of the differences in gene expression levels between the needle types were done through high-thoughput microarray analysis which is a procedure that permits the simultaneous scrutiny of thousands of genes under different conditions including changes in transcript levels in cells responding to environmental stresses (Zelena et al. citing Gasch et al, 2005) and examination of gene expression during developmental and growth processes (Zelena et al. citing Girke et al., 2005). Using this procedure and an array constructed from P. taeda which had previously demonstrated in a study by van Zyl et al. (cited by Zelena et al., 2005) to nearly efficiently hybridise with labeled cDNA from P. sylvestris as from P. taeda making it a proficient method for gene expression comparison of needles from the two species, twelve genes were identified as differentially changed in expression between normal and dwarf needles when is $P<0.01$. Five of these were genes show statistically significant 1.25- fold or greater changes in dwarf needles. Ubiquitin conjugating enzyme which is a factor in the formation of ubiquitine protein conjugates necessary in initiating the removal of abnormal and damaged proteins in the nucleus and cytoplasm, ascorbate peroxidase which is an enzyme that plays a role in the detoxification of reactive oxygen species and phenylalanine ammonialyase or PAL which is an enzyme that catalyzes nonoxidative conversion of L-phenylalanine into trans-cinnamate in secondary metabolism of higher plants and is believed to be related to ozone tolerance, defence against pathogens and metabolism of exogenous compounds were all upregulated. On the other hand, the researchers found that glutathione peroxidase which is vital in other areas of oxidant metabolism involving the removal of lipid and alkyl peroxides and AP2-domain transcription factor which is a plant regulatory protein that affects plant development are both down regulated.

The materials used for the high-throughput micro-array analysis were needles collected during the winter of the December 2000. Only five of the twelve genes found differentially expressed were statistically significant. These genes were classified as functional in information storage and processing, cellular operations, metabolism or otherwise poorly characterized functional groups. This is parallel with the findings of the study by Danchenko et al. (2009) wherein gene expression concerned with metabolism, energy, cell and growth/division, protein destination and storage, transporters, and disease and defense were differentially expressed in mature soybeans.

This research was concerned with the comparison normal and aberrant forms but at a level which eliminated the extraneous factor of difference in biological material. The detection of differences in gene expression between normal and dwarf needles were significant but in a limited capacity. It had not been stated whether it is possible that the limitation is affected by the strength of the stimulus which declined with temporal passage. The research was conducted a few years after the accident. Whether the level in the difference of up or down regulation of gene expression is more pronounced in the years immediately after the ChNPP accident. The researchers conjectured that the differential expression of very few genes during early development is adequate for the conversion of normal needles to dwarf ones. They did, however, not rule out the possibility that the abnormal morphogenesis could have been induced by the modification of cellular processes such as DNA methylation or by radiation-induced transposition of transposable genetic elements.

The mitotic abnormalities resulting into the co-existence of dwarf and normal needles in the same branch may be explained by circumstances of the exposure. Occurrences of mutations sufficiently early in development of seeds result into genetic uniformity throughout wherein the whole plant exhibits the mutation (Nybom et al., 2010). On the other hand, mutations after fertilisation will have chimaerical qualities wherein only parts of plants are mutated. The difference between mutation uniformity and chimaerical mutation could possibly be explained by rapid cell division which characterises early development. Mutation is uniform when the original cell from which DNA is replicated to form other cells is mutated before any cell division process can occur. On the other hand, chimerical mutation can manifest when some cell division had already occurred because some of the cells will be replicated from the unmated one while other cells will come from the mutated. Nybom et al. (2010) in this case was discussing crops. In the case of trees which have long life spans and retain meristems or undifferentiated cells, it is possible that these cells have undergone some form of division before being exposed to irradiation.

Gene expression is vital in the performance of various plant processes. The up- or down regulation of these expressed genes means some functions are not being done or accomplished properly. It is, however, unknown whether the differentiated gene expression reinforces dwarfism in needles or it is the result of the dwarfism of the needles. Energy is needed in the transcription of genes to produce enzymes The dwarf needles, which are functional in the production of photosynthase which provides energy in the transcription of genes are limited in their capacity to do so. This limited amount of photosynthase must then be maximized in its utilization in that genes which are necessary in repair and defense are prioritized in production as opposed to enxymes which for housekeeping or maintaining normal plant functions.

Highlighting Cat and GPx Gene Regulation

SN10 further explored gene regulation by highlighting the difference of Cat and GPx. It was a study by Vornam et al. (2012) which utilised specimens from the same demes of trees from SN8 and SN9. It is related to SN6 as well since it also focused on gene regulation effect on morphology. Procedure for association of genetic analysis of single nucleotide polymorphism, somatic mutation and gene expression with ionising radiation analysis which allowed possible connection of changes in morphology with gene expression was piloted. It tested for catalase (Cat) and glutathione (GPx) which are ROS-scavenging genes essential to the activation of plant defense systems. The conditions where the specimens for experimentation were collected were the same with SN8 and SN9.

The authors included an analysis of the radioactive materials contribution to internal dose where it was revealed that Pu and Am series contribution did not exceed 0.003% and 0.006%, respectively.

This study hinged on the concept of phenotypic plasticity which results from the activation of particular genes when stress is perceived due to altered environmental condition. Schlichting and Smith (2002) posited that the differential changes in gene regulation is crucial for phenotypic reactions to occur as environmental conditions change. The study hypothesised that the expression profiles of Cat and GPx in chronic and acute irradiated demes would differ from the expression profiles of said nucleotides in controls due to its role in defense against oxidative stress. It built upon the previous study (Zelena et al., 2005) which compared of dwarf pine needles with normal ones to demonstrate differences in the level of gene expression. This study affirmed the results of the study by Zelena et al. (2005) in terms of the amount of production of Cat and GPx. In demes P1 and P2, Cat expression was higher by a mean factor of 9 and 11 respectively when compared to their controls while the GPx expression is down regulated by a mean factor of 0.3. The authors interpreted these results that Cat plays a meaningful role in cell protection from H_2O_2 accumulation although P2 showed greater variability. This confirms the study by Kovalchuk et al (2004) where induction of Cat expression in Arabidopsis is one of the adaptation mechanisms employed. The study also confirmed the down-regulation of the GPx from the study of Zelena et al. (2005) when compared to the control plants although this was reconciled by the authors by reasoning that the encoding of GPx is done through a gene family by which the control and reduction in expression of some genes will favor the action of others.

The researchers did not include the P3 deme in their analysis of the results or their study which seems to be an oversight that would have had revealed substantial information in the difference in regulation of gene expression between damaged trees and slightly or non-damaged trees growing adjacently. If data had been significantly different between deme P2 and P3, then this would have been a step in further establishing the connection between the regulation of gene expression and phenotypic plasticity. If the comparison of data from the two demes, however, have had no significant difference, then the relationship between gene expression and alterations in morphology can be regarded as remote. Since information from deme P3 was excluded, this remains to be a subject for further conjecture.

The authors determined the genomic region for Cat and GPx using orthologous sequences at 510 and 545 base pair of Pinus sylvestris data derived from EMBL Access No FN546173 and EMBL Access FN546175, respectively. These means that the genes involved in the coding of Cat and GPx were determined by looking at gene sequences from other plants which have already been determined to code for these specifically from a library that keeps track of these information. The genomic region 510 bp partially codes sequence of Cat with two introns and one exon while the 545 bp partially codes for GPx with two introns and two exons. Exons are sections of the DNA that code for proteins while introns are noncoding sections of an RNA transcript because the DNA encoding it are spliced out before the molecule is translated in to protein (Nature.com, 2014). The exact nature of introns and its effects in gene translation is still under investigation although there is a hypothesis that it may have a role in gene regulation. Gene fragments were amplified and examined for nucleotide diversity of both Cat and GPx.

