The Influence of Soil Fungi on the Sorption of Cesium and Strontium within Organic Layer of Soil

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Abstract

Soil contamination by nuclear accidents has led to a resurgence of interest in microbe—radionuclide interactions. Soil fungi accumulate radioactive elements from contaminated soil, and it has been hypothesized that this alters the availability of radionuclides to plants and alters their movement in particular areas. The main purpose of this research was to critically describe the role of soil fungi as a storage organism and the potential of soil fungi to retain the mobility of the radioactive elements Cs and Sr in the organic soil system. We conducted experiments employing the isolation and identification of saprotrophic soil fungi and interpreted the data to identify the microorganism communities found in forest soil. The results provide some evidence that the different fungal communities found in forest soils may be related to the tree species present: various tree species may lead to the development of distinct fungal communities during their decomposition. This may be due to differences in the nature of the litter itself as well as variation in the microbial communities found in forest floors under different tree species. In the present study, representative fungi were selected and assigned to three genera: *Fusarium*, *Trichoderma*, and *Aspergillus*.

To describe the toxicity of Cs and Sr to fungi, it was necessary to monitor the fungal responses to these elements using a growth kinetic which was fitted to the growth model. The current research mainly extended to the study of the toxicological effect of Cs and Sr on the aforementioned three genera of fungi. Recently, this field has attracted more attention, resulting in some evidence for the direct inhibitory effect of Cs and Sr on the three fungal genera. The study further indicated that Cs exerted significant direct inhibition on the fungi, with an EC_{50} of 80 mM—160 mM, whereas Sr exerted less significant direct inhibition on the fungi, with an EC_{50} of 171 mM—222 mM. However, the natural levels of Cs and Sr in soil were lower than EC_{50} values 600 to 650 times. Hence, all representative fungi being adapt or evolutionarily to survive and reproduction even in the environment extremely contaminated by Cs and Sr.

The fate of Cs in the environment is mostly influenced by the sorption process. In this study, the sorption characteristics were examined at different contact times to provide the kinetic data and fitted with two kinetic equations—the pseudo first-order and the pseudo second-order models—to represent the experimental data. These data obtained from rate kinetics were better described by the pseudo second-order model than by the pseudo first-order model for Cs in combined conditions for stable and radioactive isotopes. On the other hand, for the radioactive isotope Sr, the experimental data were better described by the pseudo first-order model than by the pseudo second-order model, as evidenced by the correlation coefficient values (r^2). Moreover, experiments to assess the potential of soil fungi for the biosorption of Cs and Sr were also conducted. The results showed that the monolayer sorption capacity for Cs ions was as follows: *Aspergillus* sp. >*Fusarium* sp. >*Trichoderma* sp. and *Fusarium* sp. >*Trichoderma* sp. > *Aspergillus* sp. However, when Sr ions were added to the system to provide the competitive condition, the sorption capacity was decreased. For stable isotopes, when the concentrations of both Cs and Sr elements were increased, the percentage of biosorption was decreased, as evidenced by the increase in the amount of each element until they reached a plateau. In contrast, for radioactive isotopes, the percentage of biosorption of either element was not changed from its initial activity. The effect of pH was demonstrated by a decrease in sorption capacity at a lower pH because H^+ also interacts with sites mediating the monovalent and divalent cation transport system.

Finally, the full potential of fungi to cycle Cs and Sr in the organic soil system was assessed in a series of experiments. Organic material was produced under laboratory conditions from leaf litter to minimize the interference from competition by clay minerals. The results conclusively showed that the living components of soil systems are of primary importance in the uptake of Cs and Sr in organic material. The single strains of *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp. showed increased amounts of Cs and Sr in a fixed form compared with those found in a biotic system. This finding may in part account for the high level of retention of Cs and Sr in upland organic soil, which is not accounted for satisfactorily by the physicochemical process alone. It may also account in part for the strong, irreversible binding observed in biotic systems.

Key words: cesium, organic soil, soil fungi, strontium

Abbreviation

A	The maximal value reached
μ_m	The maximum specific growth rate
λ	The lag time
B_f	Bias factor
P	Predicted value
O	Observed (O) values
DW	Durbin–Watson statistic
K_1	The rate constant for first order sorption
q_1	The amount of solute adsorbed at equilibrium
q_t	The amount of solute adsorbed on the surface of the fungi cell at time = t
K_2	The rate constant for second order sorption
q_e	The amount of Cs or Sr sorbed
C_e	The amount of Cs or Sr residue in the solution
q_m	The maximum amount of Cs or Sr sorbed
K_L	An equilibrium constant representing the affinity between the fungi cell and Cs and Sr solution for Langmuir isotherms
K_f	A constant that is related to sorption capacity for Freundlich isotherms
n	An empirical parameter that varies with the degree of heterogeneity
A_i	An initial activities in the solution
A_f	A final activities in the solution
M	The mass of fungal cells

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Chapter 1

Introduction

1.1 Background

Radioactive elements are the elements consist of a non-stable nucleus. These elements give off energy waves called radiation by spontaneous disintegration of the nucleus accompanied by the radiation of alpha particles, beta particles, or gamma rays, and are subsequently converted to stable elements. An elements with atomic numbers greater than 83 are radioactive. Radioactive elements have been responsible for both positive and negative events in human history. Radiation is become a concern when these particles and the associated energy come in contact with molecules inside the human body, because their interaction can result in cell damage or death. The occurrence of radioactive elements in the environment may be classified into three main categories:

1) Naturally occurring radioactive elements in the Earth's crust and atmosphere; 2) environmental radiation as a result of anthropogenic activity such as the development of nuclear weaponry involving the creation of nuclear or radioactive elements through scientific research; and 3) radiation caused by accidents at nuclear reactors or incidents involving nuclear weapons, for example Chernobyl disaster in the Ukraine in 1986 and Fukushima in Japan in 2011. Accidental radiation is a key concern because radiation from past accidents diffused into the atmosphere resulting in significant consequences to people and the environment. Cesium (Cs) and Strontium (Sr) are the dominant nuclides contaminating the environment. Based on their half-life, 2 years for ^{134}Cs, 30 years for ^{137}Cs and 29 years for ^{90}Sr, these elements show the highest presence in the soil. These elements are also characterized by high transferability and high fission yield.

The radioactive elements are deposited from the atmosphere into the soil by two major mechanisms, followed by wet deposition caused by rainfall and precipitation and dry deposition by the transfer of gases and particles. Soil is a major source of

radioactive isotopes entering the food chain. There is no general difference between radioactive and stable isotopes of a given chemical element with respect to their behavior in the environment. Soil characteristics such as the composition of the soil solution, the physical and chemical soil properties, and the soil microorganisms and fungi may play a role in the mobility of radioactive isotopes.

1.2 Topic Development

The increase in soil contamination by anthropogenic activity and nuclear accidents has caused a resurgence of interest in the role of soil mobility of radioactive isotopes. The microbe—radionuclide interactions play an important role in the mobility of radioactive isotopes. Soil fungi are the greatest living biomass in the decomposing organic layers of forest soil. To a large extent, they determine the fate and transport process of radioactive elements in the soil. The soil fungi interaction with radioactive elements has been documented extensively with various mechanisms such as bioremediation, biomineralization, bioaccumulation, and biosorption. Microbiological activity, particularly fungal activity, is likely to be responsible for the long—term retention of radioactive elements in the organic layer of the soil. It has been hypothesized that this may alter the availability of radioactive elements to plants and alter their movement in particular areas.

The accumulation of radioactive elements in fungi was found to be the most difficult to predict. The current research is not intended to provide a comprehensive mechanism of biological processes in soil systems, but to study the possibility and to critically describe the information on the role of the fungi as a storage organism, for which there are not data available.

This research provide the basic concept of inhabitant soil fungal for the proposed to eliminated the predation by the native microorganism that is the one factor for decrease in the cell number of soil fungi when it was applied to the site study. Moreover, it was fast to the adaptation to the surrounding environment. This research is focusing on the saprotrophic fungi that are the free-living fungi type. The previous studies are a majority to focus on the microbial retention of Cs and Sr on the mycorrhiza

that is a symbiotic association composed of a root plant. They tend to ignore the role of the free-living fungi types and as a result, the assessment of the interaction was underestimation. Lastly, the full potential of soil fungi interact with Cs and Sr was focused by comparison between two different systems to assess the biological activity in the organic material.

1.3 Purpose of the research

The main objective of this research was to describe the influence of the soil fungi as a storage organism and the potential of soil fungi to restrict the mobility of the radioactive elements Cs and Sr in the organic soil system, accomplished by following:

- Distinguish the representative saprotrophic soil fungi found in forest soil.
- Investigation of the effect of Cs and Sr in an extreamly concentration on growth kinetics and inhibitory effects on the representative soil fungi.
- Investigation of the soil fungi as a storage organism in terms of the rate of reaction and mechanisms of Cs and Sr.
- Assessment of the potential of soil fungi to enhance the retention of Cs and Sr in the organic soil system

1.4 Scope of the research

The experimental approaches were established to obtain data on regarding Cs and Sr accumulated in the soil fungi. In particular, the laboratory study involved:

- Isolation and identification of soil fungi
- Determination of growth kinetics and tolerance
- Determination of biosorption kinetics of Cs and Sr for both stable and radioactive isotopes
- Determination of biosorption isotherm of Cs and Sr for both stable and radioactive isotopes
- Determination of the contribution of fungi activity to the sorption of Cs and Sr in the organic soil system

1.5 Outline of this dissertation

This dissertation was written for the partial fulfillment of the requirements for the degree of Doctor of Philosophy. The dissertation consists of eight chapters:

Chapter 1: Introduction

This chapter describes the concept of this research. The research background, topic development and hypothesis are elucidated. In addition, the research objective and scope are provided to clearly understand the state of argument.

Chapter 2: Literature Review

The purpose of this chapter is to describe the current state of research in the defined area and consider whether there are any closely related areas. In addition, this chapter identifies the gaps in the literature and discusses what is required to attend to those particular research gaps. This chapter outlines the general information followed by the chemical characteristics of Cs and Sr, the fate and transport of both elements in the soil system, and the interaction between the elements and soil microorganisms.

Chapter 3: Soil fungi isolation and identification

This chapter elucidates experimental and data interpretation approaches for the isolation and identification of soil fungi. This chapter aims to determine the native soil fungi found in the forest soil and to specify the representative soil fungi used in this research. Highly sensitive and specific molecular techniques were applied, such as the sequence variability of the internal transcribed spacer (ITS) region of fungi, which is a potentially useful method for rapid and accurate diagnosis of fungal isolation.

Chapter 4: Growth kinetic and the torelance

In the previous chapter, the representative soil fungi are described. Soil fungi deal with a wide variety of potentially toxic environmental challenges. They are significantly affected when the ambiance of their environment changes. Therefore, they should be able to sense and respond to these changes survive. Beyond some limits, the

existence of fungi tends to decrease as a result of inhibition to growth caused by the occurrence of Cs and Sr in the environment. In this chapter, the growth kinetics of the soil fungi affected by the high concentration of two elements Cs and Sr are investigated using statistical evaluation of mathematical models to describe the fungal growth. The inhibitory effects of Cs and Sr on the growth of soil fungi are studied. This chapter aims to describe clearly how the growth kinetics of soil fungi are affected by various condition when Cs and Sr contamination occurs in an extreme concentration. In addition, the responses to Cs and Sr by different fungi are investigated.

Chapter 5: Biosorption kinetics of cesium and strontium

Previous investigators have studied various fungal species to define responsive signaling to the presence of Cs and Sr in the environment. The fate in the environment of both Cs and Sr are mostly influenced by the sorption process. The aims of this chapter are to apply a kinetic model of nonlinear regression for the main purpose of explaining the rate of the sorption process. The kinetic expressions are commonly used. In this chapter, both stable and radioactive isotopes of Cs and Sr are investigated, with experiments performed under various conditions

Chapter 6: Biosorption isotherm of cesium and strontium

According to the previous chapter, where the rate of the sorption process is examined, this chapter intends to clearly understand how a soil fungi accumulates Cs and Sr as both stable and radioactive isotopes. The sorption isotherm such as Langmuir and Freundlich isotherms are used to describe the sorption characteristics and to quantify the sorption capacity. Batch experiments described in this chapter were performed under various conditions.

Chapter 7: Application within organic soil system

The precise role of soil organic matter and microbiological processes in the bioavailability of radioactive elements needs to be more fully elucidated to obtain the parameters important for model approaches for predicting the fate and transport of elements in soils and soil—plant system. Majority of the previous chapters have

outlined the microbial retention of both stable and radioactive isotopes of Cs and Sr by measuring the amount of the elements stored in the fungi cell. This chapter intends to determine the contribution of microbial activity to the sorption of Cs and Sr in the organic material, which is necessary to compare the non-sterile systems with sterile systems. This chapter highlights the need to develop a new experimental approach to characterize the full potential of soil fungi to accumulate Cs and Sr in the soils.

Chapter 8: Conclusions

The final chapter summarizes the main finding of the dissertation and the contributions of this study. Moreover, a suggestion for future works is provided.

Chapter 2

Literature Review

Interest in the interactions between radioactive elements and fungi has considerably increased in recent years in the wake of the nuclear incident in Fukushima, Japan since 2011. The distribution of released radioactive elements, such as ^{137}Cs, ^{134}Cs, ^{89}Sr, and ^{90}Sr, is the most important nuclide for assessment of the long-term radiation which is related to the chemical properties of the stable isotope Cs^{+} and Sr^{2+} for the public. The purpose of this chapter is to describe current research within a defined area follow the general chemical characteristics, fate, and transport through the soil and interactions between the elements and soil and determine whether there are any closely related areas.

2.1 General chemical characteristics

2.1.1 Cesium (Cs)

Cs is an alkaline metal that exists in the solution almost exclusively as a monovalent cation (Greenwood & Earnshaw, 1984). It has the lowest boiling point, and lowest melting point, highest vapor pressure, highest density, and lowest ionization potential compared with other stable alkali metals (ATSDR, Toxicological profile for Cesium, 2004). These properties make Cs far more reactive than other members of the alkali metal group. Cs salts and most Cs compounds are highly water soluble. The physical and chemical properties of Cs and its compounds are shown in Table 2-1.

Cs has several isotopes in the natural. ^{133}Cs is the only one of stable isotope which occurs in the soil concentration ranging between 0.3 and 2.5 mg/kg (Lindsay, 1979). Radioactive isotopes have a wide range of half-life (Helmers, 1996). Given their high fission yield and relatively long half-life, the radioactive isotopes ^{137}Cs and ^{134}Cs are significant fission products. No essential biological role of Cs has been elucidated, although trace quantities have been detected in many organisms (Ghosh, Sharma, &

Talukder, 1993). Moreover, Cs in the soil is strongly influenced by the amount of K^+ in the soil solution that governs high mobility in a biological system.

Table 2-1 Physical and chemical properties of cesium and its compounds

Characteristic	Cesium; Cs (metal)	Cesium chloride	Cesium carbonate	Cesium hydroxide	Cesium oxide	Cesium nitrate
Melting Point; °C	29	646	610	272	490	414
Boiling point; °C	685	1290	No Data			
Density (g/cm3) (20 °C)	1.93	3.988	4.24	3.68	4.65	3.66
Solubility in water (kg/L) (20 °C)	Reacts violently with water	1.87	2.1	4 (15 °C)	Very soluble in water	

Source: (ATSDR, Toxicological profile for Cesium, 2004)

2.1.2 Strontium (Sr)

Sr is an element that commonly occurs in the environment and can exist in the Sr^{2+} oxidation state consist of four naturally occurring stable isotopes, ^{84}Sr (0.55%), ^{86}Sr (9.75%), ^{87}Sr (6.96%), and ^{88}Sr (82.74%), which all behave the same way chemically (ATSDR, Public Health Statement for Strontium, 2004). The ionic radius of Sr^{2+} is 1.12 Å, very close to that of Ca^{2+} at 0.99 Å. The chemical behavior of Sr^{2+} is also similar to that of $Ca^{2+.}$

Sr can also exist as radioactive isotopes and ^{90}Sr and ^{89}Sr were two of its isotopes that were released into the atmosphere by the Fukushima Daiichi incident (MEXT, 2011). The more hazardous of the two is ^{90}Sr, and it was a key environmental concern because it gives off beta particles and has a long half-life (28.1 years). Although ^{89}Sr also has a high yield, it has a short half-life (52 days) and does not persist in the environment and cause a disposal problem. In the case of the Chernobyl accident, most of the radioactive Sr was sorbed by the soil (Rigol, Roig, Vidal, & Rauret, 1999). Radioactive Sr is strongly fixed over a long period of time, similar to Cs.

2.2 Fate and transport though the soil

2.2.1 Cesium (Cs)

Although much of the radioactive Cs in the environment becomes dispersed into the atmosphere, a large amount of Cs remains in terrestrial ecosystems indefinitely after deposition. Cs is strongly fixed in the soil and shows a very low vertical mobility or plant availability when illite clay minerals were present. It has strong adsorption properties due to binding to specific sites, namely frayed edge sites (FES) (Cremers, Elsen, Preter, & Maes, 1988); (Nakao, Funakawa, & Kosako, 2009). These data led to the assumption that the fate of Cs in the soil affected bioavailability for plant uptake and also has a low rate of vertical migration (Rosen, Oborn, & Lonsjo, 1999).