Trees exposed to acute and chronic exposure (deme P1) showed higher values of nucleotide diversity in synonymous sites, non-synonymous sites and non-coding position when compared with the control trees (deme P5). Modifications at synonymous sites tend to be less critical than the ones at non-synonymous because several codons are coding for an amino acids needed to build a protein. Complications at the non-synonymous sites, on the other hand, could have detrimental effects because it tends to prematurely stop the transcription for the amino acids needed by the organism. Definitive effects of mutation on the non-coding position have not been determined but, depending on the kind of alteration, can affect coding by altering the number of nucleotide bases which are interpreted in threes. It is higher as well in non-coding regions in comparison to coding regions. P1 yielded six polymorphic sites in which were found to be monomorphic in P5 while one site was monomorphic in P1 while polymorphic in P5. Out of the six polymorphic sites in P1, three were somatic mutations of which one was found in the non-coding region while the other two were found in the coding region. The implications of these somatic mutations depends on whether a substitution or an indel has occurred. One of the mutations found in the coding region is a synonymous change wherein the amino acid it codes was unchanged while the other one exchanged the coding from Isoleucin to Leucin which are isomeric leaving the catalytic functions of the enzyme largely unaffected because isomers may have different chemical structures but the same molecular formula (Wikipedia, 2016b) This meant that translation of the gene was basically unchanged in the former since the code was unchanged while the coding in the latter was changed but not to a great extent. Nucleotide diversity measures polymorphism in a sample of gene sequences and summarises statistics used to represent patterns of molecular diversity within a sample of gene copies while polymorphism is the occurrence of two or more clearly different morphs or forms, also referred to as alternative phenotypes, in the population of a species. Clear correlation between dose rate in tree and individual mutation at Cat failed to materialise. This is attributed by the authors to the fact that radionuclide measurements and genetic analysis were made on different branches. Analysis of Cat regions for nucleotide diversity from 20-year old trees which were chronically irradiated (P2) and 20-year old trees which were not irradiated (P4, control) showed higher values in the non-coding regions than in the coding ones. In one of the trees from P2, an amino acid Lysine is changed to Arginine. In P4 which acts as control, a somatic change identified was synonymous implying a harmless mutation.

Higher nucleotide diversity within the coding regions than in the non-coding regions for GPx was observed for all demes. P1 and P5 demonstrated higher diversity in non-synonymous sites, synonymous sites and non-coding positions. Synonymous sites are multiple codons that encode amino acids for the same protein while non-synonymous sites are specific codons that encode amino acids (Loewe, 2008). Mutations at synonymous sites are not as lethal as substitutions at non-synonymous sites because the sequence of amino acid in the former is unchanged due the numerous codons encoding the same amino acid while the consequence of the latter is a different protein or termination of a protein. P1 had one polymorphic site which was monomorphic in P5 while one monomorphic site in P1 was polymorphic in P5 rendering the controls as more diverse than the irradiated counterpart P2 and P4, which were younger trees, showed more polymorphic sites than the older trees and higher diversity in the non-coding than in coding regions. Results indicate that no somatic mutation events were detected.

Gene expression is vital in the performance of various plant processes. The up- or down regulation of these expressed genes means some functions are not being done or accomplished properly. It is, however, unknown whether the differentiated gene expression is the cause of dwarfism in needles or it is the result of the dwarfism of the needles. The dwarf needles are limited in their production of photosynthase which provides energy in the transcription of genes. This limited amount of photosynthase must then be maximized in its utilization in that genes which are necessary in repair and defense are prioritized in production as opposed to enzymes which mainly maintain normal plant functions.

The up- or down regulation of genes is a viable option in revving up the defense mechanisms. Without excessive environmental stresses, the expressed genes have their own function to attend to. However, the presence of radionuclides in the environment requires that these enzymes function in other capacity such as protecting cell from oxidative damage.

7. The ChNPP Explosion and Intergenerational Impacts

The study of intergenerational impacts of exposure to radiation intersects with the study of impacts with population and genetic material. Alterations in genetic material of somatic cells in plants can get passed on to the next generation because cells do not differentiate to germ cells and somatic cells early on as animals do. These alterations passed to future generations can affect populations in the long run resulting into a reduction in population size or extinction. Plants prevent the multiplication of unstable genetic material into future generations by employing several means.

7.1 | Nucleotide Excision Repair in Pollen

The ability of the pollen to repair genetic damages impacts fertility rates and intergenerational transfer of mutation due to the rapid cell division after fertilisation. Structural genetic damages which were not repaired before division are replicated to new cells resulting into deviantly-formed morphological and physiological features.

Betula verrucosa (birch tree) pollens were studied as representative of haploid repair systems from 1987 to 1998. From the background of the study, it can be surmised that the short-lived radionuclides have decayed to their more stable counter parts at the beginning of the study but it also meant that released elements which were in the form of fuel particles were beginning to add their impact to the level of radiation. The possibility that the first batch of pollens have been affected cannot be discounted as pollen of the species takes 10 months to develop and the researchers did not specify which month of the year 1987 the samples were collected. Migration of the radionuclides have also started migrating from the crown of the trees to the leaf litter and top soil. No information regarding soil composition had been given precluding inference on the rate of radionuclide vertical migration on soil or fixation.

The study revealed that not only the dosage of radiation matters but also confirmed that the type of radiation plays a part in the accuracy of the repair systems as pollen from sites with alpha radionuclides had impaired systems. In the 11-year study, the pollen of Betula verrucosa exposed to gamma radiation dose has improved the ability to carry out Unscheduled DNA Synthesis (UDS) annually until it matched the abilities of the unexposed control for repair. The authors explained that UDS signifies repair synthesis in NER in the absence of replication and endoreplication. UDS is associated with low amount of DNA replication when compared with the amount of associated with semi-conservative replication (De Hauwere and Lombaert, 2008). Endoreplication, on the other hand, is a variant of mitotic cell cycle which replicates nuclear genome in the absence of cell division by aborting or circumventing cytokinesis leading to nuclear gene content and polyploidy (Wikipedia, 2016a). It has been suggested that endoreplication is a universal developmental mechanism by which differentiation and morphogenesis of cell types that fulfill an array of biological functions depend on. This is therefore particularly essential during germination of plants when meristematic cells must develop into various plant organs. In the pollen from exposed sites, this process had been replaced by UDS. The ability to carry out UDS was observed to be not distributed evenly.

Unscheduled DNA Synthesis (UDS) signifies a cell's ability to perform genomic nucleotide excision repair (Kelly and Latimer, 2005). This study proves that exposure to chronic radiation initiates nucleotide excision repair which had been previously associated more with damages brought about by UV radiation than by IR. NER removes short single-stranded DNA segment with lesions while leaving the undamaged strand as a template for a short complementary sequence with the help of DNA polymerase until the final ligation is carried out by the DNA ligase to complete the NER and form a double-stranded DNA (Wikipedia, 2016d). Processes like NER is essential to survival of organisms in radioactive places. This was demonstrated in a study of Vicia cracca by Syomov (1996) where the intensity of UDS under doses of 500 Gy or lower in radioresistant populations was a factor of 1.5 higher when compared to the controls. The researchers interpreted this data as the move of repair systems to dark-repair methods as a more efficient repair procedure compared to the low recombination levels resulting from the study of Kovalchuk et al. (2004). While this statement remains uncontested, they did not suggest that homologous recombination and NER responds to different types of damage and possibly could be employed and utilised simultaneously. DSBs are the most lethal kind of damage but 1 or 2 Gy can cause around only 40 DSBs in relation to 1000 SSBs from the same dose. It is possible that NER, as a repair system, employed for SSBs will be utilised more as additional SSBs are produced subject to the kind of radionuclide damage involved as disturbance concentrated on one area of the cell seems to be more damaging than the ones dispersed around it.

NER is differentiated from BER as a more flexible damage-repair mechanism able to repair almost any large change in the structure of the DNA double helix. Large changes or bulky adducts are created when large carbohydrons covalently reacts with DNA bases as well as when sunlight causes pyramidine dimers (T-C, T-T and C-C) (Lodish et al. 2000). It is initiated when damaged regions are diagnosed based on the presence of abnormal structure as well as abnormal chemistry. Diagnosis is followed by multi-protein complex binding at the damaged site. Both sides of the 5’ and 3’ sides are incised from the damaged strand several nucleotides away from the damaged site. The damage-containing oligonucleotide from between the two nicks is then removed creating a gap filled in by a DNA polymerase which is then ligated to the nicks (Kowalski 2009). Boubriak et al. (2008) experiment on haploid and diploid cells proved that the NER system can repair different types of DNA damages including those caused by IR because conformational changes are recognized instead of a particular types of damage making it the most versatile system for dealing with DNA damage. The NER pathway is further divided into the global genomic repair (GGR) and the transcription-coupled repair (TCR) where the latter is selective for the transcribed DNA strand in expressed genes while the former repairs DNA damage over the entire genome (Gill et al., 2014).

Observation of UDS in this study was done during germination when meristematic cells have not yet differentiated to form cells which will comprise either dermal, ground or vascular tissues. Plants retain these dividing, undifferentiated cells through out their life in certain areas like the tips of shoots and roots. The frequency of mitotic abnormalities in meristematic cells at the base of the dwarfed needles when compared to normal ones is statistically significant at $P<0.001$ (Zelena et al., 2005). Since increases of meristematic cell repair activities result in mitotic abnormalities (Kalaev and Butorina, 2006), the probability that there is a relationship between the process of NER and dwarf needles exists especially when it is taken into consideration that UDS is not carried out evenly and endoreplication, which is connected with morphogenesis, is bypassed. This is, not, however, indubitable. The possibility that a different form of cell repair mechanism was utilised by the Pinus sylvestris, the specimen studied by Zelena et al. (2005), exists. It is also possible that if NER was involved, the capacity to conduct repair may grow weaker as the organism grows older.

It was not distinguished whether different types of cells employ different types of repair mechanism.

The repair mechanism of pollen from sites which contained both alpha and beta/gamma radiation remained malfunctioned as demonstrated by the comparison with the control when subjected to additional 750 Gy followed by a 3-hour germination, extraction and fractionation of DNA and Southern hybridisation of a rDNA repeat. The presence of nucleosome multimers also indicated nuclear instability as the accumulation of nucleosome represents a cell state of crisis which progresses to programmed cell death (Broubriak et al. citing Coupe et al. and Tiwari et al., 2007).

Data confirmed organism differentiation in reacting to alpha and beta/gamma radiation. Alpha particles wreak greater havoc due to their densely ionising nature which causes bigger cellular damage than gamma or beta radiation. While under ordinary circumstances, a paper or 3 cm layer of air is enough to usually deter alpha particles, their impact in penetration could be surmised to be stronger when deposited in leaves where physical barriers are nonexistent due to direct contact. Another possibility is that the damage caused by smaller, lighter particles like beta and gamma which could penetrate cell layers make it easier for alpha particles to penetrate surfaces which are impenetrable under the absence of other types of radiation. Since alpha particles in its short flight can knock about 450 000 electrons out of the surrounding atoms (oasisllc.com, n.d.), the damage incurred from them would be larger in scope and more difficult to repair especially as they have the tendency to inflict concentrated damage in one area which could result to paralysis in the functioning of said area. Larger particles have a tendency to settle faster than lighter ones limiting transport to shorter distances. The effects observed in the trees of red forest in Chernobyl had been exacerbated by being more exposed to alpha-particles than the rest of the Pinus sylvestris especially as majority of the radionuclides from the Plutonium series are purported to be have been deposited within the 5-km vicinity of the reactor.

7.2 | Epigenetic Defense and Global Hypermethylation

The epigenetics study published in 2003 by Kovalchuk et al. constituted two parallel experiments conducted simultaneously to compare progenies of the "Red

Forest" which are pine trees that died gradually months after exposure to 60 Gy dose and the progenies of the control group to investigate intergenerational hypermethylation. For the first experiment, there were three groups of seeds. The first group of seeds, which served as control, was from non-exposed trees planted in clean soil. The second group of seeds were collected from trees exposed to the acute and chronic radiation in the Red Forest that perished gradually. These were planted in clean soil with radionuclide content of 0.01 Ci/km^2. The last group of seeds were also collected from trees which had never been or exposed to radiation planted in burial sites which highly were contaminated. They tested for global methylation of the plants grown from these seeds.

The other experimental design is an intergenerational investigation of global hypermethylation patterns. The first generation is composed of three groups of 40-year-old. The first group served as control and was composed of unexposed trees growing in non-contaminated soil whose ten-year old 2nd generation served as control 2. The second group of trees which had adsorbed 40 Gy of acute and chronic radiation (C1G1) growing in soil with 600 Ci/km2. The 10-yr old progenies of this group (C1G2) was calculated to have soaked up 12 Gy of acute and chronic radiation. The third group was a group of trees (C2G1) were also planted in soil with 600 Ci/km2 but exposed to only 20 Gy acute and chronic radiation. The offsprings (C2G2) took in 5 Gy acute and chronic radiation.

Global cytosine extension assay which utilises methylation-sensitive restriction enzyme HpaII was used to study the DNA methylation. The HpaII frequently detects sequences of CCGG at randomly distributed CpG sites throughout the plant genome and leaves a 5' overhang after DNA cleavage used for subsequent single nucleotide extension with labeled [^{3}H] dCTP. [^{3}H] dCTP is directly proportional to the number of number of cleaved and unmethylated CpG sites which means it is inversely proportional to the levels of methylation. The researchers themselves, however, stated that there is a weakness in using the global cytosine extension assay as a method for detecting methylation. Cytosine residues other than CmCGG are methylated. This applies particularly to the sequence mCXG. The weakness is that there is no known method to differentiate this kind of methylation from the methylation of CpG sites. While locus-specific methylation can be measured through different procedures depending on purpose, global methylation patterns can only be measured by only one known procedure called global cytosine extension assay (and Waterland, 2007). Results of the first experiment indicate that there is a significant decrease in the incorporation of [^{3}H] dCTP in the DNA of exposed pine group 2 after the HpaII treatment. On the other hand, the results of the [^{3}H] dCTP incorporation in the DNA of exposed group 1 was interpreted to be hypermethylated even the seeds were planted in clean soil.

The study confirmed the difference in the tolerance threshold of plants when it comes to acute and chronic radiation. It has been established in the study by Arkhipov et al. (1994) that above 60 Gy is the threshold of lethally absorbed dose for Scots pine trees when exposed to acute radiation but a 100 Gy for chronic exposure. In this study, the first experiment tested the hypermethylation of plants grown from seeds of trees from the Red Forest and seeds of unexposed trees grown in highly contaminated soil. The Red Forest trees died upon receipt of more than 60 Gy. However, the exposed group 2 of 10-year-old trees which originally came from seeds of non-exposed parent trees planted in burial sites but received 80 Gy were still able to thrive.