Several studies showed how the behaviors of radioactive isotopes in the environment changed after contamination from a nuclear incident. The two main factors were the time elapsed since the initial contamination and drying-wetting cycles (Rigol, Roig, Vidal, & Rauret, 1999). However, studies around Chernobyl following the 1986 accident found that the mobility of radioactive Cs in the soil profile was slower than that expected. More than a decade after the fallout, radioactive Cs was still localized in organic soil layers. It was likely that it contributed substantially to the long-term retention of radioactive elements in the organic soil layer (Yoshida, Marumatsu, Dvornik, Zhuchenko, & Linkov, 2004); (Striner, Linkov, & Yoshida, 2002). In the natural soil, the soil horizon contains a highly organic layer (referred to as the O horizon) that is composed of decaying plant and animal tissue and microorganisms.

The role of this organic matter is more significant than the inorganic phase, and the organic components may affect the interaction of radioactive Cs. As a result, radioactive Cs may be persistently available for plant uptake. Previous studies showed that the reversibility of the radioactive Cs sorption process was greater in organic soils than that in mineral soils (Hird, Rimmer, & Livens, 1995). However, the interaction of radioactive Cs with organic acids may decrease the radioactive fraction directly involved in the equilibrium of the soil solid-liquid phase (Nisbet, Mocanu, & Shaw, 1994). This has been attributed to the high availability of radioactive Cs for plant uptake

in organic soils compared with mineral soils. It also boosts the efficient recycling between plants and soil in unproductive ecosystems.

2.2.2 Strontium (Sr)

Sr is one of the most important radioactive elements because it is characterized by a high ability to migrate. Chemically, Sr is more similar to Ca, which has a much higher mobility than Cs. The Sr sorption behavior in soil was largely influenced by the organic matter, and a large proportion of radioactive elements in the soil was bound to fulvic acids, one of the natural acidic organic polymers that can be extracted from soil humus (Sysoeva, Kanopleva, & Sanzharova, 2005). The Sr—humic complex formation occurred at a high pH and was induced by the sorption rate. The main mechanism of Sr adsorption by the solid soil phase is through ion exchange, which is poorly fixed by soil. Thus, the major factors that influence Sr sorption are the adsorption capacity, cation composition of the soil complex, soil organic matter, and exchangeable Ca^{2+} content (Heald, 1960); (Juo & Barber, 1970). Calcium ion competes with strontium ion for exchange sites. The transportation rate of Sr to the soil horizon is up to several centimeters per year due to the low fixation rate.

2.3 Interactions between the elements and soil microorganisms

Previous studies have assumed that microbial and fungal activity is likely to be responsible for the long-term retention of radioactive elements, such as Cs and Sr, in organic layers of the forest soil. (Reisinger, 1994) focused on the interactions between microbes and the mineral-radionuclide system, particularly soil fungi, which comprise the highest living biomass in the decomposing litter and were predominant in the upper part of the organic layer. These fungi accumulate radioactive elements through physicochemical and biological mechanisms, including extracellular binding by metabolites and biopolymers, binding to specific polypeptides, and metabolism-dependent accumulation. A variety of organic ligands at the microbial cell surface facilitate the association of cations with the cell through biosorption (Brookshaw, Patrick, Lloyd, & Vaughan, 2012). This interaction between the elements to the biomass through processes that do not involve metabolic energy or transport. Although such

processes may occur simultaneously if either living or dead biomass was used (Tobin, Cooper, & Neufeld, 1990). The soil microorganisms and fungi can affect the soil solution composition through a variety of mechanisms. For example, it can alter the soil pH through the production of organic material, which is known to influence the rate of transfer of radioactive elements from the solute to the solid phase. It can alter the soil structure through the formation of mineral aggregates; thus, affecting radioactive elements binding sites. The radioactive elements may also become attached to microbial cell walls or may be actively taken up from the soil solution (Tamponnet, et al., 2008). According to (Griffin, 1981) fungi take up nutrients from the soil in the aqueous phase. They use enzymes to break down macromolecular complexes for uptake. Once broken down, most substances are thought to move into the fungal hyphae, bound to specific carrier molecules.

Several studies investigated the difference in the ability of saprophytic and symbiotic fungi to accumulate radioactive Cs. Their results indicated a higher concentration of radioactive Cs in symbiotic species. In view of the pronounced vertical profile of radioactive Cs, especially during the first years following the Chernobyl accident, these observations can, at least in part be explained by the different vertical distributions of soil mycelium (Rommelt, Hiersche, Schaller, & Wirth, 1990); (Heinrich, 1993); (Yoshida & Marumatsu, Accumulation of radiocesium in basidiomycetes collected from Japanese forests, 1994). Research on radioactive Cs uptake by fungi and their ability to retain it within their mycelium was investigated in laboratory studies (Clint, Harrison, & Howard, 1991). The authors concluded that fungi are potential accumulators of radioactive Cs and they also suggested that most of Cs was biologically bound within the tissue. As a result, the fungi might have the capacity to hold all potentially labile Cs in the soil (Dighton, Clint, & Poskitt, 1991).

Understanding radioactive element accumulation by fungi is essential for the development of remediation technologies for contaminated areas. The biological process for radioactive elements in fungi was found to be the most difficult to predict. However, at present their are scarce to known about the mechanisms involved in the

uptake and retention of radioactive elements especially Cs and Sr by fungi, although the radioactive element concentration varies considerably by fungal species.

2.4 Conclusion

Due to the nuclear accident in Fukushima since 2011, Cs is the one of significantly volatile radionuclides that was deposited from the atmosphere and contaminated in the large areas of eastern and north-eastern of Japan. However, for Sr the contamination level was relatively low and did not exceed the standard range. In this study, both Cs and Sr were focused. Because base on their long half-life of the radioactive isotope, they can persistence in the environment. Moreover, they are potentially dangerous to the organism through bioaccumulation process. Soil is a major source of Cs and Sr entering the food chain. The characteristics of soil play a role in mobility of Cs and Sr. For example, the composition of the soil solution such as pH, inorganic ion, physical and chemical of soil properties such as temperature, clay content, and the present of microorganisms and the soil fungi including their interaction to Cs and Sr. In the natural soil contained with highly organic layer, hence when Cs and Sr from atmosphere was deposited. Instead of labile to the downward of soil, they are mostly localized in the organic layer which it was focused in this study. It may be explained by the high amount of microbial activity that is referred to soil bacteria, soil fungi including both a free-living and a symbiotic association composed of a fungus to the plant root such as mycorrhiza in the organic layer may play a role to Cs and Sr retention. Neither Cs nor Sr are the redox sensitive, so their fate in the environment is mostly influenced by the sorption mechanism. Especially Cs, it was likely substantially to the long-term retention in the organic layers. Likewise, Sr showed the behavior in soil was largely influenced by bound to the organic substrate such as fulvic acid. Therefore, this study was to assess the biological activity in the organic material by focusing on full potentially of native free-living soil fungi which the previous studies tend to ignore and as a result it may underestimate assessment of the retention. This study can be extended to apply for the high level of Cs and Sr contamination in the in-situ upland soil, which cannot be satisfactory accounted by physicochemical process or induce the potentiality for the biological process.

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Chapter 3

Soil Fungi Isolation and Identification

This chapter describes the experimental and data interpretation approaches employed in the isolation and identification of soil fungi. This chapter aims to distinguish the representative saprotrophic soil fungi found in the forest and to identify the microorganisms used in this study. Highly sensitive and specific molecular techniques were employed, such as sequence variability in the internal transcribed spacer (ITS) region of fungi, which is a potentially useful method for rapid and accurate identification of fungi. Finally, evidence is presented to demonstrate that tree species influence the composition of microbial number in decomposing litter, forest floor, soil, and rhizosphere.

3.1 Objective

- To distinguish the representative saprotrophic soil fungi found in the forest.
- To isolate soil fungi using dilution and plating techniques.
- To sequence DNA fragments and identify soil fungi using Basic Local Alignment Search Tool (BLAST) analysis.

3.2 Theory and significance

Microorganisms comprise fungi, bacteria, actinomycetes, protozoa, microfauna, and algae, and they are among the most important components with roles in the organic soil system. Fungi are heterotrophic eukaryotic organisms, and they are aerobic, with the exception of yeasts. They are abundant in surface soils and are important because of their roles in nutrient cycling and decomposition of organic matter and organic contaminants (Ste-Marie & Pare, 1999). Saprotrophic fungi are free-living in soil, and they are capable of decomposing dead organisms or organic residues.

Soil microorganisms are critical links between shifts in the composition of the dominant tree species and fundamental shifts in ecosystem functioning (Wardle, Bardgett, Klironomos, Setala, Putten, & Wall, 2004). The composition and functioning

of soil microorganism number can be influenced by tree species via changes in the production of litter, production of root exudates, and the microclimate such as shading, frost protection, through fall, and uptake or transpiration of soil water. It is also possible that plant and microorganism number are not linked directly, and that they may respond to different environmental factors; for example, soil microorganisms may be more sensitive to soil factors such as texture, moisture, and the organic matter content (Fierer & Jackson, 2006) Thus, these site-specific factors may overwhelm the influence of tree species on the structuring of microbial number. However, the numbers of organisms that are responsible for decomposition are known to decompose the litter faster when placed in their native environments. This may be explained by litter microbial number being better adapted physiologically or evolutionarily to the decomposition of the characteristic litter found in their ecosystem (Ayres, Dromph, & Bardgett, 2006); (Gholz, Wedin, Smitherman, Harmon, & Parton, 2000).

Soil contains many millions of fungi per gram soil. A dilution series of soil is prepared by suspending a given amount of soil in a dispersing solution and transferring aliquots of the suspension into fresh solution until the suspension is diluted sufficiently to allow individual discrete fungal colonies to be grown on agar plates. After inoculating several replicate agar plates, the plates are incubated at an appropriate temperature and counted after macroscopic fungal colonies have formed. In addition, in the pour plate techniques, the plate is gently swirled to distribute the agar and inoculums across the bottom of the plate, which is useful because some fungi can grow rapidly through agar, whereas bacteria cannot. Dilution and plating techniques are used most widely for the isolation and detection of fungi, but they are not suitable for specific identification, while they also lack sensitivity and require a long time for detection. At present, the polymerase chain reaction (PCR) is a molecular technique that is used commonly for isolation and detection (Sakai, et al., 1988); (Mullis & Faloona, 1987). PCR is a method for analyzing a short sequence of DNA or RNA by amplifying a selected subsequence of nucleotides. It is possible to delineate a specific region in a template sequence by adding a nucleotide sequence called a primer.

The entire ITS region can potentially be useful for the rapid and accurate identification of fungi because it evolves faster compared with other regions, while it may vary among species within genus (Turenne, Sanche, Hoden, karlowsky, & Kanabi, 1999); (White, Bruns, Lee, & Taylor, 1990). Figure 3-1 shows the locations of the primers used for the ITS region.

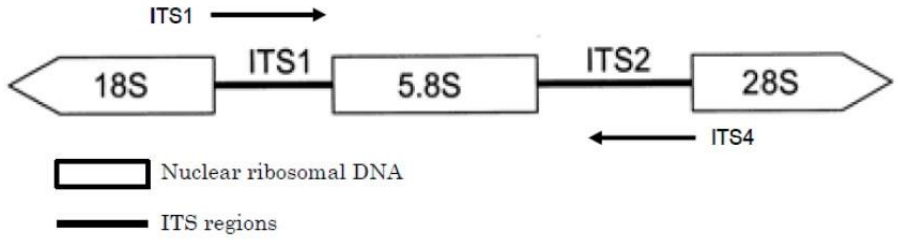


Figure 3-1 Schematic representation of fungal ribosomal genes and PCR primers.

Source: A figure has been adapted from (Turenne, Sanche, Hoden, karlowsky, & Kanabi, 1999)

After a sequence of amplified DNA or RNA has been determined, it can be used to identify fungi at the genus or species level by searching a computerized sequence database with BLAST via the National Center for Biotechnology Information (NCBI) website. Thus, a sequence from an unknown soil isolate can be compared to all known sequences in the database to determine which shares the closest relationship with the isolate.

3.3 Procedure

3.3.1 Materials

- 10 g fresh soil of each soil type
- Potato dextrose agar (PDA): 24 g of potato dextrose broth and 20 g agar in 1 liter of distilled water
- 95 ml sterile distilled water: 1 bottle per soil type
- 9 ml sterile distilled water: 3 tubes per soil type
- Sterile Petri dishes and sterile pipette tips

- Benchtop balance
- Incubator
- Needle

3.3.2 Method

1. Fresh soil samples were collected from two types of forest soil: coniferous forest where Japanese cedar (Figure 3-2; a) was the main tree species and deciduous forest where Japanese Oak (Figure 3-2; b) was the main tree species. The samples were collected in Takizawa Research Forest, Iwate University, Japan, ($39^{o}46'32N$, $141^{o}9'25E$ and $39^{o}46'40N$, $141^{o}8'10E$) during October 2012 (Figure 3-3). The surface soil was sampled at a depth of 0–2 cm because this layer contains the highest amount of microorganisms. The basic soil characteristics were determined previously under laboratory conditions. The data are shown in Table 3-1.

Table 3-1 Basic soil characteristics in the coniferous and deciduous forest types

Characteristic	Deciduous	Coniferous
pH	5.4 ± 0.1	5.8 ± 0.2
C/N ratio	16.83	15.16
Organic matter (%)	34.8 ± 8	31.4 ± 6.1
CEC (cmol/kg)	70.9 ± 11.2	84.0 ± 16.1
K (cmol/kg)	0.7 ± 0.2	0.4 ± 0.1
Mg (cmol/kg)	7.1 ± 0.7	4.5 ± 0.7
Ca (cmol/kg)	41.7 ± 2.5	40.9 ± 2.5
Sand (%)	26.2 ± 5.7	25.3 ± 6.0
Silt (%)	47.1 ± 11	51.3 ± 7.1
Clay (%)	26.6 ± 5.5	23.3 ± 2.7
Soil type	Loam	Silt loam

Source: (Kang, 2014)

Figure 3-2 The forest types: (a) coniferous forest and (b) deciduous forest

Figure 3-3 Locations of the soil sampling sites

Source: Google earth V7.1.5.1557. Morioka, Japan. http://www.earth.google.com [January 26, 2015]

2. Each of the moist soil samples (10 g) was added to 95 ml of sterile purified water and shaken well to disperse any microorganisms (Figure 3-3). In addition, 10 g of soil occupied approximately 5 ml, so 10 g of soil was suspended in a total volume of 100 ml, thereby yielding a 10^{-1} (w/v) dilution. Next, 1 ml of the suspension was removed from the bottle and added to a tube containing 9 ml of sterile purified water. A dilution series was prepared as described in Table 3-2

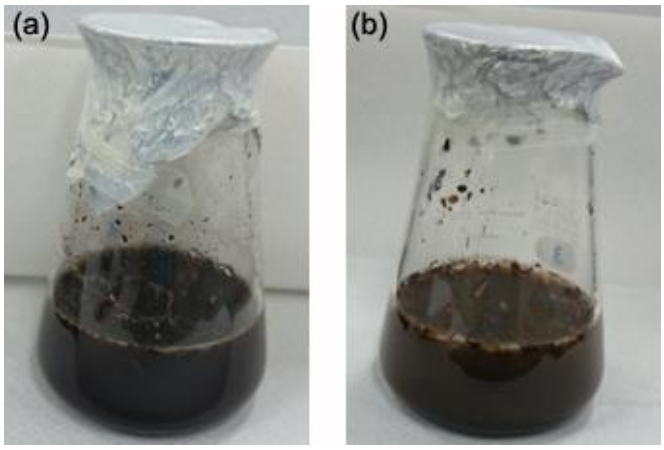


Figure 3–4 Soil samples from (a) coniferous forest and (b) deciduous forest, dispersed in sterile purified water

Table 3-2 Preparation of the soil dilution series

Solution	Process	Dilution
Solution A	10 g soil + 95 ml sterile purified water	10^{-1} (w/v)
Solution B	1 ml solution A + 9 ml sterile purified water	10^{-2} (v/v)
Solution C	1 ml solution B + 9 ml sterile purified water	10^{-3} (v/v)
Solution D	1 ml solution C + 9 ml sterile purified water	10^{-4} (v/v)
Solution E	1 ml solution D + 9 ml sterile purified water	10^{-5} (v/v)

3. The culture medium (PDA) was prepared by mixing 24 g potato dextrose broth powder, 20 g agar in 1 L distilled water for 30 min. The final pH was measured (pH 5.5 – 6.2).

4. The plates were incubated at a temperature of 25°C–30°C for 7 days and colony counts were determined for each soil dilution. The plates were counted only when they had discrete countable colonies.

5. Representative colonies with different morphologies were selected using the hyphal tip isolation technique to isolate single colonies of representative soil fungi. The representative colonies were then cultured on fresh PDA plates.

6. DNA extraction and PCR amplification were performed by Macrogen Inc., Korea The entire ITS regions of the fungal isolates were sequenced. ITS1 (5′—TCC GTA GGT GAA CCTTGC GG—3′) and ITS4 (5′—TCC TCC GCT TAT TGA TAT GC—3′) were used as the forward and reverse primers. Next, the sequences were compared with the NCBI database using BLAST, and a phylogenetic tree was created (Kumar, Tamura, & Nei, 2004).