Chronic irradiation is distinguished from acute irradiation in terms of strength and duration. Acute irradiation is depicted as exposure at a high-dose rate over a short period of time relative to which the obvious effects occur while chronic irradiation is exposure at usually a sufficient low-dose rate which lasts continuously in time spanning over a significant portion of an organism's lifetime and does not produce acute effects (Hinton et al., 2011). This difference in stimulus elicits different responses when it comes to damage repair. Plants have a tendency to react to acute irradiation similarly as to other abiotic stresses while chronically irradiated plants employ epigenetic regulation, genome stabilisation as well as post-translational regulation (Danchenko et al., 2009). Kovalchuk et al. (2000) explicates more on this dichotomy in their study of homologous recombination in acutely and chronically irradiated plants. They state that mechanisms of protection and DNA repair for combatting any damage are mobilised during and immediately after acute exposure to IR of which ionised water produces around 65% of the DSBs. Scavenger mechanisms which inactivate most of the produced radicals by converting them into non-mutagenic form are activated as a response to the creation of reactive oxygen species. On the other hand, chronic exposure to IR also means the constant production of free radicals which may be lower in number when compared to acute irradiation but induces more DSBs due to the longer exposure which then requires repair processes like homologous recombination. Since repair mechanisms is a factor in the radioresistance or radiosensitivity of plants, the mechanisms being employed dictate the threshold levels of the absorbed doses that the plant can tolerate.

The study reports hypermethylation as an adaptive response dependent on the absorbed dose which has implications on the concept of methylation as an inheritable trait. Since epigenetics deals with the modification of gene expression as environmental factors assert their influence on an organism without changing the basic gene sequence, the results of this study querries how hypermethylation in plants is triggered and whether the weakening of the stimulus which triggered it can cause demethylation thus returning the gene expression to its original state. In their first experiment, there was methylation in seeds from the Red Forest trees even if they were planted in clean, uncontaminated soil prompting the assertion that it can be inherited although the results of comparison between the 10-year old exposed group 1 and control is akin to the results of their second experiment in which there was methylation in the C2G2 but it was not statistically significant from the control. The seeds from exposed group 2 were collected from trees which were originally not exposed to radiation, it could be assumed that the parental trees from which these trees came from were not methylated as this was the case for the control group. It seems, therefore, that the plants grown from this seeds developed hypermethylation on their own as an adaptive response to chronic irradiation. In their second experiment, the progenies of the non-control trees were less hypermethylated than the parental trees which is taken as a result of having absorbed lesser dose which implies that the variation in gene expression in the parental trees are also lessened in the second generation. Zelena et al. (2005) affirmed this when half-sib progenies of trees in the Chernobyl region exposed to various cumulative doses of radiation totaling 16 Gy were grown in controlled environment of optimum growth conditions in order to determine the extent of the changes induced by exposure to radiation on the next generation of seeds. The results indicated that genome methylation is reversed when seeds were grown in clean soil. The understanding being given here is that the pattern of methylation might be inherited but it is also an activated response to the presence of stimuli.

The inheritability of traits throughout a specific number generations irrespective of environmental conditions which stimulated the original trait had changed was concluded from the experiment of flax which exhibited a three-fold heavier weight when grown under nutrient rich conditions and progenies which did not change this particular characteristic even when the nutrient-rich environment had changed (Sano citing Sano et al., 1999). Sano (1999) in his mini-review of DNA methylation in plants confirms the reverse relationship between cytosine methylation and gene expression and answers the question of inheritability through the distinction of higher and lower plant classification. In higher plants, the location of the methylation occurs mostly at cytosine residues which is a post-replication event where the status is reset after each cell cycle and eventually in each generation making it a generation-specific feature favourable for reversible control of gene expression during growth. This pattern of methylation in plants, however, is not completely reset since plant reproduction can be conducted through different systems making the transmission of methylation pattern from parents to offspring more likely.

In the second experiment, it has been noted that while the values derived are almost similar, the difference in the results between C1 and C2 trees in the first generation (G1) raises some interesting questions. While the second generation (G2) C1 and C2 followed the pattern where C2G2 is less hypermethylated than C1G2 which was expected due to lower absorbed dose of 5 Gy and 12 Gy, respectively, this pattern is reversed in the parental generation where in C2G1 which has received less acute and chronic radiation of 20 Gy is more hypermethylated than C1G1 which had 40 Gy.

Table 7.1 | **Experiment 2: Incorporation of [^{3}H] dCTP**

	Control	**Chistogalovka #1 (C1)**	**Chistogalovka #2 (C2)**
1st Generation (G1)	542.4 ± 73.0	403.1 ± 59.3 (40 Gy)	383.8 ± 47.2 (20 Gy)
2nd Generation (G2)	539.0 ± 104.8	411.8 ± 44.5 (12 Gy)	471.8 ± 92.5 (5Gy)

dpm/1 µg of DNA
Data extracted from studies of Kovalchuk et al. (2003)

This disparity questioned the assertion of the authors that hypermethylation is dependent on the dose absorbed but neither is it a confirmation that the pattern of methylation is inherited. The progenies of C2G2 which received 5Gy has statistically insignificant hypermethylation results when compared to the controls. There, then, seems to be a discrepancy in the results. The dependence of hypermethylation on the absorbed dose seems to work only when comparing intergenerational absorbed dose wherein the parent is more hypermethylated than the progeny because it absorbed more dose but it does not apply when comparing organisms with different genetic material. This is the case when C1G1 is compared with C2G1 where the incorporation of [3H] dCTP is higher in C1G1 which has a higher absorbed dose than C2G1. Since the incorporation of [3H] dCTP is inversely proportional to the level of methylation, then this means that C1G1 (40 Gy) is less hypermethylated than C2G1 (20 Gy).

8. The ChNPP Explosion and the Recovery Analysis Discussion

Table 8.1 | **Summary of Significant Findings**

SN	Significant Findings
1	The extent of damage depends on the dose of radiation absorbed. Refer to Table 4.1 for more details
2	• Developmental instability was 3-4 times larger in areas near the ChNPP than in uncontaminated zones. • The elevated level of developmental instability was determined to be from a common cause, ^{137}Cs. • Fourfold increase in developmental instability associated fivefold increase in radiation.
3	• Frequency of the anticlinal divisions increased threefold 2 years after the accident and slowly decreased but not as slow as before the accident in the 30-year old tree (16 at the time of the accident). In the 42 year old (30 at the time of the accident) the anticlinal division was two times higher three years after the accident. • Intrusive growth in the fusiform cells in 1986 increased twofold the observed rate before the accident but the highest rate was observed 2 years after . • Frequency of fusiform cambial cell elimination increased after the accident but highest rate was reached 2-years after. • Increase in the length of the tracheid was observed.
4	• Steepening of microfibril angles (MFA) did not occur after the accident for trees aged 16 and 30 at the time of the study. For the 16 y.o. tree, reversal of orientation was observed. • Cell length of the tracheid became longer rather after the ChNPP explosion instead of becoming shorter which normally occurs as a reaction to stress. • The S_2 and S_3 layers of tracheid walls changed after the accident.
5	• **Experiment** 1: ~30% increase of global hypermethylation levels in plants grown from seeds of unexposed trees planted in burial sites. Levels of hypermethylation in the DNA of plants grown from seeds of trees which died in the Red Forest were retained. • **Experiment 2:** Methylation was found to be dependent on the absorbed dose as parental trees were more hypermethylated than their progenies.
6	• Dwarf needles had significantly reduced length and weight when compared to normal needles at $P<0.001$ (Table 6.3). • No significant difference of radioactive content in normal and dwarf needles • Difference in mitotic abnormalities frequency were highly significant at $P<0.001$ with dwarf needles at 36% compared to 23% frequency in normal dresses • Five genes expressions changed statistically significant at $P<0.01$between normal and dwarfed needles.
7	• UDS occurred in the first 3h after germination of the pollen. • The ability to carry out UDS and repair γ radiation damage improved annually until it nearly matched the control abilities.