3.4 Results and discussion

3.4.1 Comparison of microbial number in soil

Soil dilution series were prepared and used to inoculate agar plates. The plates were incubated and macroscopic fungi colonies were counted (Figure 3-5)

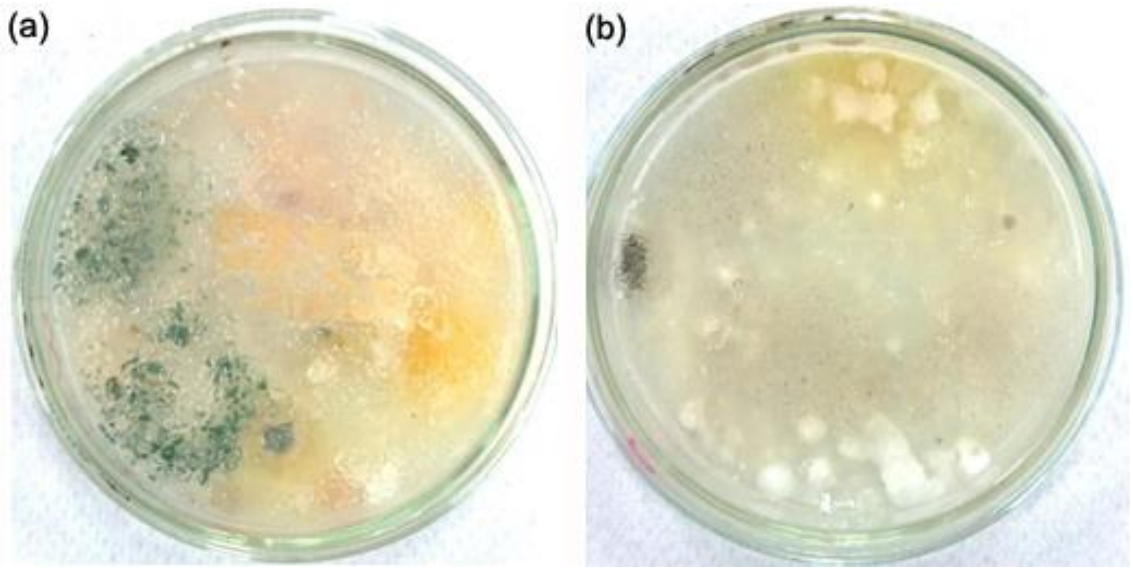


Figure 3–5 The plates showing the macroscopic colonies produced from (a) coniferous soil and (b) deciduous soil after incubation for 7 days.

Under plating condition, the microbial number was express by colony-forming unit in soil are critical links between shifts in the composition of the dominant tree

species and fundamental shifts in ecosystem functioning. For instance, differences in the nature of the leaf litter, mycorrhiza fungal associations, and the exudates released into the rhizosphere with different tree species may yield distinct microbial number in the soil. The total microbial count (colony-forming units) for each forest type was plotted against the time in days (Figure 3-5).

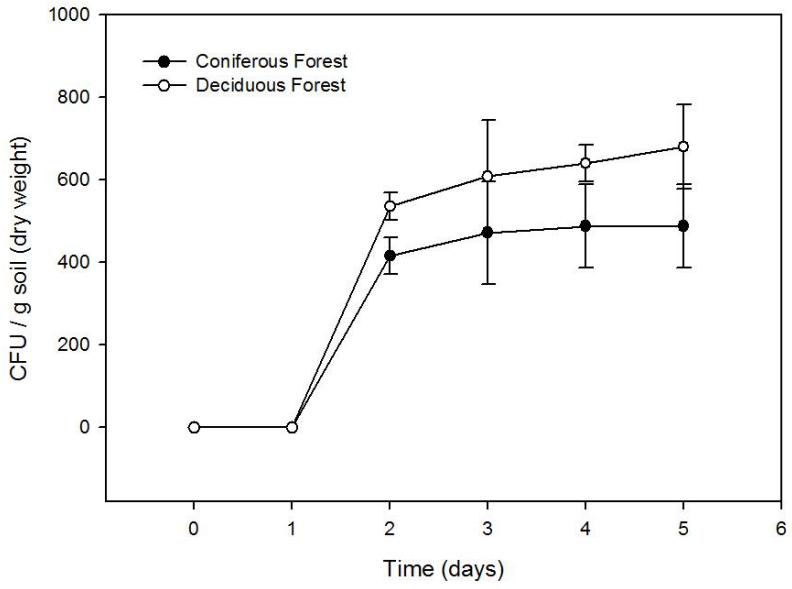


Figure 3-6 Changes in the plate counts (colony-forming unit per gram) over time in the two soil samples.

Figure 3-6 shows the changes in the plate counts for the soil samples from each forest type during incubation for 5 days. The plots demonstrate that the plate counts were higher for the deciduous forest soil sample than the coniferous forest soil sample. Previous studies suggest that the microorganism number are distinct in each forest type, where they are found in the leaf litter, forest floor, soil, mycorrhizae, and rhizosphere. Previous research has demonstrated that the biogeography of soil microorganisms is strongly related to factors such as the pH, soil texture, organic matter content, and the C/N ratio in the soil (Fierer & Jackson, 2006); (Fierer, Lauber, Zhou, McDonald, & Costello, 2010); (Rousk, et al., 2010). The results obtained in the present study indicated that the soil characteristics was not show the significantly difference for both forest

type. Thus, site-specific factors such as decomposing litter, forest floor, and rhizosphere might have been more important than the soil factor in structuring the microbial number. (Grayston & Prescott, 2005) showed that the most distinct microbial number in humus layers under Western Red Cedar had lower fungi: bacteria ratio and greater utilization of most C substrates than those under other species.

The native soil microorganism has been interpreted as a consequence of the litter fungi genera being adapted to decompose litter. This may have been related to the calcium concentration and pH in the cedar forest floor, rather than whether the forest was deciduous or coniferous, and thus the variation in the microbial number might have been determined by the influence of calcium on the soil fungal genera (Reich, Peterson, Wedin, & Wrage, 2001); (Hobbie, Diepen, Lilleskov, Ouimette, Finzi, & Hofmockel, 2014). Several studies have reported differences in the microbial number in bulk forest floor samples collected from below different tree species, particularly when conifers and broadleaves are compared. (Ushio, Kitayama, & Balser, 2010) reported distinct microbial number in separate upper and lower layers below coniferous and broadleaf species in a mixed species tropical montage forest. However, the soil characteristics can be expected to be influenced less by the tree species than the forest floor composition, which is largely created from the leaf litter produced by trees.

Several studies have also compared the fungal genera in different litter types. For example, the succession of fungi in the leaf litter produced by trees such as *Betula*, *Corylus*, *Fraxinus*, and *Quercus* demonstrated the similarity of the dominant species of fungi in all litter types (Frankland, 1998). The fungal genera in decomposing litter were also compared in mixed conifer forest, such as pine, spruce, and fir. The results showed that some fungal species were occasionally missing from one of the litter types, but the fungal genera were generally similar in all litter types (Hayes, 1965). This may also indicate that these were the most common fungi in this particular forest. In fact, greater differences in the fungal flora have been observed within a single type in adjacent but different habitats. (Kubartova, Ranger, Berthelin, & Beguiristain, 2009) compared the fungal genera in the decomposing tree litter produced by *Fagus*, *Quercus*, *Picea*, and *Pseudotsuga* in separate plots of each species on a single site. The results showed that

the majority of fungal species were detected in several or all litter types, but their relative abundances differed among the litter types.

3.4.2 Selection of representative soil fungi

The fungal genera derived from each soil type (Figure 3-4) were used to isolate single strains with the hyphal tip method. The hyphal tip method is employed to obtain pure cultures of fungi (Tutte, 1969) by taking a single hypha from the edge of the culture and cutting the hyphal tip immediately before the last branching point using a scalpel and a minutien pin. Next, the tip is transferred to a new culture plate and incubated at a temperature of 25°C–30°C. This method is economic because antibiotics are not needed in the culture plate as the precise manipulation means that the isolated spores are free of bacterial contamination. Unlike other isolation approaches, this method can reduce the excessive use of sterile pipettes, agar plates, and other sterile items, as well as spore dilutions. This method is useful because it can be modified to isolate various fungi from different taxonomic groups. For example, (Hsieh & Goh, 1990) used this method to successfully isolate hyphomycetes from their conidia, for example, *Cercospora* and allied genera. However, this method is also complex when selecting a single hypha if the fungal growth is dense and compact.

This method cannot be used to distinguish all of the species of microorganisms found in different forest soils. However, some of the fungi with the most abundant growth were selected as representatives, and their macroscopic colonies are shown in Tables 3-3 and 3-4.

Table 3-3 Representative soil fungi selected by the hyphal tip isolation technique from the coniferous forest soil

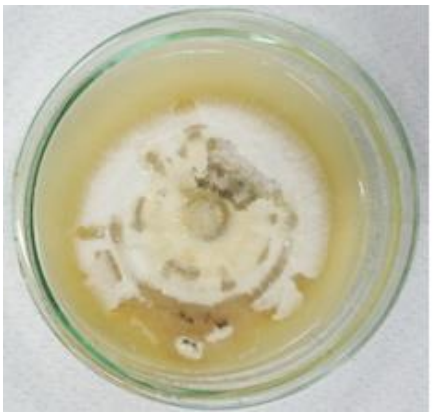
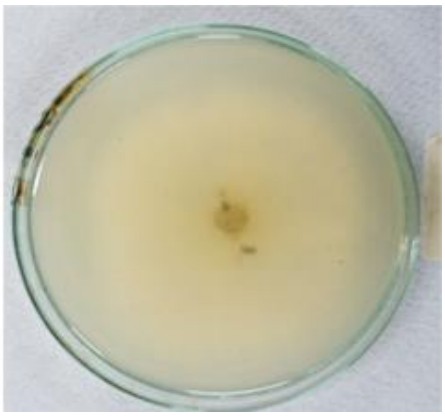
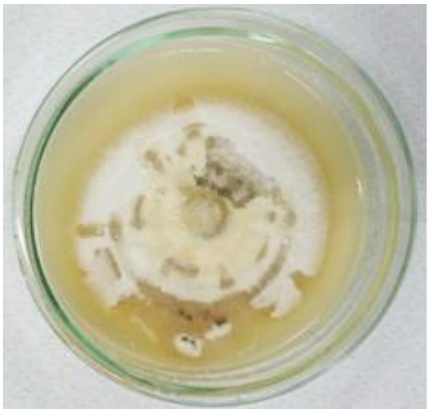
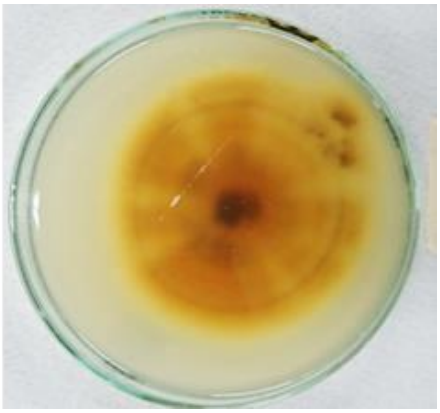
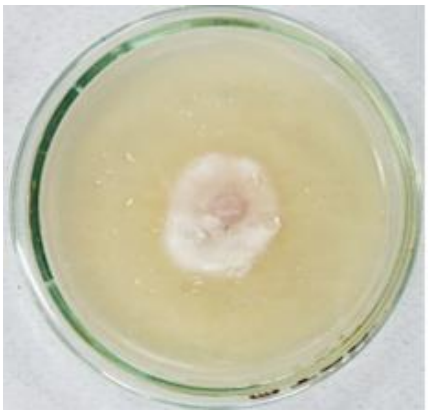
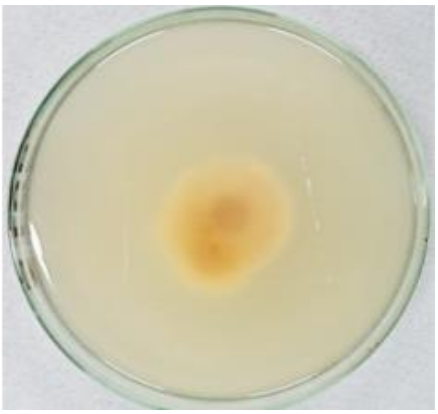
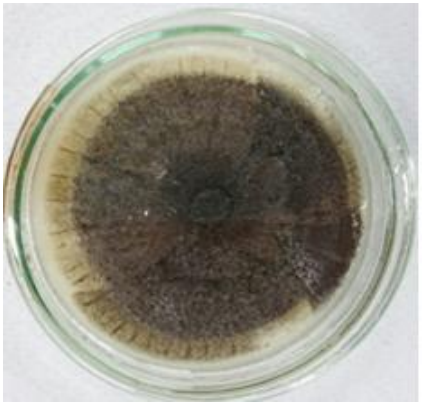
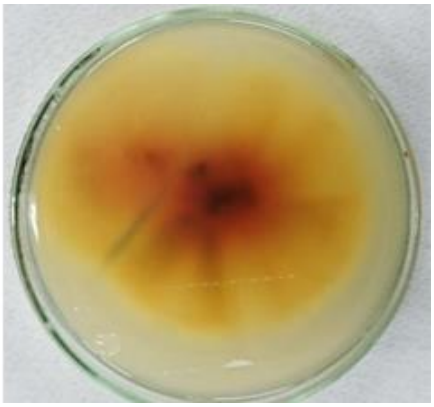
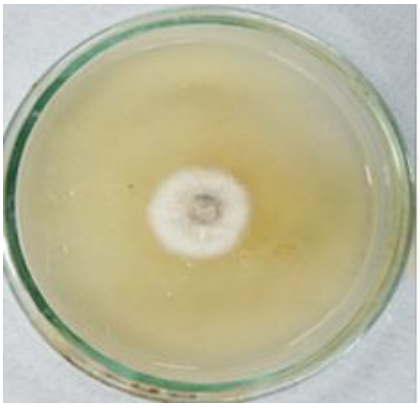
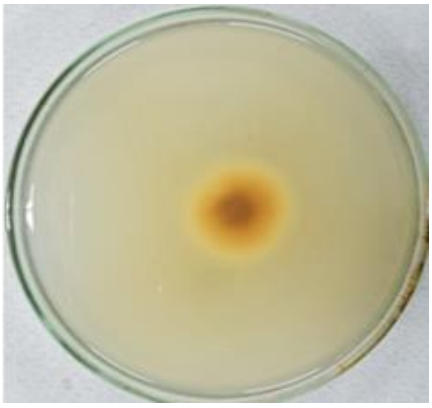
Sample No.	Front	Back
C1		
C2		
C3		

Table 3-4 Representative soil fungi selected by the hyphal tip isolation technique from the deciduous forest soil

Sample No.	Front	Back
D1		
D2		
D3		

3.4.3 DNA sequencing

The pure fungal cultures were subjected to DNA sequencing. ITS region of each fungal isolate was sequenced using ITS1 and ITS4 as the forward and reverse primers.

Table 3-5 DNA sequences of ITS regions from the fungal isolates from the coniferous forest soil

Sample No.	**Contig**
C1	TCGATTACCC AACCCAATGT GAACGTTACC AAACTGTTGC CTCGGCGGGA TCTCTGCCCC GGGTGCGTCG CAGCCCCGGA CCAAGCGCC CGCCGGAGGA CCAACCAAAA CTCTTTTGTA TACCCCCTCG CGGGTTTTTA TATCTGAGCC ATCTCGGCGC CTCTCGTAGG CGTTTCGAAA ATGAATCAAA ACTTTCAACA ACGGATCTCT TGGTTCTGGC ATCGATGAAG AACGCAGCGA AATGCGATAA GTAATGTGAA TTGCAGAATT CAGTGAATCA TCGAATCTTT GAACGCACAT TGCGCCCGCC AGTATTCTGG CGGGCATGCC TGTCCGAGCG TCATTTCAAC CCTCGAACCC CTCCGGGGGG TCGGCGTTGG GGATCGGCCC TTTACGGGGC CGGCCCCGAA ATACAGTGGC GGTCTCGCCG CAGCCTCTCC TGCGCAGTAG TTTGCACACT CGCATCGGGA GCGCGGCGCG TCCATTGCCG TAAAACACCC AACTTTCTGA AATGTGACCT CGGGTAGGAC CCT
C2	AAAGTCTACA ACTCATCAAC CCTGTGACAT ACCTAAACGT TGCTTCGGCG GGAACAGACG GCCCTGTAAC ACGGGCCGCC CCCGCCAGAG GACCCCCTAA CTCTGTTACT GTAAATGTTT TTTCTGAGTA AACAAGCAAA TAAATTAAAA CTTTCAACAA CGGATCTCTT GGCTCTGGCA TCGATGAAGA ACGCAGCGAA ATGCGATAAG TAATGTGAAT TGCAGAATTC AGTGAATCAT CGAATCTTTG AACGCACATT GCGCCCGCCA GTATTCTGGC GGGCATGCCT GTTCGAGCGT CATTACAACC CTCAGGCCCC CGGGCCTGGC GTTGGGGATC GGCGGAGCCC CCGTGGGCA CACGCCGTCC CCCAAATACA GTGGCGGTCC CGCCGCAGCT TCCATTGCGT AGTAGCTAAC ACCTCGCAAC TGGAGAGCGG CGCGGCCACG CCGTAAAACA CCCAACTTCT GAATGTGACC TCGAATCAGG AATCCCT
C3	GGCTCGGTCT AACTCATCAC CCTGTGACAT ACCTAAACGT TGCTTCGGCG GGAACAGACG GCCCTGTAAC ACGGGCCGCC CCCGCCAGAG GACCCCCTAA CTCTGTATCT GTTATGTTTT TTCTGAGTAA ACAAGCAAAT AAATTAAAAC TTTCAACAAC GGATCTCTTG GCTCTGGCAT CGATGAAGAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA GTGAATCATC GAATCTTTGA ACGCACATTG CGCCCGCCAG TATTCTGGCG GGCATGCCTG TTCGAGCGTC ATTACAACCC TCAGGCCCCC GGGCCTGGCG TTGGGGATCG GCGGAGCCCC CCGTGGGCAC ACGCCGTCCC CTAAATACAG TGGCGGTCCC GCCGCAGCTT CCATTGCGTA GTAGCTAACA CCTCGCAACT GGAGAGCGGC GCGGCCACGC CGTAAAACAC CCAACTTCTG AATGTGACCT CGAATCAGGT AGATCCT

Table 3-6 DNA sequences of ITS regions from the fungal isolates from the deciduous forest soil

Sample No.	Contig
D1	
D2	
D3	

The contig sequences of the fungi shown in Table 3-5 and 3-6 comprise a set of overlapping DNA segments, which together represent a consensus region. The ITS regions were found using the Bioedit program. This program was used to eliminate the code "N," which is used to represent missing data or gaps to prevent the designed primers from binding to sites with intraspecific variation (Villard & Malausa, 2013).

The results showed that each DNA contig had a length of about 500 base pairs. Each contig was compared with the sequences in the NCBI database using BLAST. The percentage similarity of each fungal contig sequence is shown in Table 3-7.

Table 3-7 Percentage similarity of fungal contigs compared with the NCBI database

Fungal genus	**Similarity (%)**					
	C1	**C2**	**C3**	**D1**	**D2**	**D3**
Rhizopus sp.	58.3	56.2	56.9	63.9	60.2	56.9
Mucor sp.	58.6	60.4	60.3	64.3	60.2	56.9
Pythium sp.	55.8	56.2	58.1	62.6	57.6	58.7
Penicillium sp.	36.3	39.1	33.9	41.3	38.6	38.4
Geotrichum sp.	57.2	58.0	57.3	64.7	58.7	59.4
Trichoderma sp.	98.5	88.2	73.5	86.0	86.1	87.0
Chaetomium sp.	81.2	82.9	75.8	85.2	82.1	83.5
Verticillium sp.	79.8	80.0	71.3	80.4	79.1	80.7
Aspergillus sp.	76.6	77.2	71.3	96.9	78.8	78.3
Alternaria sp.	71.3	74.0	70.5	82.6	74.4	75.5
Fusarium sp.	84.0	90.7	78.8	86.0	91.2	89.1
Rhizoctonia sp.	65.7	72.9	66.7	73.0	70.4	70.6

Table 3-7 shows that the representative fungi selected by the hyphal tip isolation technique belonged to three genera: *Fusarium*, *Trichoderma*, and *Aspergillus.* C1 shared 98% similarity with the genus *Trichoderma*, D1 shared 97% similarity with the genus *Aspergillus*, and the others shared 78%–91% similarity with the genus *Fusarium*. It can

be assumed that these were saprotrophic fungi, which are free living in the soil with the ability to decompose dead organisms and organic residues. Most of the fungi belonged to the genus *Fusarium*, which is a large genus of filamentous fungi with widespread distributions in soil, and they are also associated with plants. *Trichoderma* is a genus of saprotrophic fungi that produces various types of secondary metabolites, and *Aspergillus* is a genus that was originally isolated from soil. In this study, *Aspergillus* was found only in the deciduous forest soil. This genus comprises a few hundred species. Table 3-5 shows some of the DNA contig sequences of the fungi (C2, D2, C3, and D3) from the genus *Fusarium*, which includes 13 important species. Figure 3-6 shows the phylogenetic tree obtained using the neighbor-joining method from the DNA contig sequences within the ITS segment to describe the relationships among fungi.

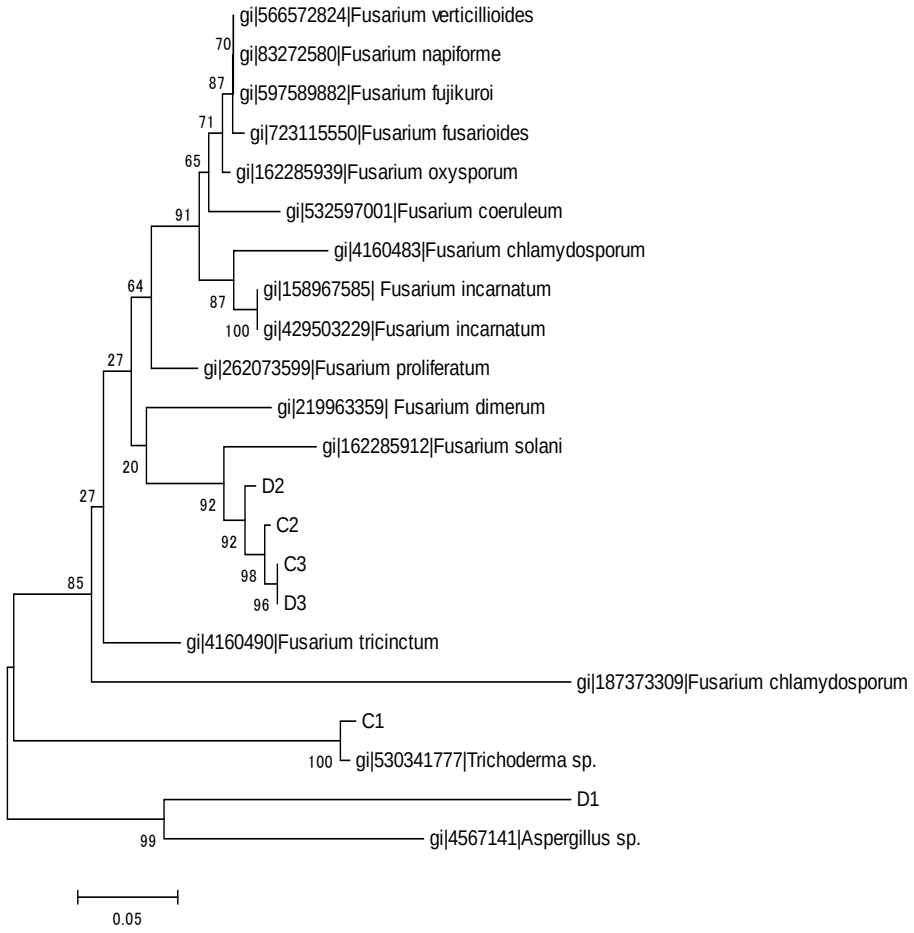


Figure 3-7 Neighbor-joining tree based on the DNA contig sequences of fungi. The number at each node indicates the percentage shared similarity.

3.5 Conclusions

This study provides some evidence that the different fungal genera found in forest soils may be related to natural factors such as soil factor and site factor associated with the tree species present. The leaf litter produced by different tree species may lead to the development of distinct fungal genera during their decomposition. This may be due to differences in the nature of the litter itself as well as variation in the microbial number found in forest floors under different tree species. Distinct fungal genera have been reported in forest floors under different tree species in various forest types. In this study, the differences in fungal genera in the upper mineral soil were determined under different tree species.

Assessing the extent to which soil microbial number are influenced by the tree species under which they occur is necessary to understand how tree species influence ecosystems. The results of this study can be expanded to further analyses of ecosystem functioning, but it is difficult to characterize microbial number and to understand how changes in their composition might affect ecosystem processes. The specialization of microbial number under different tree species may be important for physiologically restricted ecosystem processes performed by a limited suite of organisms. Thus, it is important to understand the mechanisms that underlie the effects of forest types and different tree species on soil microorganisms. Molecular techniques are facilitating this type of research. In the present study, representative fungi were selected using the hyphal tip isolation technique, and they were assigned to three genera*: Fusarium*, *Trichoderma*, and *Aspergillus*. Under plate culture technique condition, showed some fungal genus were occasionally missing from one of the forest soil types, but the fungal genera were generally similar in both coniferous and deciduous forest such as *Fusarium* sp. which was found in both forest type. However, it can be assumed that these are all saprotrophic fungi and free living in the soil, where they have the ability to decompose dead organisms and organic residues. Hence, not only the organic residues but also nutrient element and toxic element may associate with the fungi cell through physicochemical and biological mechanisms.

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Chapter 4

Growth Kinetics and Tolerance

The representative soil fungi are described in the previous chapter. Soil fungi deal with a wide variety of potentially toxic environmental challenges. They are significantly affected when the ambience of their environment changes. Therefore, they should be able to sense and respond to these changes to survive. Beyond certain thresholds, the persistence of fungi tends to decrease as a result of growth inhibition due to the tolerance caused by the presence of cesium (Cs) and strontium (Sr) in the environment. In this chapter, the growth kinetics of soil fungi affected by Cs and Sr are investigated using the statistical evaluation of mathematical models to describe the fungal growth. The inhibitory effects of Cs and Sr on the growth of soil fungi are studied. This chapter aims to clearly describe how the growth kinetics of soil fungi are affected by various conditions when Cs and Sr contamination occurs. In addition, the responses to Cs and Sr by different fungi are investigated.

4.1 Objective:

- To investigate how the growth kinetics of soil fungi was affected by Cs and Sr
- To evaluate the suitability of mathematical models to describe the fungal growth curve using statistical evaluation measures.

4.2 Theory and Significance

Certain anthropogenic activities, such as the development of nuclear weaponry, results in the creation of nuclear energy or radioactive elements and has typically led to the discharge of radioactive cesium (Cs) or strontium (Sr) to the environment. Both Cs and Sr were released in the environment during the Fukushima nuclear incident in 2011. The radioactive fallout mainly spread to the eastern areas of Japan through direct deposition from the atmosphere into the soil and subsequent movement caused by washing of rainfall and precipitation. Isotopes of Cs and Sr are among the most hazardous radioactive contaminants of the environment (Mosquera, Carvalho, Veiga,

Mangia, & Anjos, 2006). The oxidation states of these two elements under natural conditions are Cs^{+} and Sr^{2+}. The chemical behavior and bioavailability of Cs and Sr are very close to those of potassium (K^{+}) and calcium (Ca^{2+}), respectively. Contamination by Cs and Sr may have an adverse impact on the activity, ecology, and population dynamics of soil fungi. In environmental studies, there has not been much focus on the influence of physiochemical factors on the toxicity of a pollutant to soil fungi. (Zhdanova, Zakharchenko, Vember, & Nakonechnaya, 2000) detected fungal growth at various locations near the Chernobyl nuclear power plant during 1997–1998. Their study showed that among the 37 species of fungi observed, ranges of radioactive tolerance by different varieties of fungi were broad, with some species found in areas of high contamination and others found only in locations with weak contamination. It was shown that the level of radioactive contamination was the main ecological factor distinguishing the presence of different species of microorganisms.

However, impacts caused by sterilization and re-inoculation prevailed in the microcosms of the current study, which featured ^{137}Cs or ^{90}Sr contamination to levels of up to 50-fold of that of hotspots occurring in Chernobyl. The results show minor changes in soil microbial functions in response to the contamination, suggesting a strong resilience of natural soils to radioactive contamination (Niedree, Vereecken, & Burauel, 2013). Soil pollution is an accumulation of a substance that may be native or introduced at a level harmful for the growth and health of microorganisms, including fungi. Thus, as a first step within the current study, interactions among soil fungi and pollutants (Cs and Sr) were studied to understand the behavior of complex biogeochemical ecosystems and the tolerance of soil fungi isolated from forest soils to these pollutants. The tolirance of Cs and Sr to soil organisms has been assessed by various studies. The previous study on the tolerance of Cs to soil microorganisms by counting the number of soil microorganisms which Cs concentration increasing over the range 0 - 100 mM CsCl, showing that filamentous actinomycetes are more sensitive to Cs than planktonic bacteria. The results obtained by (Kuwahara, et al., 2005) demonstrated that filamentous actinomycetes could not grow when exposed to Cs concentrations of 25 mM, whereas planktonic bacteria could grow in concentrations as high as 100 mM. However, the growth of planktonic bacteria did show a progressive

decrease over the range of 25, 50, and 100 mM. The tolerance of Sr to the microorganisms *Micrococus* sp. and *Cupriavidus metallidurans* tends to decrease following an increase in Sr concentration over the range of 0–400 mM; however, inhibition of growth appears from a concentration of 300 mM and above (Salem, et al., 2012). Sr was shown to be considerably toxic to cyanobacterium, with toxic effects including a decrease in dry weight, protein, and carbohydrate content (Khalil, 1997).

Growth kinetics is a phase in which the specific growth rate starts at a zero and then accelerates to a maximal value within a certain time period. When the growth curve is defined as the logarithm of the number of organisms plotted against time, these growth-rate changes result in a sigmoidal curve, with a lag phase just after t = 0, followed by an exponential phase, and then by a stationary phase (Zwietering, Jongenburger, Rombouts, & van't Riet, 1990). The predictive modeling used in the current study describes only the number of organisms and does not include the consumption of substrate as a model based on the Monod equation would do. There are a number of sigmoidal functions that have been used for modeling somatic growth and population dynamics (Thornley & France, 2007), which could be applied to fungal growth. The fungi grow exponentially and can be represented by the general equation (4-1).

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \mu N \tag{4-1}$$

The Gompertz (GMP) model was found to produce better results by a fitting procedure when compared with alternative models, such as the Logistic (LOG) model (Gibson, Bratchell, & Roberts, 1988). The Logistic model can be represented by the equation (4-2).

$$\mathrm{y} = \mathrm{a}\,.exp[-exp(b - cx)] \tag{4-2}$$

In addition, others nonlinear function used in this study to evaluate the suitability of several mathematical functions for describing fungi growth curves was Logistic model; LOG. The equation was written as equation (4-3).

$$y = \frac{A}{1 + e^{[b - cx]}} \tag{4-3}$$

The equation describing a mathematical model contains parameters of mathematical rather than biological significance. Therefore, the model used in the current study was rewritten to substitute the mathematical parameters with more suitable parameters of biological significance: A, μ_m, and λ (Zwietering, Jongenburger, Rombouts, & van't Riet, 1990). The equation was rewritten as equation (4-4) for the GMP model and equation (4-5) for the LOG model.

$$y = A \cdot exp\left\{-exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1\right]\right\} \tag{4-4}$$

$$y = \frac{A}{\{1 + e^{\left[\frac{4\mu_m}{A}(\lambda - t) + 2\right]}\}} \tag{4-5}$$

It is often useful to plot the logarithm of the relative population [$Y = \ln (N/N_0)$] against time [x = t]. The fungi growth curve can be described by three phases with three parameters, with μ_m representing the maximum specific growth rate and defined as the tangent in the inflection point, λ representing the lag time and defined as the x-axis intercept of this tangent, and A is the maximal value reached. A can be calculated as [$\ln (N_\infty/N_0$], where N represents fungi biomass and has mostly been expressed in terms of fungi cell number using an indirect measurement, namely optical density (OD_{600}).

The prediction of the microbial metabolic lag is important as neglecting an existing lag may result in significant overestimation of the amount of substrate degraded. In particular, for predicting bioremediation in contaminated environmental systems, a delay in microbial activity caused by pollutants in response to substrate

arrival has not been incorporated into a microbial kinetic model. Therefore, the current study in addition described the delaying impact of both Cs and Sr fungi response.

4.3 Procedure

4.3.1 Material

- Fungi cell culture (Chapter 3); *Fusarium* sp., *Trichoderma sp.*, *Aspergillus* sp.
- Cesium Chloride (CsCl)
- Strontium Chloride ($SrCl_2$)
- Erlenmeyer flask (100 mL) 12 units per cell cultures
- 50 mL potato dextrose broth (PDB) per experiment conditions
- Spectrophotometer (Photolab 6100 VIS), Figure 4-1
- Shaker
- Incubator
- Sterile pipette tips and syringe

Figure 4-1 Photolab 6100 VIS

4.3.2 Method

1. Cs^{+} and Sr^{2+} solutions were individually prepared in 50 mL of fresh PDB with concentrations ranging from 5, 10, 25, 50, and 100 mM and 0 mM as a control.

2. A total of 1 mL of each solution of cell culture, *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp., was then inoculated into the PDB solution, to which various concentrations of Cs or Sr were added (1). All experiments were conducted in duplicate. The solutions were incubated on a shaker at 110 rpm with temperature maintained at 25°C until the end of the incubation around 168 h.

3. Approximately 5 mL of the suspension of each treatment was sampled at time intervals of 0, 24, 48, 72, 96, and 168 h with a sterilized syringe.

4. The fungi response was expressed in terms of fungi cell number, which was indirectly determined by measuring the optical density at 600 nm (OD_{600}) with a spectrophotometer (Photolab 6100 VIS)

5. All models were fitted to the data using nonlinear regression in Sigmaplot version 10.1. The initial values supplied were different for each data set, and the selection of starting values was on the basis of the visual inspection of the plot, which was automatically calculated in the software.

4.4 Results and Discussion

4.4.1 Effect of Cs and Sr on the growth of fungi

The data derived from the experiments were used to construct three growth curves for different fungal species in which growth was expressed as optical density units. The nonlinear functions used were the LOG and GMP models. The data fits obtained with all models were statistically evaluated taking into account the fitting behavior, examination of residuals, and statistics for goodness of fit. In general, all models provided an adequate fit without major problems, although the initial estimates of parameters to reach convergence within a reasonable number of iterations were different for each curve.