	• Pollen exposed to both α- and γ/β less able to achieve full genetic repair capability than pollen exposed only to γ/β after additional 250 Gy experimental irradiation.
8	• Genetic differentiation between P2, P3 and P4 populations were low • Comparison of loci detected no significant comparisons between P2 and P3. • Fifteen different loci showed higher differentiation at 99% confidence level, 8 of them above the 99% confidence level indicating candidates for outliers potentially under selection.
9	• One microsatellite mutation was found in P1, three were discovered in P2/P3 and none in control. Difference in microsatellite mutation events for all populations including control was statistically insignificant. • 12 AFLP loci mutation events were found in P4, 34 in P2 and 32 in P3. Difference between P2 and P3 was not statistically significant but P2/P3 compared with control P4 group was significant with 3.8 and 3.5 times higher.
10	• Deme P1 demonstrated higher diversity of Cat nucleotide in synonymous, non-synonymous sites and non-coding positions when compared to P5. • Six polymorphic sites in deme 1 was revealed to be monomorphic in deme P5 while one monomorphic site in deme 1 was polymorphic in deme 5. • Higher GPx nucleotide diversity for all populations was present in coding regions than non-coding ones. • One polymorphic site in deme 1 was revealed to be monomorphic in deme P5 while three monomorphic sites in deme 1 were polymorphic in deme P5 while • No clear correlation between absorbed dose and individual mutation rate was established for Cat for GPx. • Up-regulation of Cat expression in P1 and P2 was 9-fold and 11-fold when compared to their respective controls while GPx was down-regulated in both cases by 0.3 factor.
11	• Variation in growth was significant among years with variance significantly different among years; Variance during 1986-2008 significantly greater than 1914 to 1985. • Growth was severely depressed from 1987-1989 with mean annual values of -0.10 • 13% of the variance in standardized tree growth is caused by background radiation while 23% of the variance in standardized tree growth is accounted by the level of radioactivity in wood. • Weather and temperature affected growth rate which improved when it was warm or rainy or both. Effects of temperature and drought mediated the consequences of radiation exposure.
Data extrapolated from the studies enumerated in Table 2.1	

There are numerous impacts of the ChNPP Lenin V.I. reactor incident on biological material but the most fundamental ones concerned population, cellular and subcellular, genetic damage, transfer of genetic damages to the next generation and the transfer of radioactive materials from exposed organisms to non-exposed organisms.

Forests trees are just one component of the environment. Table 8.1 summarized significant findings of the studies included in this study.

The ChNPP aftermath simulated quasi-laboratory conditions in natural settings. Consequences which used to be studied solely under laboratory supervision manifested and were investigated in all sorts of biological materials including Pinus sylvestris. The damaging nature of radiation was further confirmed as trees in plots near the reactor exhibited deviant morphology and physiology than those farther away. The response from forest trees, however, was unpredicted as forest species rebounded from damages and employed defense mechanisms to protect genetic material and avoid excessive rearrangement of the DNA. This is not to say that the events in Chernobyl would not have long-lasting consequences. After all, the studies reviewed and assessed in this thesis focused on just one component of the environment.

The first level of assessment was population impacts. It was assessed through reduction of population size, reduction in longevity, rate of fertility and extinction. The first study systematically observed and categorized radiation impacts on Scots pine trees. In the Red forest zone, the death of the coniferous trees meant that other trees like deciduous ones had space to flourish. In other zones, the severe damage meant that insects which are considered harmless under normal circumstances can cause death. The death and recovery of the pine trees have consequences on the population of other species.

On the other, the study by Moller investigated the presence of symmetrical fluctuation as indications of developmental instability. This was classified under population impacts because asymmetric profiles allegedly affects the fitness of plants thereby resulting into reduced fertility rates and population size in the long run. Selection has been known to act against developmental notable phenotypes. This is contentious as asymmetry had been known to exist in other species which flourished.

The various morphological and physiological malformations in both types of trees have consequences in the trees themselves and on other species which are either dependent or competing with them for resources. Mortality in deciduous trees had not been noted but fluctuations in symmetry are indicative of disturbance in underlying development processes. These malformations can have repercussions in the population in the long term if it affects the pollination process or the longevity of the plants.

Cellular and subcellular impacts affected the survival of the organisms. The studies classified under this section used tree rings in analysing the effect of the external environment and the specimens under study. SN11 utilized growth which are very good indications of tree health. The severe depression of growth, especially in locations where competition for resources may be tight, is indicative of disturbances in plant processes affecting the health of an organism. For example, small trees, when surrounded by taller trees, have less capacity to catch sunlight. The authors accounted that added stress of a mutagenic stimuli exacerbates undesirable weather and temperature effects resulting into weak trees. It is uncertain whether this assertion by the authors is but the results of SN3 and SN4 gave more clarity into processes that occur in the tree rings of pines in the exclusion zone.

Cambial events result into secondary growth of trees where the trunk and the branches become wider. These events were classified into anticlinal divisions, intrusive growth of fusiform cells and cell elimination. The results of this study regarding the increase in frequency of anticlinal division was interesting given that SN11 reported that the years after the ChNPP accident marked by severe growth depression coincided with the years when anticlinal divisions were reported to be highest. These years should have shown strong growth patterns as anticlinal divisions results into secondary growth. It is conjectured that anticlinal division is offset by the increase in the frequency of cell elimination. Cell elimination is a normal process in the cambium even under ordinary circumstances. Under CEZ conditions, it is conjectured to eliminate mutated or damaged cells resulting into a seemingly depressed growth. The excessive elimination, in turn, encourages intrusive growth resulting into lengthened tracheid cells.

Intrusive growth is the active advancement of cell tips. Spaces created around cells due to the increased rate of cell elimination gave room for the cells to grow. Shorter cells, according to research, were more probable candidates for elimination resulting into the prominence of longer cells (Hejnowicz citing various authors, 1961).

Longer cells have higher chances of surviving the process of cell elimination but the unusual length of the tracheids in the specimens collected also had disadvantages. The unusual orientation of the MFA was expected to have a negative impact on the survival of the organism. It is, however, prudent to consider that the tracheids are part of the water transport system of the trees. Delayed reaction, enzyme appropriation, or a new environmental stimuli may account for it although the latter reason is given more credence. In the examination of the radionuclide cycle, the disintegration of the fuel particles which often have alpha particles was estimated to have occurred 1-2 years after the incident. The failure of the MFA to steepen could be a result of enzymes needed for wood formation being utilised in their other capacities as defense against pathogens but it also had the added benefit of sieving damaging radionuclides from the soil to prevent it from reaching more sensitive plant parts such as undifferentiated cells or the buds.