Models were statistically compared to evaluate and discriminate between them. The bias factor used was developed by (Ross, 1996) as an index of the model

performance in terms of the average deviation between predicted (P) and observed (O) values, as shown in equation (4-6).

$$B_f = e^{[\sum \ln\left(\frac{P}{O}\right)/n]} \tag{4-6}$$

No disagreement between predicted and observed values would result in a bias factor equal to 1. However, a bias factor >1 indicates a failed model as predictions are greater than observed. The serial correlation of residuals was examined with the Durbin–Watson (DW) statistic to test whether a model has been successful in describing the underlying trend (Draper & Smith, 1981).

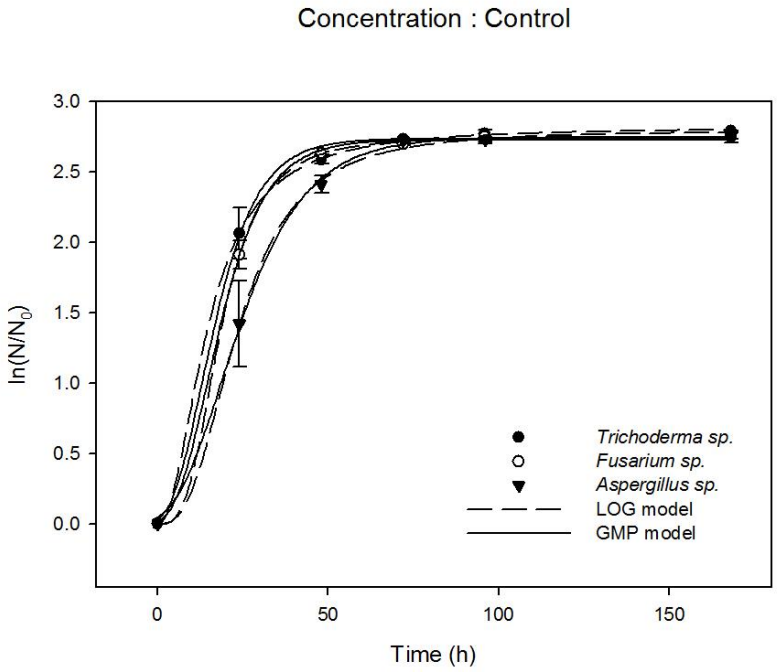

Figure 4-2 Cell growth kinetics under control concentrations (without added elements) for *Trichoderma* sp., *Fusarium* sp., and *Aspergillus* sp. fitted with the Gompertz (GMP) model and the Logistic (LOG) model

Figure 4-2 shows the growth kinetics of three cell genera under a control condition, in which no elements were added. The same trend is evident for all fungi genera. The results indicate that the cell population reached a maximum growth around 3.0, after which growth plateaus, indicating that the occurrence of a negligible delay in

microbial activity in response to substrate arrival has not been incorporated into the microbial kinetic model.

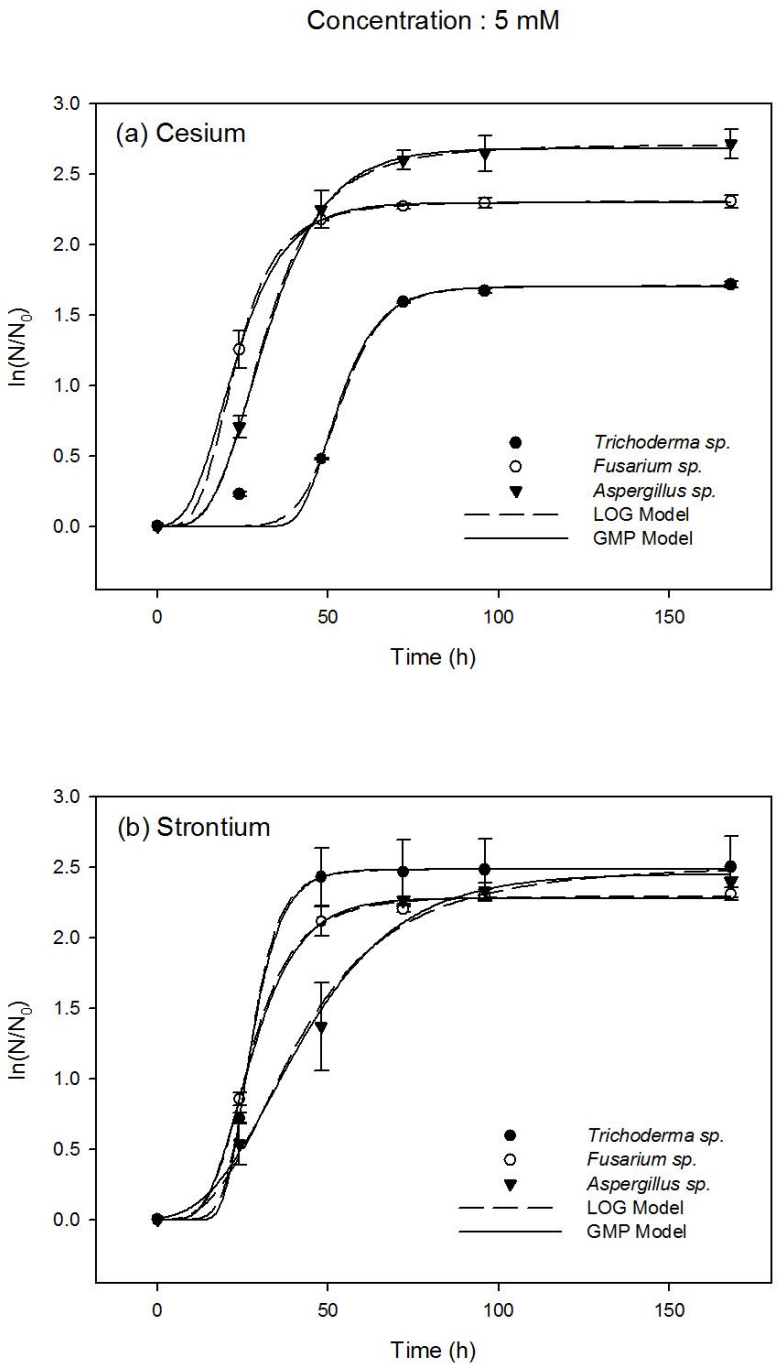

Figure 4-3 Cell growth kinetics under concentrations of 5 mM (a) cesium and (b) strontium fitted with the Gompertz (GMP) model and the Logistic (LOG) model

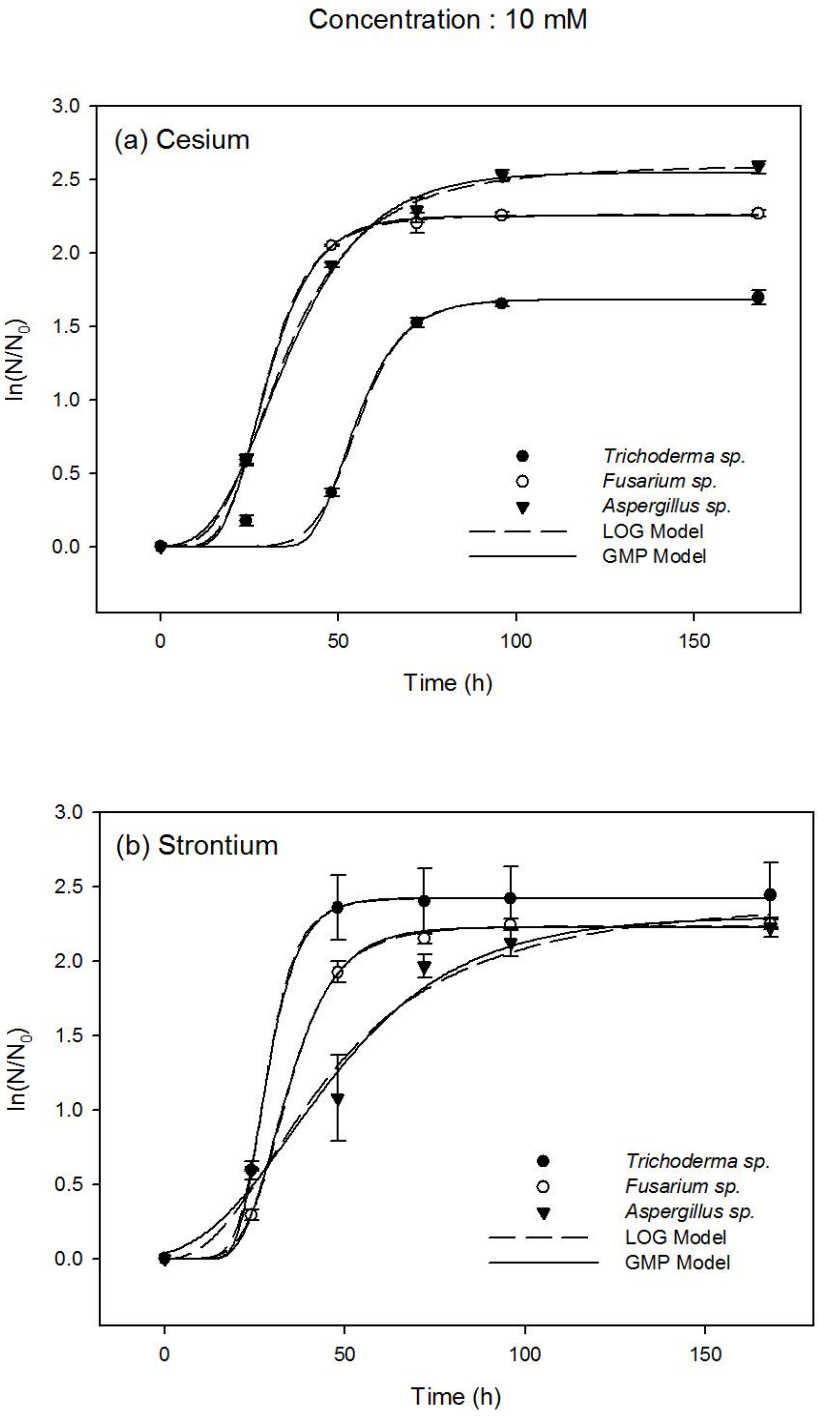

Figure 4-4 Cell growth kinetics under concentrations of 10 mM (a) cesium and (b) strontium fitted with the Gompertz (GMP) model and the Logistic (LOG) model

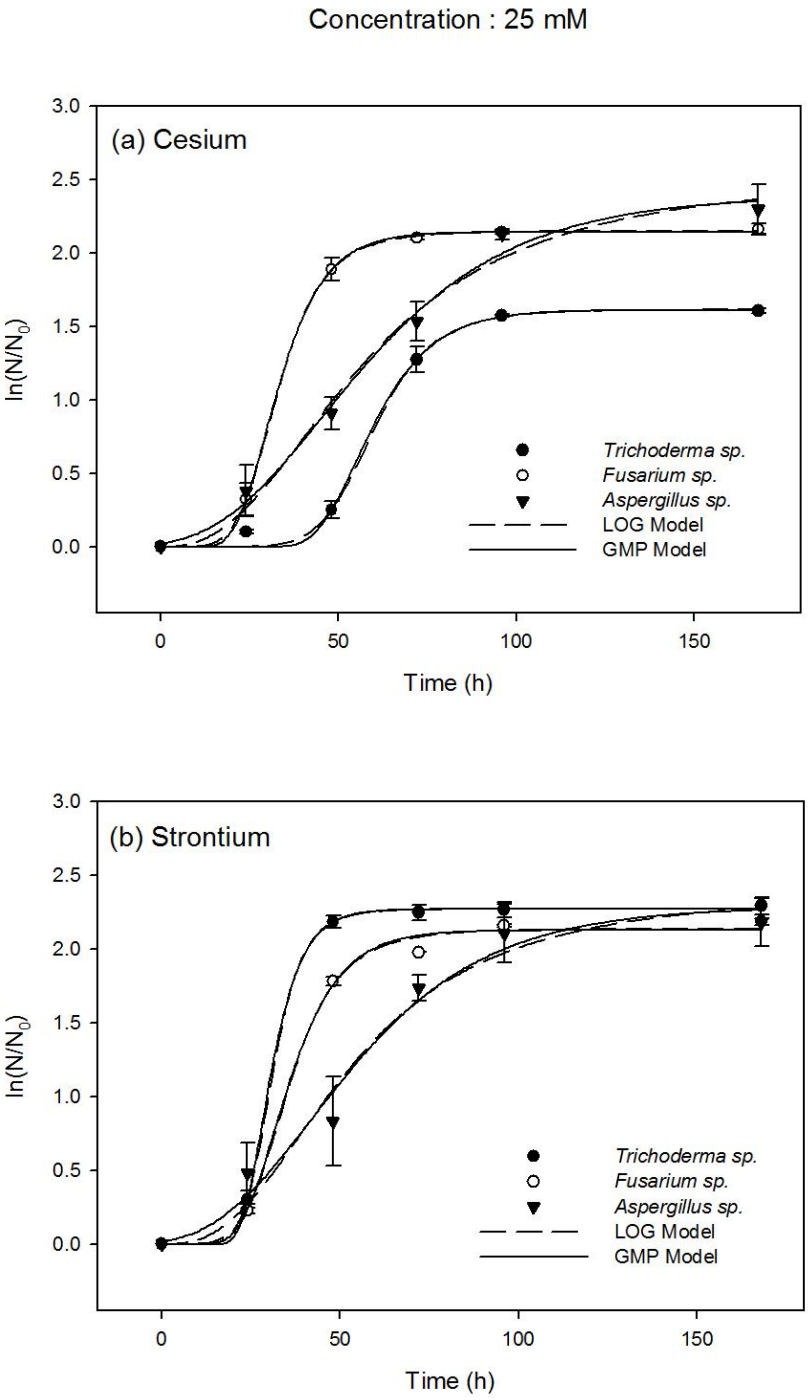

Figure 4-5 Cell growth kinetics under concentrations of 25 mM (a) cesium and (b) strontium fitted with the Gompertz (GMP) model and the Logistic (LOG) model

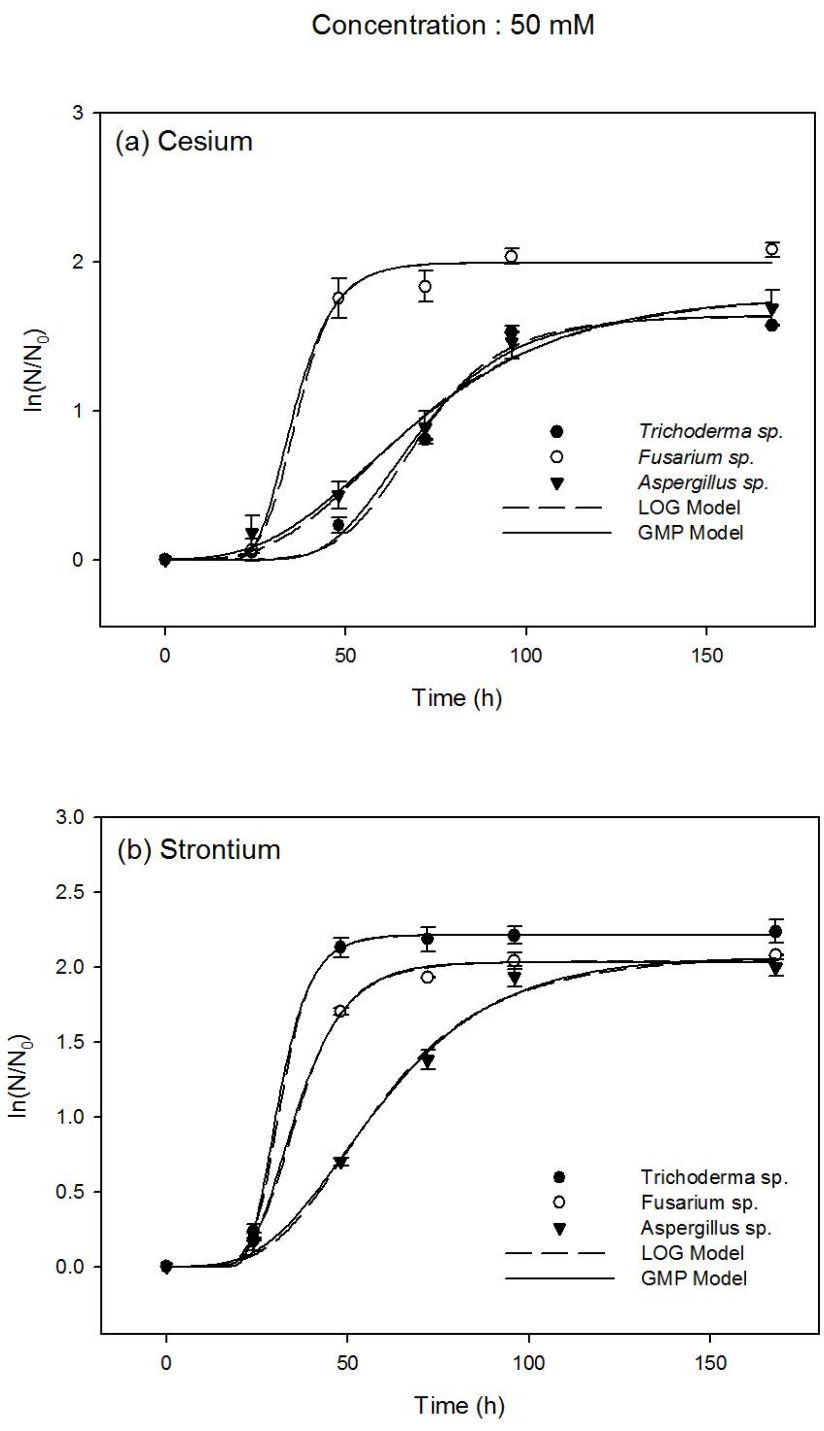

Figure 4-6 Cell growth kinetics under concentrations of 50 mM (a) cesium and (b) strontium fitted with the Gompertz (GMP) model and the Logistic (LOG) model