Genetic impacts are significant issues of concern encompassing a wide-range of subjects. Gene or point mutations are changes that take place within a single gene and maps to one chromosomal locus. The two common causes of point mutational changes are substitutions, and indels (insertions and deletion). Substitution ensues when wrong base matches happen. Typical base matches pairs adenine with thymine and cytosine with guanine. When adenine matches up with cytosine or guanine with thymine, substitution happens. Ionising radiation can cause the substitution of nucleotide pairs, conventionally called base pairs, of which several outcomes can ensue as direct consequence of genetic code degeneracy and codon translation-termination existence (Griffiths et al, 2000). Silent substitutions where one codon for an amino acid is changed into another codon for that same amino acid, missence mutation where the codon for one amino acid is exchange by a codon for another amino acid, and nonsense mutation where the amino acid codon is replaced by a translation-termination codon leading to premature cessation of the translation (Lodish et al, 2000; Griffiths et al, 2000). Silent substitutions are nonthreatening since they never alter the amino acid while the last two mentioned varies in severity depending on a case-to-case basis (Griffiths et al., 2000). Missence mutations can either be synonymous substitution where the protein's structure and function are less affected and non-synonymous substitutions which could have severe repercussions in the process (Griffiths et al., 2000).

In the course of assessing and reviewing selected studies, the result of study on mutations in coding sections as a means of selection response were statistically insignificant as a defense mechanism while the results of studies in gene regulation were more promising. Selection response is the differentiation of adaptive genes with main functions and close associated genomic regions as a feedback to environmental stimuli. The SN8 experimented on needles to examine genetic differentiation as an explanation for the difference between trees which were heavily damaged against those which exhibited minimal damage did not yield significant statistical data. Selection through genetic differentiation would require rearrangement of the genome. Point mutations are possible but more often than not, mutations manifested physiologically and morphologically are accumulated effects of small mutations. Since the goal of the organism is to preserve DNA integrity, defense mechanisms that do not alter coding sections of the genetic data are preferred. Further study of microsatellite mutations and AFLP was undertaken in SN9. SN9 is a follow up to SN8 which used specimens from the same group of trees. SSRs of one to six-base nucleotides were used for the microsatellites markers while AFLP detected deviation from the absence or presence of polymorphisms. Polymorph is the association of several phenotypic forms with alleles of one gene or homologs of one chromosome occurring in a population. The change of SSR at loci involved in adaptation would be preempted by selection. However, the statistically significant data failed to materialize when all populations were compared. The AFPL comparison between controls and exposed trees yielded statistically higher percentage of polymorphism but the P2 and P3 groups did not differentiate making it unlikely that these deviation were the reason for the difference in their appearance.

The up- or down regulation of genes is a viable option in revving up the defense mechanisms. Without excessive environmental stresses, the expressed genes have their own function to attend to. However, the presence of radionuclides in the environment requires that these enzymes function in other capacity such as protecting cell from oxidative damage.

In the case of mutation reported in SN10 when Isoleucin amino acid was changed to Leucin, the mutation happened in a coding section but it did not result in a debilitation because the resulting amino acid was isomeric as well therefore the catalytic function which used to be the role of Isoleucin was unaffected (Vornam et al., 2011).

The change of Isoleucin to Leucin production, however, was observed in only one specimen making it a single incident of mutation rather than a population response. The arbitrary difference between a mutation and polymorphism in terms of frequency of occurrence in the population is >1%. (Twyman, 2003). The least common allele must appear in at least 1% of the population to be regarded as polymorphism instead of a mutation. A mutation affects a number of individuals while polymorphism have population and biodiversity impacts involving a part or the whole ecosystem. This is evident in the Red Forest zone when the demise of the Pine Scots trees was replaced by the growth of other trees.

Foliage of trees in the Red Forest zone gradually turned red months after the ChNPP accident. Change in the color of leaves is typical in the aftermath of exposure to irradiation. Chlorophyll mutations were the first genotypic changes recorded. These had been categorised as albino, viridis, chlorina, xantha, alboxantha, alboviridis, tigrina, striata, maculate, zonata which manifest different colors and markings on leaves depending on the mutation (Lundquist citing Gustaffson, 1992). Of these variations, only viridis is not lethal. Xantha mutations, characterised by predomination of carotenoids turning leaves into light yellow or orange, seemed to have occurred in the trees which died. Predominance of carotenoids discontinues production of chlorophyll which is essential for manufacture of photosynthase. Trees from the plots which did not absorbed the lethal dose threshold appeared to have been recovering gradually on the surface but the extent of genetic modifications needed further assessment.

It is likely that chlorophyll mutations are a form of frameshift mutations resulting from indels which refers to insertions and deletions. The insertion is the addition of an extra base while deletion is the removal of an extra base. The process of protein synthesis is altered because it reads a unit of genetic code in the DNA or RNA molecule in codons, which are groups of three adjacent nucleotides. The frameshift changes the total number of bases increasing the tendency to mess up how the bases are interpreted after the alteration. The ensuing frameshift might mean that the new group of three bases will code for a different product which could carry consequences on polypeptide sequence that extend beyond the site of mutation itself by disturbing the downstream translation of the entire amino acid from the mutant site that the product bears no relation to the original sequence. Complete loss of normal protein structure and function is, therefore, exhibited by frameshift mutations (Griffiths et al., 2000).

Mutation also threatens the stability of the genome which fundamental to survival of plant and animal species (Hinton, 2014). When excessive radiation is absorbed which corresponds to extreme damages, sterility ensues. Induce mutation studies revealed that increased mutation rate is manifested in sterile progenies (X_1) of plants which had been exposed to radiation as seeds (X). Fertile progenies (X_1) maintain a constant rate of mutation (Lundquist, 1992).

Mutations can occur in coding or non-coding sections of the genes. Coding sections correspond functionally-active regions of the DNA while non-coding sequences do not code for polypeptides but may contain crucial functional information. Ramifications of mutations at this sites are less obvious than the coding ones. Generally, the outcome depends on the location and whether it disrupts a functional site potentially changing the patterns of gene expression in terms of amount or time of release as response to certain environmental cues or in certain tissues (Griffiths et al., 2000).

Nybom et al. (2010) noted that exposure to the highest doses below the threshold of lethality have different repercussions for sterility which is bound to directly affect fertility rates of individual specimens and in the long run, population size. Complete sterility results from irradiation during early embryo formation. In the aftermath of the ChNPP accident, some trees did not produce seeds for several years. Further effects on the reproductive processes of the affected trees are manifested by small or absence of male flowers, low number of seeds in cones, increase of unfilled seeds and decrease in seed weight (Kal’chenko and Fedotov, 2001). Dosage and physiological state of the cytoplasm were determined to factors that affect sterility. The high percentage of sterility in trees can be inferred to have suffered from meiotic irregularities (Lundqvist, 1992). In crops which live for a short period, sterility meant that the lineage is kept alive through heterozygotes. Sterility in trees which survived acute exposure even with far-reaching damages seemed to ensure that highly unstable genome are not passed off to the next generation.

In Scots pines trees, passing off hypermethylation patterns to the next generation is a measure of protection. Hypermethylation, as a defense mechanism, however, is not without consequences. It is an epigenetic mechanism triggered by acute and chronic radiation exposures which tends to protect not only the existing organism but the progenies as well through inheritance. Epigenetics covers molecular events that occur on the DNA but not within the DNA sequence (Chial, 2014). Mechanisms responsible for these changes may last through multiple or infinite generations of cell divisions and have the potential to be inherited by future generations (Tabatabaei, 2009; Khin et al., 2011; Chial, 2014).

The absence of corresponding change in the underlying DNA sequence due to chemical modifications that do not change the identity of the base pairs but access to these sequences have lead researchers to regard these alterations as relatively more stable gene expression adjustments. This is not to say, however, that these changes do not have adverse effects. Several diseases in mammalian organisms have been traced to have epigenetic roots. In plants, the effects may not be as harmful as these but the boundaries of differences and variations in mechanisms have yet to be completely understood. Stable gene expression alterations are reported to be more prominent during development and cell proliferation and are, therefore, associated with early life development but random change or environmental influence can give rise to epigenetic processes in mature humans and mice (Jaenisch and Bird, 2003). Differences in epigenetic controls are reported to vary in between taxonomical kingdoms (Gratchev, 2006) and had been given consideration during the assessment of the studies.

Methylation of the DNA is used by cells to lock genes in the “off’” position that had been discovered as an important component in numerous cellular processes and had been determined to play a key roles in both development and diseases (Chial, 2014) including genomic imprinting, X-chromosome inactivation, embryonic development and preservation of chromosome stability (Phillips, 2008). One of the prerequisites of transcriptionally active regions is the absence of DNA methylation.

The DNA methylation processes occur at CpG sites when a methyl group (–CH_3) is inserted on the 5-carbon ring whereby it changes cytosine with the effect of changing gene expression (McGarty, 2013; Chial, 2014). It must be noted that CpG islands are unmethylated. The process of conversion utilises DNA methyltransferases (DNMTs) enzymes which converts a basic cytosine with a single oxygen and two NH group at the opposite poles to 5-methylacytosine through the replacement of carbon by a methyl group (Mcgarty, 2013; Phillips, 2008). CpG sites are sites where C is 5′ in position to G connected by a phosphate group (Simmons, 2008). Since the cytosine is adjacent to guanine and guanine bases are normally paired with cytosines, the process results into two diagonally methylated cytosine residues on opposing DNA strands (Phillips, 2008). The insertion is an enzymatic process that regulates which of the genes are actively transcribed through modification of molecule shape (Chial, 2014). In plants however, it had been discovered that methylation is not restricted to CCG sequences but also in CNG and CNN sequences where N stands for any of the four nucleotide bases (Gehring and Henikoff, 2007). Plantae are the most highly methylated eukaryotes, with up to 50% of their cytosine residues exhibiting methylation (Phillips, 2008).

The main function of DNA methylation revolves around silencing transcription of transposable elements and thus protects host genomes from deleterious effects (Gehring and Henikoff, 2007). Jaenisch and Bird (2003) adds that DNA methylation is just one but a major contributor to a wider system responsible for stable maintenance of gene expression patterns during mitotic cell division and establishment of silent chromatic state through the interaction of proteins that modify nucleosomes. Gratchev (2006) differentiates prokaryotic systems which utilise DNA methylation as a form of cell protection and a measure of control for replication fidelity. DNA methylation as a form of cell protection from foreign DNA introduction by recognising methylation signatures. Unrecognized methylation signatures are then eliminated by cleavage. Replication fidelity is conserved when newly synthesised strands after DNA replication are analysed by the mismatch repair system before being methylated. Correction in the event of a mutation then takes place on the nonmethylated strand. In higher organisms or eukaryotes, the role as protection had been found to be similar where the machinery for recognition and/or eliminating of foreign DNA seemed to have been conserved. He also observed that due to the higher complexity of eukaryotic genome, it is possible that some other role or function had not been defined yet. The establishment and maintenance of DNA methylation patterns require three DNMTs plus additional DNMT enzymes with specialised functions (Jaenisch and Bird, 2003). Maintenance of established patterns is done by DNMT1 which means copying methylation from an existing DNA strand to its new partner after replication while DNMT3a and DNMT3b mediates the formation of new or de novo patterns which consists of putting initial methyl group patterns in place on a DNA sequence (Phillips, 2008).

Hypermethylation, however, is not without consequences. It protects the DNA but it also has the capacity to silence genes which can be manifested through cellular and morphological changes. In properties like symmetry which can affect pollination processes, it might not matter whether an alteration is caused by a mutation or hypermethylation. The physiological change can still alter the relationship between the population of trees under study and other organisms.

In all these, the energy expenditure of the plants had not been considered as contributing factor to the size malformation of plant organs like leaves. While it is true that the chromosome aberration is a predicting factor in development of abnormal leaf size (Zelena et al., 2005), it may be that demand for adenosine triphosphate (ATP) or energy expenditure reinforces the presence of these aberrations. Half of all photosynthase or carbohydrates, as the sugar and starch products of photosynthesis, are used for respiration like maintenance of trees living parts which includes taking up water and nutrients to produce chemicals to deter herbivores, adapt to changing temperature and water availability and execute cell repairs (Franklin and Mercker, n.d.). Stresses brought about by insects, diseases, poor weather or an unfamiliar environment uses up a greater proportion of that photosynthase. The plants needed more than the usual amount of sustenance and energy in executing housekeeping and maintenance in the presence of environmental stresses which compete for energy in the production of new plant organs into the upkeep of the old existing cells reinforcing (but not causing) the malformation of budding organs. The existence of a plant mechanism directing energy expense in stressful environment remains to be an avenue for investigation.

9. The ChNPP Explosion and the Conclusions

This thesis was undertaken to partly answer the question of resettlement and redevelopment of areas outside the CEZ but near enough to the ChNPP to have been affected heavily by the fallout. In the 20^{th} year of the ChNPP explosion, the assertion that poverty and economic distress caused more harm than the actual fallout battled concerns for both the known and unknown consequences of extremely high radiation to biodiversity, migration of mutation from affected to non-affected species and increased rates of genetic damage causing accumulation of mutation from generation to generation. As the 30^{th} year of the accident passed, the same issues resurfaced as news of the emergence of natural animal sanctuary circulated (Baker and Chesser, 2000). This review and assessment highlighted forest trees as a component of the environment with a major role in these concerns.

Based on the different studies assessed and reviewed listed in Table 2.1, related studies and other published materials examined, the forest trees species slowly recovered from irradiation. No hardwoods species were recorded to be extinct from the ChNPP accident. For coniferous species, several literatures mentioned that Picea abies became extinct in the vicinity of ChNPP (Sokolov, 1992) but no studies focusing on the said specie had been readily accessible during the conduct of the review and assessment. It is unknown whether there was any endemic specie of forest trees at the time of the incident.

The population size of coniferous trees were affected depending on the amount of radiation received in the immediate aftermath of the accident but most areas had been reported to recover except the Pine scots located in the Red Forest zone. Absorbed acute doses of 60 Gy had been verified to reduce lifespan of pine trees in this zone. New ecosystem dynamics consisting of hard wood forest species and grasses emerged in the Red Forest zone.

Fertility had been affected to the extent that trees in sublethal zones had stopped producing seeds for several years but unlike in crops where sterility is permanent, gradual recovery of trees through the years had been observed.

No studies had been found to focus solely on reduced lifespan of deciduous trees although developmental instability, which is thought to affect growth and fitness, had been proven for black locusts and rowan trees. Growth and fitness are essential for the survival and fertility of trees. Both were hardwood species.

Cellular and subcellular studies concentrated mainly on tree rings. Increase in the frequency of cambial events, longer tracheid walls, and unusual MFA orientation had been recorded. The steepening of MFA is related to the resistance of the tree to lateral wind forces, gravitational, and bending at a critical angle. These effects were observed to peak two years after the accident. It is posited that the unusual orientation of the MFAs occurred as a result of enzyme appropriation to its role in pathogenic metabolism but it also prevents damaging radionuclides from the soil and disintegrating fuel particles to reach more sensitive parts of the plants like undifferentiated cells and buds.