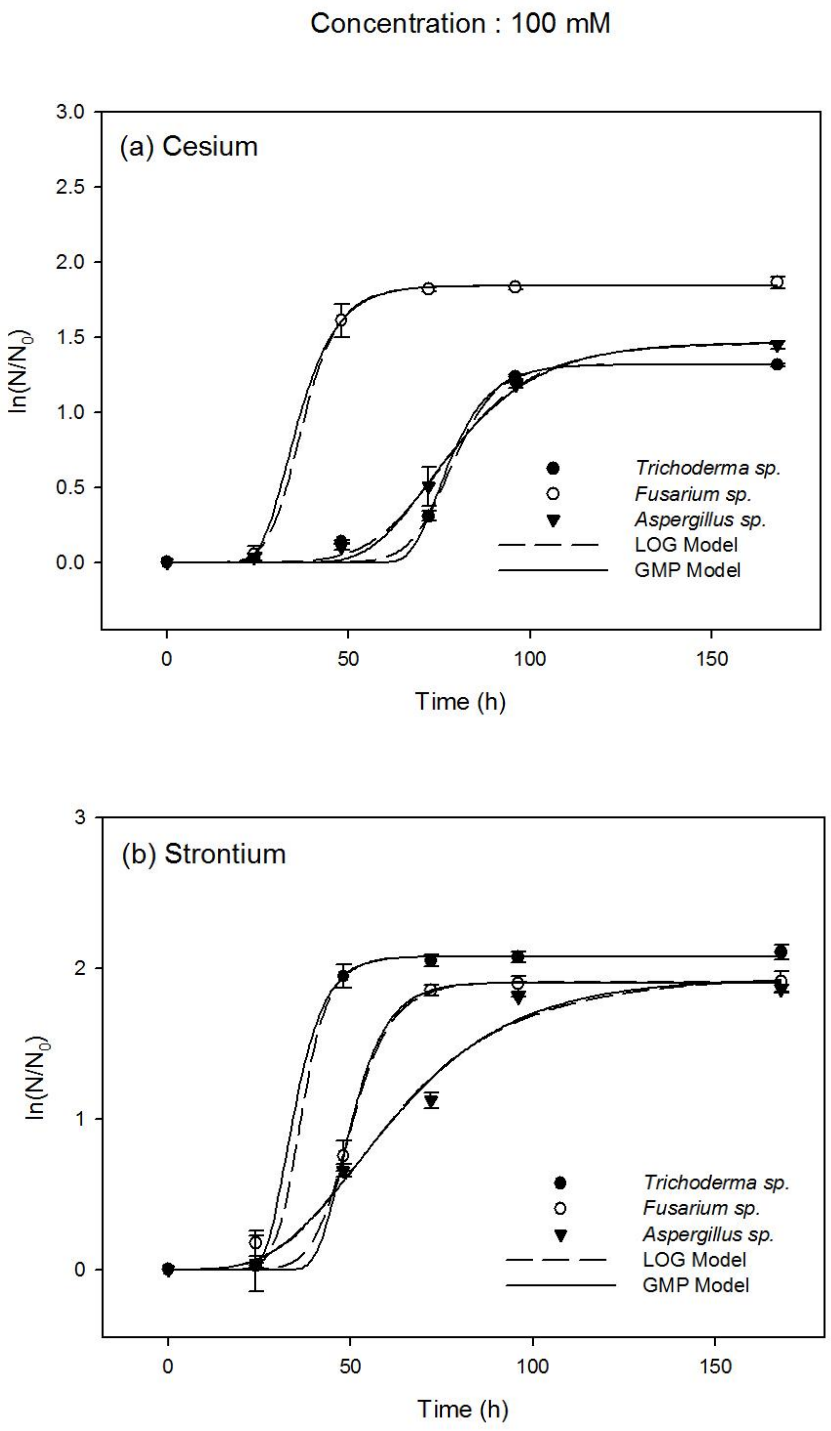

Figure 4-7 Cell growth kinetics under concentrations of 100 mM (a) cesium and (b) strontium fitted with the Gompertz (GMP) model and the Logistic (LOG) model

The examination of residuals obtained for each of the curves depicted in Figures 4-2 to 4-7 was based on analysis of systematic bias between observed and predicted values. The statistical analytical data are shown in Table 4-1.

Table 4-1 Statistical analytical data for growth curves of cells of three fungi genera

Fungi Genus	Ele.	Conc.	DW		R^2	
		(mM)	LOG	GMP	LOG	GMP
Trichoderma sp.	Control	-	2.32	1.77	0.9999	0.9974
	Cs	5	2.05	2.01	0.9832	0.9829
		10	2.04	2.02	0.9903	0.9901
		25	2.03	1.99	0.9965	0.9964
		50	2.91	3.16	0.9886	0.9860
		100	1.54	1.50	0.9890	0.9887
	Sr	5	1.58	1.50	0.9999	0.9999
		10	1.59	1.51	0.9999	0.9999
		25	1.55	1.50	0.9999	0.9998
		50	1.58	1.53	0.9998	0.9998
		100	1.49	1.50	0.9997	0.9997
Fusarium sp.	Control	-	3.34	1.51	0.9999	0.9995
	Cs	5	1.92	1.67	1.0000	0.9999
		10	2.48	2.14	0.9999	0.9997
		25	1.84	1.76	0.9999	0.9998
		50	2.07	2.04	0.9938	0.9935
		100	1.64	1.61	0.9998	0.9998
	Sr	5	2.70	2.36	0.9992	0.9988
		10	2.72	2.46	0.9996	0.9994
		25	2.47	2.30	0.9970	0.9964
		50	2.13	2.07	0.9986	0.9983
		100	2.04	2.00	0.9925	0.9923

Table 4-1 Statistical analytical data for growth curves of cells of three fungi genera (continued)

	Control	-	2.72	2.81	0.9992	0.9991
		5	2.93	1.93	0.9999	0.9997
		10	3.35	2.82	0.9990	0.9979
	Cs	25	2.86	2.97	0.9877	0.9925
		50	2.71	2.87	0.9851	0.9874
Aspergillus sp.		100	1.87	2.10	0.9978	0.9952
		5	3.03	3.17	0.9868	0.9914
		10	3.07	3.28	0.9760	0.9833
	Sr	25	2.97	3.04	0.9706	0.9789
		50	2.91	2.94	0.9920	0.9935
		100	3.54	3.54	0.9833	0.9852

The growth phase of the three fungi genera focused on in the current study can be divided in three sub-phases, namely the lag phase; LAG (0–24 h), exponential phase; EXP (48–72 h), and stationary phase; STA (96–168 h). The bias factor of each condition was calculated and is shown in Table 4-2.

Table 4-2 Bias Factor values obtained when fitting the model to each growth condition

Condition	**LAG**	**EXP**	**STA**	**LAG**	**EXP**	**STA**
	LOG Model			**GMP Model**		
Control	1.00	1.00	1.00	0.99	1.01	0.99
Trichoderma sp.						
Cs 5 mM	0.01	1.00	1.00	0.00	1.00	1.00
Cs 10 mM	0.01	1.00	1.00	0.00	1.00	1.00
Cs 25 mM	0.02	1.00	1.00	0.00	1.00	1.00
Cs 50 mM	0.04	0.81	0.98	0.00	0.89	0.98
Cs 100 mM	0.00	0.12	1.00	ND	0.00	1.00
Sr 5 mM	1.00	1.00	1.00	1.00	1.00	1.00
Sr 10 mM	1.00	1.00	1.00	1.00	1.00	1.00

Table 4-2 Bias Factor values obtained when fitting the model to each growth condition (continued)

Sr 25 mM	1.00	1.00	1.00	1.00	1.00	1.00
Sr 50 mM	1.00	1.00	1.00	1.00	1.01	0.99
Sr 100 mM	1.05	1.01	0.99	1.01	1.01	0.99
Fusarium sp.						
Cs 5 mM	1.00	1.00	1.00	1.00	1.00	1.00
Cs 10 mM	1.00	1.00	1.00	1.00	1.01	0.99
Cs 25 mM	1.00	1.00	1.00	1.00	1.00	1.00
Cs 50 mM	1.21	1.03	0.97	1.09	1.04	0.97
Cs 100 mM	1.03	1.00	1.00	1.01	1.00	1.00
Sr 5 mM	1.00	1.01	0.99	1.00	1.01	0.99
Sr 10 mM	1.01	1.01	0.99	1.01	1.01	0.99
Sr 25 mM	1.06	1.02	0.98	1.03	1.02	0.98
Sr 50 mM	1.05	1.01	0.99	1.03	1.01	0.99
Sr 100 mM	0.01	1.00	1.00	0.00	1.00	1.00
Aspergillus sp.						
Cs 5 mM	1.00	1.00	1.00	1.00	1.00	1.00
Cs 10 mM	1.02	1.01	0.99	1.03	1.01	0.99
Cs 25 mM	0.70	1.06	0.98	0.81	1.05	0.98
Cs 50 mM	0.33	1.05	0.97	0.48	1.08	0.97
Cs 100 mM	0.02	0.72	1.00	0.00	0.45	0.99
Sr 5 mM	0.83	1.02	1.01	0.89	1.01	1.01
Sr 10 mM	0.79	1.05	1.00	0.82	1.04	1.01
Sr 25 mM	0.57	1.09	0.99	0.69	1.09	0.99
Sr 50 mM	0.48	1.03	0.98	0.60	1.04	0.98
Sr 100 mM	1.59	1.01	0.97	1.63	1.01	0.97

A perfect agreement between the experimental value and the model value would be represented by a bias factor of 1. Higher or lower values indicate a systematic over- or under- simulation of experimental values, respectively. Table 4-2 shows that a

perfect agreement between the experimental and predicted values mostly occurred for control conditions and when growth rates of fungi reached a plateau. During lag and exponential phases, exposure to elements affects the adaptation of fungi. As you can see in Table 4-2, the response of more sensitive fungi, such as *Trichoderma* sp., to Cs exposure during adaptation could not be agreeably predicted by a model. In certain cases, as the concentrations of element were increased, observed values diverged further from model predicted values. Serial correlations for residuals were examined using the DW statistic (Table 4-1) to test residuals for their independence to each other. The DW values were obtained by fitting models to the growth data.

The results show that DW was in the range of 1.5– 2.5, indicating that residuals did not correlate: a result obtained when the serial correlation is small and residuals are randomly distributed around the zero line when plotted against time. The goodness of fit (R^2) values shown in Table 4-1 are in most cases close to unity; however, some differences were detected among models, indicating that this statistic can be used as a basis for model ranking. Discrimination among models can be achieved by a statistical comparison. In this case, using only the R^2 statistic does not provide sufficient information as different models are associated with different numbers of parameters. Therefore, data fits obtained by using various models were statistically compared using the F ratio test. The results are shown in Figures 4-8.

The F-statistic is the test statistic for the analysis of variance approach to test the significance of the model. It is to compare standard deviations between 2 data sets. Differences between the results of two models and experimental data are evident while they generally followed the same trend; however, the F test indicated a suitable model fit when the condition was not affected by elemental exposure or tolerant fungi species were exposed. The first one gives critical values of F which is given the confidence levels (0.05), the numerator degrees of freedom, and the denominator degrees of freedom. The results showed when the F critical values smaller than F values indicated that the model was rejected because the growth of fungi was affected by toxic elements, particularly for sensitive fungi genera.

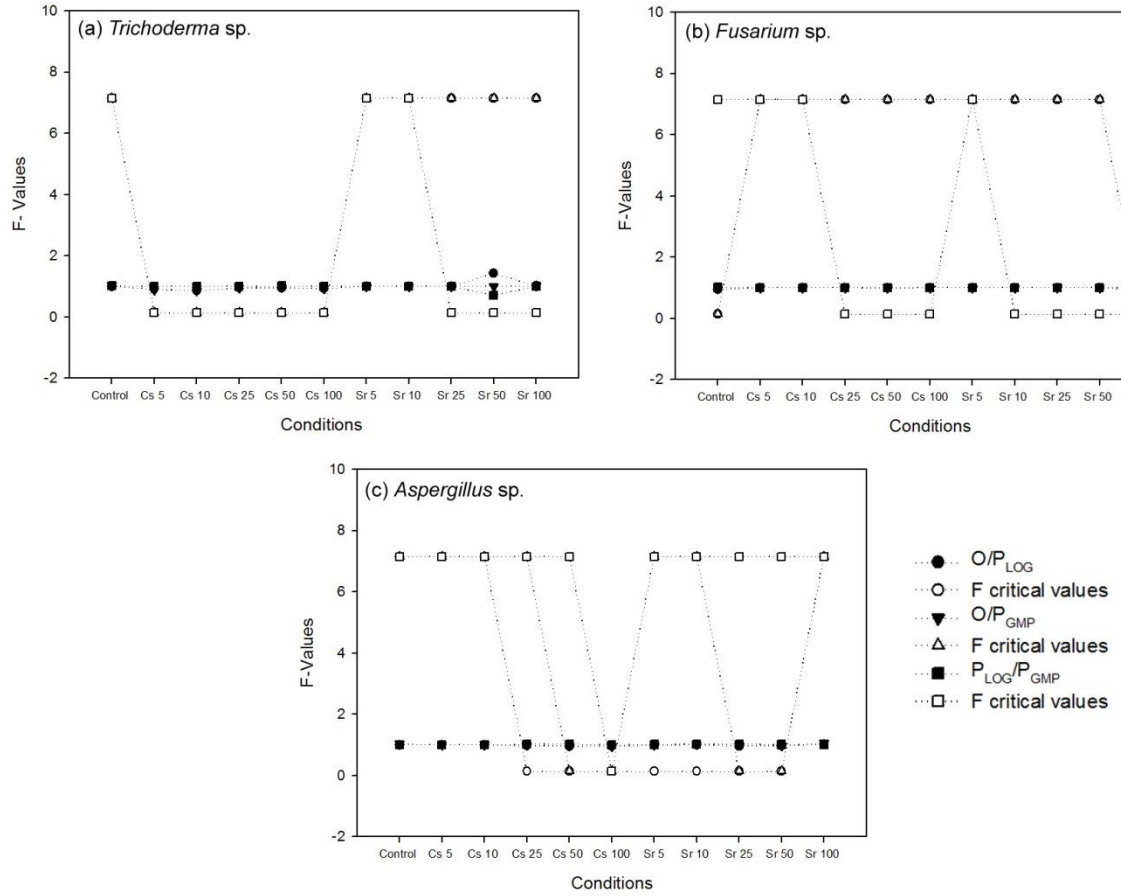

Figure 4-8 Model fits of (a) *Trichoderma* sp. (b) *Fusarium* sp. (c) *Aspergillus* sp. growth data assessed using the F ratio test. The Gompertz and Logistic models are compared.

Table 4-3 Statistics and parameter values obtained when models are fitted to the growth data.

	Cesium (Cs)			Strontium (Sr)		
	A	**λ**	**μ_m**	**A**	**λ**	**μ_m**
Trichoderma sp.						
Control	2.74	0.05	8.90	2.74	0.05	8.90
5 mM	1.70	0.62	5.11	2.48	0.55	5.42
10 mM	1.68	0.55	5.44	2.42	0.55	5.36
25 mM	1.61	0.33	6.85	2.27	0.61	5.06
50 mM	1.64	0.14	10.72	2.21	0.65	4.82
100 mM	1.32	1.14	3.72	2.08	0.81	4.40
Fusarium sp.						
Control	2.73	0.06	9.21	2.73	0.06	9.21
5 mM	2.30	0.09	8.41	2.28	0.15	8.06
10 mM	2.25	0.21	7.54	2.23	0.25	7.55
25 mM	2.14	0.26	7.04	2.13	0.23	7.67
50 mM	1.99	0.45	5.52	2.03	0.26	6.99
100 mM	1.84	0.47	5.02	1.91	0.89	4.75
Aspergillus sp.						
Control	2.75	0.03	13.54	2.75	0.03	13.54
5 mM	2.68	0.11	11.78	2.45	0.03	18.19
10 mM	2.55	0.06	14.61	2.30	0.02	21.27
25 mM	2.39	0.02	25.91	2.29	0.02	22.61
50 mM	1.77	0.03	19.49	2.07	0.05	17.24
100 mM	1.47	0.20	8.93	1.93	0.05	16.41

The sigmoidal models to describe growth data can be constructed from the fungi growth parameter, which is shown in Table 4-3

Growth curves of three fungi genera for different initial Cs or Sr concentrations. In the case of the control, there was no exposure to elements. The results show a similar trend among cells for all genera, where the number of cells was inversely correlated with the concentration (Table 4-3).