Genetic damage and repair mechanisms continued to be an interesting focus of experiments and investigations. Results from the studies suggested that the adaptation mechanisms of the trees that survived strong exposure had been strengthened even if malformations were still observed. Some of these means discussed in the preceding section were selective response, alteration of gene expression, NER and hypermethylation. SN9 observed that the DNA of 50-year-old Scots pine trees exposed to high acute radiation below the lethal threshold which gradually tempered into chronic one were found to have less AFLP and microsatellite mutations than 20 year old trees which had been planted in the aftermath of the accident. These suggested that mechanisms aimed preserve the integrity of the DNA are preferred repair systems and mending of genetic damage due radiation for long–lived species is probable.

The studies on intergenerational radiation impact was also promising in terms of limiting the damage being passed on to future progenies. Improved efficiency of the dark repair mechanism NER was concluded from the study in the pollen of Betulla verrucosa except those which had been exposed to alpha, beta and gamma particles. Hypermethylation was established in the DNA of exposed trees Pinus sylvestris trees which not only protected the integrity of the DNA structure of the parent the organisms but of future generations as well through inheritance. No studies were readily accessible concerning the migration of mutation of species from affected to non-effected ones.

Further studies and examination of biodiversity dynamics is recommended before resettlement and redevelopment can be entertained despite the seeming and obvious recovery of the forest trees. Survival and adaptation of the majority of forest trees exposed to the worst of extremely high radiation to a nuclear power plant explosion had been concluded from the studies but the secondary degree of impact in the biodiversity and ecosystem needed more research. Hypermethylation, for instance, protects the DNA but it also silences the expression of some genes. It is unknown whether these genes code for anything that would have an impact on phenotypic characteristics.

Observed damages lessened with increasing distance from the V.I. Lenin reactor but further investigations specific to the area being considered for resettlement or redevelopment is advised due to differing circumstances and conditions in terms of the levels of contamination and extent of biological damage due to the difference in radiation levels caused by dry and wet deposition. Forest trees are also just one aspect of the whole dynamics. There still remains the question of the migration of mutation from affected to non-effected species in fauna. It is also expected that the mechanisms involved in maintaining the integrity of faunal DNA structure is vastly different from those in plants. Therefore, the review and assessments of reports concerning components of the areas in the vicinity of the ChNPP is advised.

10. The ChNPP Explosion, the Gaps and Potential Investigative Approaches

The process of review and assessment revealed some gaps in the body of knowledge that can be used as directions for further studies.

1. Set parameters for assessment of primary and secondary impacts.

The challenging nature of radiation impacts demands that parameters be set in evaluating its primary and secondary impacts. Primary impacts are those that affect specie under examination and investigation. Secondary impacts are the effects of the primary impacts on other species that have an ecological relationship with the specie under under scrutiny.

2. Inventory of all flora and fauna around existing and future NPPs.

While architectural designs, structures, construction methods and materials of future NPPs are continuously improving and preemptive actions are taken to prevent incidents with the same magnitude as the ChNPP, a detailed list of all flora and fauna should be compiled to understand the ecosystem surrounding any nuclear power plant. The catalogue should include and indicate which species are endemic.

3. Differentiation of repair mechanisms utilized by plant organisms with different radioresistance.

Another potential avenue of investigation is the differentiation of repair mechanisms employed by radiosensitive and radioresistant species. There are several mechanisms utilised by plants upon exposure to radiation. In one of the studies evaluated, it had been determined that meristematic cells of Betula verrucosa employed NER while another study, outside the limits of this thesis, experimented on Arabidopsis thaliana and Nicotiana tabacum exposed to internal chronic radiation and found that these plants exhibited higher frequency of homologous recombination (Kovalchuk et al, 2000). If the ability of the cell to repair structural damage to DNA contributes to resistance or sensitivity to irradiation, it would be noteworthy to differentiate the mechanisms are being exercised by plants with various level of sensitivity.

4. Increasing the lethal threshold of radiosensitive plant species through prior irradiation

Some of the studies assessed and reviewed hinted that the integrity of the DNA structure is strengthened and the efficiency of the repair mechanisms of plants is improved although no studies had been found to actually test this assertion. It would be interesting to find out if prior irradiation can increase the lethal threshold dosage of radiosensitive plant species.

5. Investigation of energy expenditure of the plants as a factor in defense mechanism employment.

The production of different enzymes for stress response and developmental processes is supposed to consume more energy than normal production. It is possible that excessive production of some enzymes results in the underproduction of other enzymes.

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DEFINITION OF TERMS

Adaptation – a complex process by which populations of organisms respond to long-term environmental stresses by permanent genetic change (Kovalchuk et al., 2003)

AP sites – apurinic or apyrimidinic site resulting from the loss of a purine or pyrimidine residue from the DNA (Griffiths et al., 2000)

DNA Polymerase – an enzyme that can synthesize new DNA strands from a DNA template; several such enzymes exist (Griffiths et al., 2000)

Epigenetic – the study of how cells express genes through cellular and physiological trait variations which results from external and environmental factors that activate or deactivate genes. Research aims to describe potential transcription alterations in the cell (Wikipedia, 2016)

Homologous – having the same alleles or genes in the same order of arrangement; being one of a pair of chromosomes, one from the female parent and one from the male parent, that have genes for the same traits in the same positions. Genes on homologous chromosomes may not have the same form (Dictionary.com, n.d.)

Ligation – the joining of two DNA strands or other molecules by a phosphate ester linkage

Morphological mutations – changes in the outward appearance of individuals which can often be explained as due to mutation in the biochemical pathway (McClean, 2009)

Phosphodiester bond – a bond between a sugar group and a phosphate group; such bonds form the sugar-phosphate backbone of DNA. (Griffiths et al., 2000)

Phenotype – ensemble of observable characteristics displayed by an organism resulting from the interaction of its genotype with the environment (Wikipedia, 2016)

Phenodeviant – an individual whose phenotype differs significantly from that of the typical phenotype in the population (TheFreeDictionary.com, 2016).

Polymorphism – the occurrence in a population (or among populations) of several phenotypic forms associated with alleles of one gene or homologs of one chromosome (Griffiths et al., 2000)

Proteomes – the complete set of proteins produced by the genome at any one time. Transcription of genes is the first stage of gene expression and is followed by the translation of messenger RNA to produce proteins

Mutation Types

Biochemical mutations – lesion in one specific step of enzymatic pathway which is often connected with morphological mutations. (McClean, 2009)

Conditional mutations – mutations that occur only when restrictive conditions, which are certain factors in the environment, exist. In the absence of these factors called permissive condition, the wild type phenotype is expressed (McClean, 2009)

Gain of function mutation – mutation that creates a new allele which encodes a new product with a new function. The new allele will be dominant when any heterozygote with the new allele is combined with the original wild type allele which leads to the expression (McClean, 2009)

Lethal mutations – mutations that affects the longevity of an organism leading to the death, which may take several months or years (McClean, 2009)

Loss-of-function mutation – occurrence of a mutation in a wild type allele which encodes a product necessary for a specific biological function. The mutation in the allele results in the lost of the product for which it also encodes. Null mutations happen when the function is entirely lost. Leaky mutations are when some of the function may remain but not at the level of wild type allele (McClean, 2009)

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