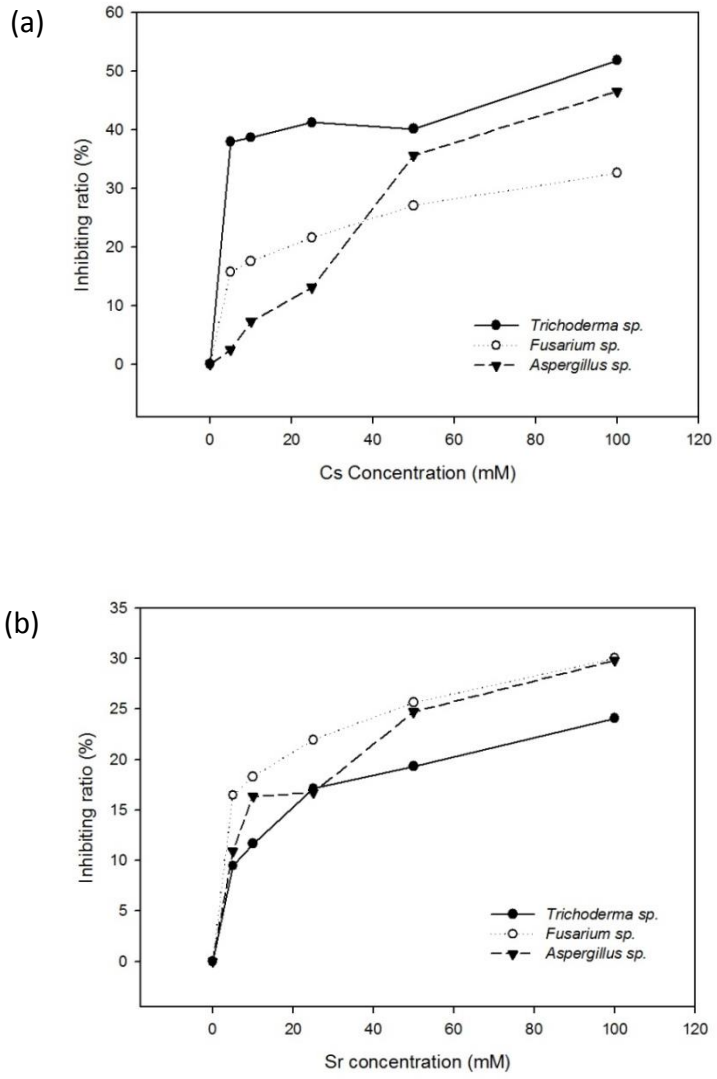

Figure 4-9 Inhibitory effect of (a) cesium (b) strontium on the growth of fungi

Figure 4-9 shows the results of the inhibitory effect of (a) Cs and (b) Sr on fungi growth, including *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp. that were cultured for 7 days at 25°C. The inhibiting ratio (IR) was calculated by Equation (4-7).

$$\mathrm{IR}(\%) = \frac{A_c - A_t}{A_c} \times 100\% \qquad (4\text{-}7)$$

Where A_c is A of control and A_t is the specific growth rate at time ∞ for each condition derived from the Sigmoidal model (Table 4-3).

Different species of fungi had different responses to exposure to Cs and Sr. For instance, the inhibiting ratio to *Trichoderma* sp. and *Aspergillus* sp. is >40% when concentrations of Cs are >50 mM. The values of the inhibiting ratio were transformed to probability value. EC_{50} values which is the concentration of a Cs and Sr that gives half-maximal response, were then calculated according to linear regression (Mu, Zhou, Hu, Zhang, Zhang, & Cui, 2006). The results are shown in Tables 4-4.

Table 4-4 Tolerance of Cs and Sr to fungi

Fungi Genus	**Cesium**		**Strontium**	
	EC_{50} (mM)	**R^2**	**EC_{50} (mM)**	**R^2**
Trichoderma sp.	80.17	0.4012	222.28	0.7292
Fusarium sp.	157.24	0.6939	178.48	0.6045
Aspergillus sp.	96.73	0.9385	171.74	0.7577

The experimental results show the tolerance of Cs to the assessed fungi are *Trichoderma* sp. >*Aspergillus* sp. > *Fusarium* sp., whereas the tolerance of Sr to the assessed fungi are *Aspergillus* sp. >*Fusarium* sp. >*Trichoderma* sp. These results showed that fungi genera are more sensitive to Cs than Sr although, the natural levels of Cs and Sr in soil were about 20 mg/kg or 600 to 650 times lower than EC_{50} values. Hence, all representative fungi can survive even in the environment extremely contaminated by Cs and Sr.

Similar results were in addition observed for various microorganisms in previous studies (Nies, 1999). For example, (Monsieurs, et al., 2011) examined the metal resistant *on Cupriavidus metallidurans* which is the soil bacterium involved in the

resistance and processing of heavy metals. The result showed minimum inhibitory concentration is 125 mM for Cs^+ and 200 mM for Sr^{2+}. (Kuwahara, et al., 2005) reported that filamentous actinomycetes are more sensitive to Cs than planktonic bacteria. (Perkins & Gadd, 1993) examined toxicity screening of several yeast strains, showing that *Rhodotorula rubra* and *Saccharomyces cerevisiae* are more sensitive than *Candida albicans*.

Most Cs^+ in the cell of a microorganism is maintained in a soluble form because of its high solubility and weak coordinating ability in either the cytoplasm or the vacuole. The relative distribution of intracellular Cs^+ between the cytoplasm and vacuole is largely determined by the activity of the vacuolar membrane H^+—ATPases, which serve to drive transport of monovalent cations from the cytoplasm into the vacuole (Jongbloed, Clement, & Borst-Pauwels, 1991). The fraction of the cell volume occupied by vacuoles considerably varies in different microorganisms. In fungi, vacuoles occupy between 25% and 95% of the cell volume, and the ratio of total K^+ to cytoplasmic K^+ is approximately 4:1 (Duffus and Paterson, 1974). Compared with other toxic metal elements such as Cu^{2+} or Cd^{2+} (Gadd, 1993), Cs has relatively low tolerance. For example, 1 mM of Cs solution reduced the final growth yield of cyanobacterium in a batch culture by only 70% (Avery, Codd, & Godd, 1993) and 80 mM, 97 mM, and 157 mM for *Trichoderma* sp., *Fusarium* sp., and *Aspergillus* sp., respectively, indicating that fungi can reduce their growth yield by 50%. (Perkins & Gadd, 1993) studied whether vacuoles play an important role in intercellular Cs^+ detoxification in microorganisms. In addition to intercellular detoxification, microbial tolerance to elements can result from decreased intracellular uptake. It is primarily the weak coordination characteristic displayed by Cs^+ and consequently its low tendency to exert such potentially toxic effects by blocking of functional groups on biologically important molecules, causing conformational modification, denaturation, and inactivation of enzymes and disputing cellular and organelle membrane integrity. An additional organelle, which may be important to intracellular target for Cs^+, is the ribosome because Cs^+ can be irreversible dissociation of protein from 50S and 30S ribosome (Lerman, Spirin, Gavrilova, & Golov, 1966)

4.5 Conclusions

It is necessary to measure the growth curves in order to use models to describe the growth of fungi in an environment affected by high concentrations of certain Cs and Sr. To reduce measured data to interesting parameters, such as the growth rate, it is recommended that data be described using a model instead of using linear regression over a subset of data. In the present study, fungi growth kinetic models were applied to simulate fungi growth and lag time in the presence of two elements, Cs and Sr, and were proposed and validated using data obtained for pure cultures of representative soil fungi follow by *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp. data. Sigmoidal models to describe the growth data can be constructed with three main parameters. There is not a single, simple statistical method to evaluate similarities and differences between sigmoidal models and to deal with the question of which model should be used. The statistical performance obtained led to some suggestion for the evaluation of models, as most of the statistical tests performed were consistent when rating models, although an overall assessment is required. According to analyses of residuals, the GMP and LOG models showed behavior that was accepted by the F test, provided the fungi were not affected by elemental conditions or tolerant fungi species were assessed. The model was rejected when the growth of fungi was affected by the toxic status of elements, in particular, the more sensitively fungi genera.

The research conducted in the current study mainly relates to the tolerance to study the effect of Cs and Sr on the evaluated three fungi genera. Recently, more attention has been focused on this field, and as a result, some achievements have been made, including the evidence of the direct inhibitory effect of Cs and Sr on three fungi genera. The present study further indicated that Cs has a significant direct inhibition effect on fungi, with an EC_{50} of 80–160 mM, whereas Sr has a less significant direct inhibition effect on fungi, with an EC_{50} of 171–222 mM. The tolerance of Cs to fungi in order of severity is as follows: *Trichoderma* sp. >*Aspergillus* sp. >*Fusarium* sp., whereas the toxicity of Sr to fungi was contradictory in order of severity as follows: *Fusarium* sp. > *Aspergillus* sp. > *Trichoderma* sp. However, the natural levels of Cs and Sr in soil were lower than EC_{50} values 600 to 650 times. Hence, all representative fungi

being adapt or evolutionarily to survive and reproduction even in the environment extremely contaminated by Cs and Sr. As a result, it induced an opportunity to interact with Cs and Sr in the soil solution.

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Chapter 5

Biosorption Kinetic of Cesium and Strontium

Previous investigators have studied various fungal species to define responsive signaling to the presence of cesium (Cs) and strontium (Sr) in the environment. The fates in the environment of both Cs and Sr are largely influenced by the sorption process. Therefore, the main mechanism has been hypothesis that depending on the biosorption mechanism. The aim of this chapter is to apply kinetic models based on nonlinear regression employing commonly used kinetic expressions to determine the sorption rates of Cs and Sr. Both stable and radioactive isotopes of Cs and Sr are investigated using experiments performed under various conditions.

5.1 Objective:

- To assess the potential of soil fungi for the sorption of Cs and Sr in term of contact time
- To perform nonlinear regression for pseudo first-order and pseudo second-order kinetic models.

5.2 Theory and Significance

Soil contamination by cesium (Cs) and strontium (Sr) from nuclear accidents has led to a resurgence of interest in microbe-radioactive interactions. Cs is the rarest of alkaline metals that is strongly adsorbed onto clay minerals (Cremers, Elsen, & De Preter, 1988); (Atsushi, Shinya, Hirofumi, & Takashi, 2012). Sr is one of the most important radionuclides because it is characterized by a high ability to migrate. An investigation of the Chernobyl fallout found that Cs was still localized mainly in the organic soil layer more than a decade after deposition, indicating that Cs has a low rate of vertical movement and low bioavailability for plant-uptake (Rosen, Oborn, & Lonsjo, 1999); (Steiner, Linkov, & Yoshida, 2002). It is likely that the effects of fungal and microbiological activity have substantially contributed to the long-term retention of Cs and Sr in the organic layer. Thus, the higher microbial biomass in biotic systems plays

an important role in accumulating both nutrient elements and radionuclides. It has also been shown that the fate of Cs and Sr in the environment is largely influenced by the sorption process (Newsome, Morris, & Lloyd, 2014) (Newsome et al., 2014).

The nature of a given sorption process depends on the physical and chemical characteristics of the adsorbent system and the system conditions. Thus, the prediction of batch sorption kinetics is necessary to describe the sorption process, and kinetic expressions are commonly used to explain how fast the rate of sorption occurs. It is important to determine the time needed to reach equilibrium and the examination of the rates of adsorption that can be used to develop models and to understand the solutes on the adsorbent surface. The pseudo-first order rate has long been widely applied for these types of systems. For example, (Seki & Suzuki, 1997) reported that a pseudo first-order rate best fit a lead sorption system. The expression is shown in equation (5-1)

$$\frac{\mathrm{d}q_t}{\mathrm{d}t} = k_1(q_1 - q_t) \tag{5-1}$$

This expression can be rearranged for linearized data plotting by integrating for the boundary conditions t = 0 to t = t and $q_t = 0$ to $q_t = q_t$, as shown by equation (5-2):

$$\log(q_1 - q_t) = \log(q_1) - \frac{k_1}{2.303}t \tag{5-2}$$

Where K_1 is the rate constant for first order sorption, q_1 is the amount of solute adsorbed at equilibrium, and q_t is amount of solute adsorbed on the surface of the fungi cell at time = t.

The pseudo first-order kinetic rate equation often provides an excellent fit with experimental kinetic data for various sorption processes. However, in some previous studies, it has failed to theoretically predict the q_1 values, indicating that these sorption processes deviate from theory (Allen, McKay, & Khader, 1989). The pseudo second-order rate equation has also been found to fit most experimental data for

sorption systems (Ho & McKay, 1999), and in particular, well explain the kinetics of the sorption of heavy metals (Hui, Chao, & Kot, 2005) and other inorganic matter (Mahmut, I. Ayhan, engil, & Harun, 2008). For adsorption systems following pseudo second-order kinetics, the Cs or Sr ions are assumed to be adsorbed onto two surface sites. Thus, the sorption kinetics following pseudo second-order kinetics can be expressed by rewriting equation (5-1) as equation (5-3):

$$\frac{\mathrm{d}q}{(q_1 - q_t)^2} = K_2 \mathrm{d}t \tag{5-3}$$

Integrating equation (5-3) for the boundary conditions t = 0 and t = t and q = 0 and q = q affords equation (5-4):

$$\frac{1}{(q_1 - q_t)} = \frac{1}{q_e} + K_2 t \tag{5-4}$$

The nonlinear pseudo second-order and pseudo first-order kinetic models and their linearized expressions are summarized in Table 5-1.

Table 5-1 Linear and nonlinear forms of the pseudo first-order and pseudo second-order kinetic models

Type	Nonlinear	Linear
pseudo-first order	$q = q_1 (1 - e^{-K_1 t})$	$\log(q_1 - q_t) = \log(q_1) - \frac{k_1}{2.303} t$
pseudo-second order	$q = \frac{K_2 q_1^2 t}{1 + K_2 q_1 t}$	$\frac{t}{q} = \frac{1}{K_2 q_1^2} + \frac{1}{q_1}$t

Note that the nonlinear regression model was found to be more appropriate than the linear model for predicting the optimum rate and kinetic parameters for both the pseudo-first and second-order kinetics of methylene blue sorption onto an activated carbon system (Kumar, 2006).

In the present study, regression of the pseudo first-order and pseudo second-order kinetic models for Cs^{+} and Sr^{2+} sorption on fungi cells was performed. In addition, the sorption behaviors of the radioactive isotopes of both elements (Cs^{134} and Sr^{85}), which exhibit no general differences from their stable isotopes with respect to their behavior in the environment, were evaluated. The obtained data were used to determine how fast soil fungi accumulate Cs and Sr via the sorption process.

5.3 Procedure

5.3.1 Material

- Fungi cell culture: *Fusarium* sp., *Trichoderma* sp., *Aspergillus* sp.
- Cesium Chloride (CsCl)
- Strontium Chloride ($SrCl_2$)
- Radiotracers: ^{134}Cs and ^{85}Sr
- Centrifuge tubes
- Filter paper (Whatman no.1)
- 50 mL Potato dextrose Broth (PDB) per experimental conditions
- Inductively coupled plasma mass spectrometry (ICP-MS; XSeries 2, Thermo Scientific), Figure 5-1
- Gamma spectrometer with an HPGe detector (GMX series, EG&G, Ortec)
- Shaker
- Benchtop balance

5.3.2 Method

1. Active cells of the fungi (Chapter 3) *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp. were separately cultured at 25°C in PDB with shaking at 110 rpm until the late exponential growth phases were reached. Resting cells were then obtained by washing the cultured cells three times with sterile purified water to halt cell growth.

2. To investigate the sorption of both the stable and radioactive isotopic forms of Cs and Sr, the resting cells (1) were re-suspended in 25 mL of sterile

Figure 5-1 ICP-MS XSeries 2 instrument from Thermo Scientific.

distilled water. The experimental conditions are shown in Table 5-2. The fungi cells were incubated at 25°C with shaking at 110 rpm for a given time interval (0, 0.5, 1, 3, 6, 9, or 24 h) and filtered through dried filter paper (Whatman no.1). The Cs and Sr remaining in the aliquot and the dry weight of cell pellets were then determined. In this experiment, the effect of pH has been negligible. The initial pH of each solution was approximately 5, and no pH adjustment was performed.

Table 5-2 Experimental conditions for the evaluation of Cs and Sr sorption

No.	Elements	Concentration
1	Cs^{+}	5 μg/L
2	Sr^{2+}	5 μg/L
3	Cs^{+}/ Sr^{2+}	10 μg/L
4	^{134}Cs/ ^{85}Sr	^{85}Sr; 4 kBq/L; and ^{134}Cs; 20 kBq/L*

* ^{85}Sr 4 kBq and ^{134}Cs 20 kBq are equal $4.6x10^{-6}$ and $4.2x10^{-4}$ μg, respectively

3. Cs^{+} and Sr^{2+} are stable isotopes and their concentrations were determined using inductively coupled plasma mass spectrometry; ICP-MS (XSeries 2, Thermo Scientific). Samples were prepared following the ISO17294-2 and EPA 6020a standard methods (Henk, 2003). The γ-ray spectra of the radioactive isotopes ^{134}Cs and ^{85}Sr were obtained for 30 to 60 min using a gamma spectrometer with an HPGe detector, and a channel analyzer was used to detect the gamma radiation from the samples.

5.4 Results and Discussion

5.4.1 Kinetics of the stable isotopes of Cs and Sr

Two types of experiments were performed to determine the sorption kinetics of the stable isotopes of Cs and Sr: individual studies, wherein the sorption rate for each element was determined separately in batch experiments, and a combined study, wherein the competitive sorption of the two elements was evaluated. The quantities of the elements adsorbed onto the fungi cells were fitted with the nonlinear regression results for the pseudo first-order and pseudo second-order models (Figure 5-2 to 5-3).

The results for both experimental conditions indicate that increase of the contact time above the equilibrium time did not result in any significant increase in the sorption of Cs or Sr by the fungi cells. In addition, the sorption rates were rapid when the concentrations of the element ions were high, such as during the initial period. However, as the contact time increased, the rates, and consequently the sorption efficiencies for the element ions by the fungi cells, decreased and eventually became nearly constant, likely due to saturation of the ions on the cell surfaces.

The batch experiments were first performed using each element by itself in order to optimize the sorption conditions. The starting concentration of each element was 5 μg/L, and sorption was evaluated as a function of time to determine the optimal contact time. The results followed the same trend for bathched systems studied, with the values for the correlation coefficients (r^2) for the pseudo first-order fittings higher than those for the pseudo second-order fittings. On the other hand, the calculated q_1 values

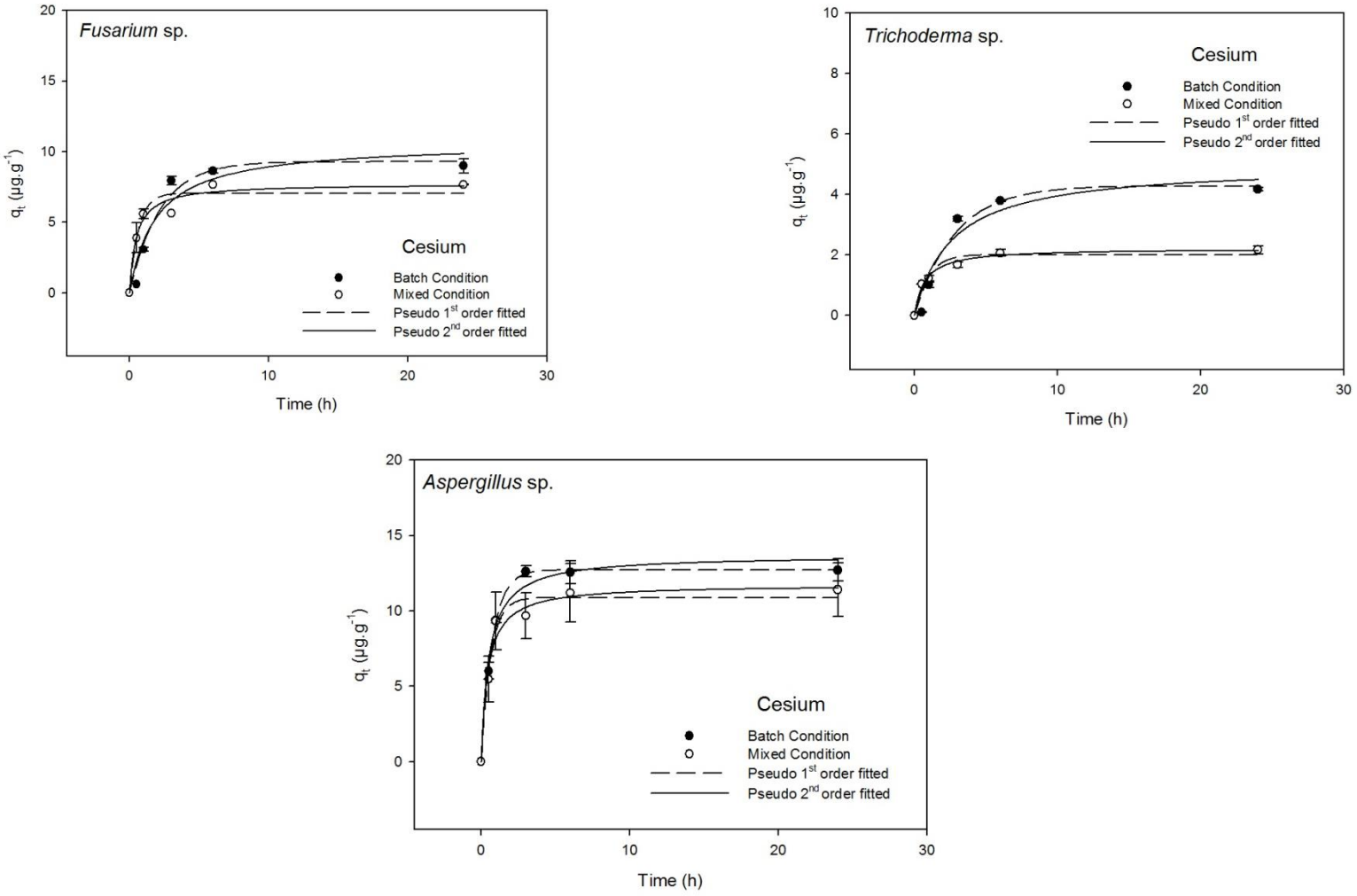

Figure 5-2 Concentration–time profiles for the biosorption of Cs onto resting of fungi cells

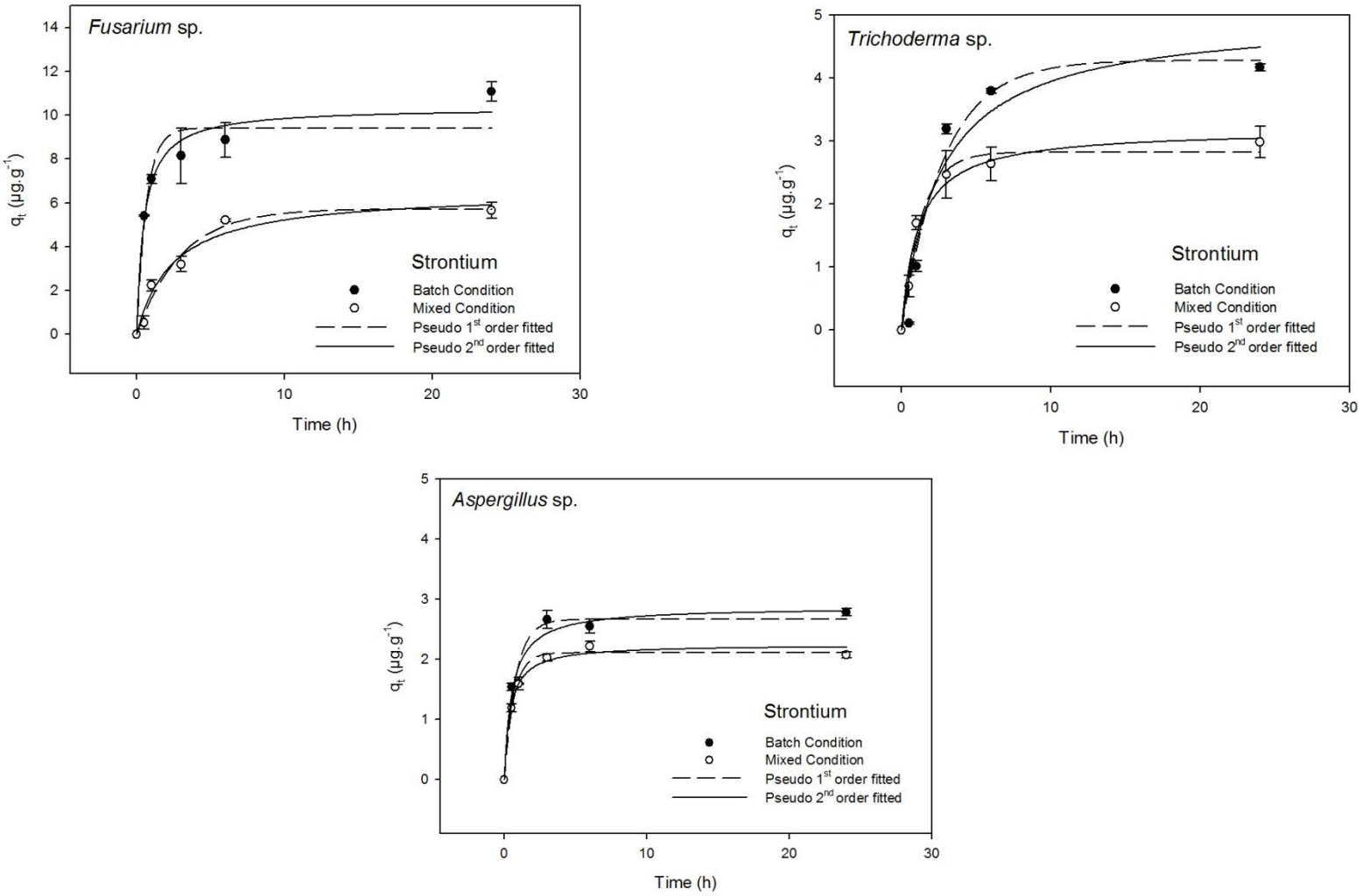

Figure 5-3 Concentration–time profiles for the biosorption of Sr onto resting of fungi cells

were higher for the pseudo second-order model than those for the pseudo first-order model, $(q_1)_2 > (q_1)_1$, and the theoretical values obtained using the pseudo second-order model were closer to the experimental values.

Table 5-3 Kinetic parameters and correlation coefficients for nonlinear regression of the pseudo first-order and pseudo second-order models for the adsorption of Cs and Sr

Fungi cell	pseudo-first order			pseudo-second order		
	k_1 (min^{-1})	$(q_1)_1$ ($\mu g.g^{-1}$)	r^2	k_2 ($\mu g.g^{-1}\ min^{-1}$)	$(q_1)_2$ ($\mu g.g^{-1}$)	r^2
Batch Condition						
Cesium						
Fusarium sp.	0.46	9.28	0.97	0.05	10.63	0.93
Trichoderma sp.	0.35	4.28	0.97	0.08	4.98	0.94
Aspergillus sp.	1.31	12.71	0.99	0.14	13.66	0.99
Strontium						
Fusarium sp.	1.52	9.41	0.94	0.19	10.34	0.97
Trichoderma sp.	0.35	4.28	0.97	0.09	4.28	0.94
Aspergillus sp.	0.02	2.66	0.97	0.01	2.86	0.97
Mixed condition						
Cesium						
Fusarium sp.	1.53	7.06	0.94	0.27	7.71	0.96
Trichoderma sp.	1.05	2.01	0.96	0.64	2.21	0.99
Aspergillus sp.	1.59	10.87	0.98	0.20	11.73	0.98
Strontium						
Fusarium sp.	0.34	5.72	0.97	0.06	6.50	0.96
Trichoderma sp.	0.75	2.82	0.98	0.29	3.18	0.98
Aspergillus sp.	0.02	2.11	0.99	0.02	2.24	0.99

The parameters and correlation coefficients (r^2) for the nonlinear regressions of the pseudo first-order and pseudo second-order equations using the data are summarized in Table 5-3. Nearly the same trends were observed for mixed between Cs and Sr system. The competitive sorption between those element was occurred as those obtained q_1 value were much lower than the individual sorption of Cs and Sr. The K represent the rate constant determined by analyzing the reacting system. The value of K depends on the operating system such as concentration of Cs and Sr. It was determined by slowest processed. For example, since it takes a longer time to reach equilibrium, K values also decreases.

Previous studies have shown that the sorption of ions follows a two-step mechanism. First, the ions are adsorbed onto the surface of the fungi cells via a physicochemical process, namely passive transport, over a relatively short contact, and thus, this process is reversible. Subsequently, the ions are taken up into the cell via a biological mechanism (Volesky, 1990) namely active transport, which requires a long contact time to reach equilibrium (Michael & Ayebaemi, 2005). In the present study, the fungi cells were washed with sterile, purified water to halt cell growth and cell activity. Consequently, their biological functions were no longer active and sorption could only take place on the surfaces of the cells. Therefore, the sorption equilibrium was rapidly reached within 60 min, and no further sorption was observed, even when each of the solutions with the different elements was mixed for a further 2 h.

5.4.2 Sorption kinetics of the radioactive isotopes of Cs and Sr

Cs and Sr are radioactive elements that are released into the environment. Thus, in the present study not only the sorption kinetics of the stable isotopes of Cs and Sr but also those of ^{134}Cs and ^{85}Sr, which are their respective radioactive elements, were evaluated. This experiment was conducted under mixed conditions with a competitive between Cs and Sr. The initial activities of the radiotracers for each element were approximately 4 and 20 kBq/L for ^{85}Sr and ^{134}Cs, respectively, and were determined using the total volume of the solution.

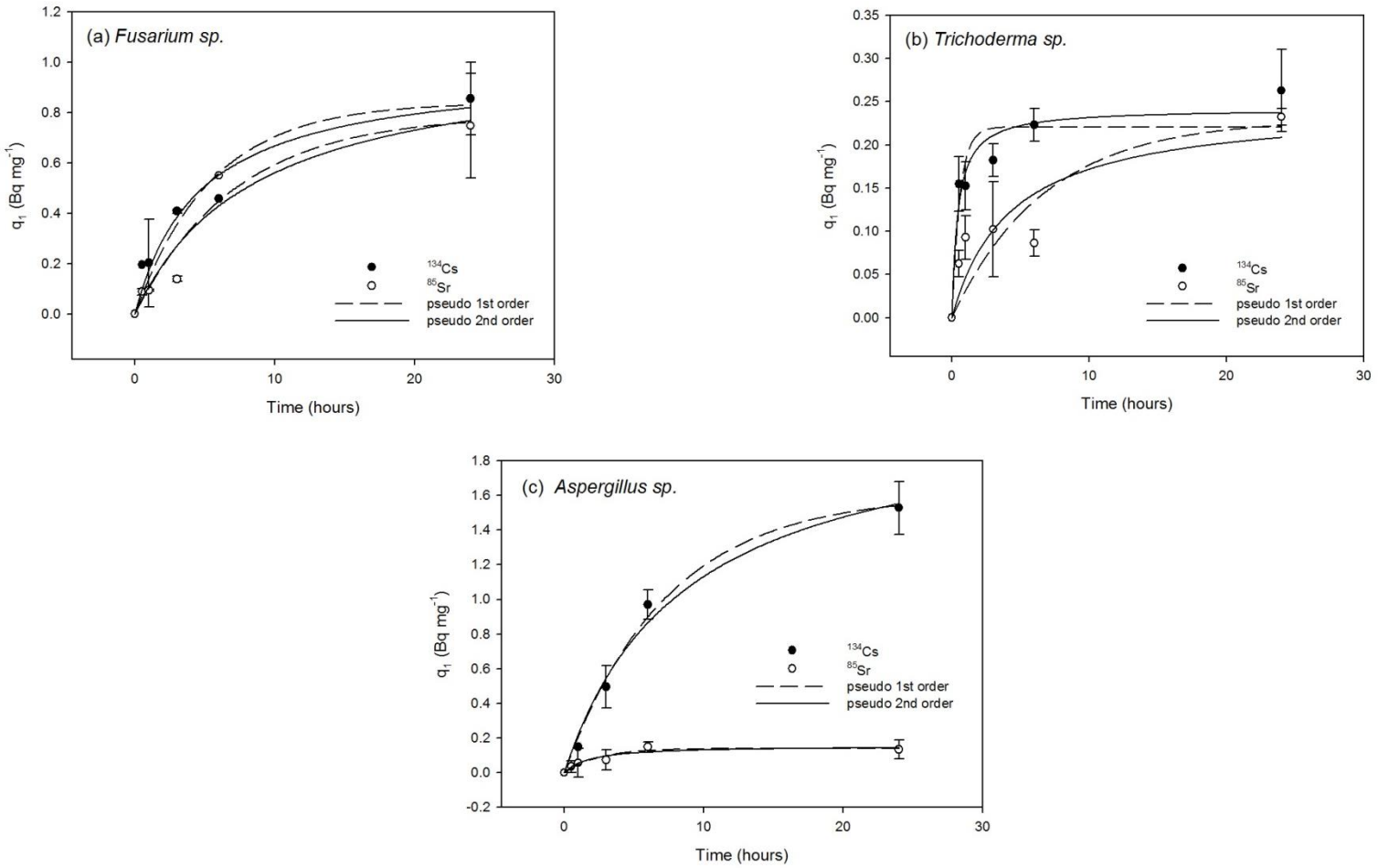

Figure 5-5 Activity - time profiles for the sorption of radioactive isotopes: ^{134}Cs and ^{85}Sr onto the resting of fungi cell (a) *Fusarium* sp. (b) *Trichoderma* sp. (c) *Aspergillus* sp.

The results indicate a similar trend to that observed for the stable isotopes: no significant increase in adsorption by the fungi cells was observed at contact times beyond the point at which equilibrium was reached. The parameters and correlation coefficients (r^2) for nonlinear regression of the pseudo first-order and pseudo second-order equations are summarized in Table 5-5

Table 5-5 Kinetic parameters and correlation coefficients for nonlinear regression of the first-order and pseudo second-order models for the competitive adsorption of the radioactive isotopes of Cs and Sr

Fungi cell	pseudo-first order			pseudo-second order		
	k_1 (min^{-1})	$(q_1)_1$ ($Bq.mg^{-1}$)	r^2	k_2 ($Bq.mg^{-1}min^{-1}$)	$(q_1)_2$ ($Bq.mg^{-1}$)	r^2
Cesium						
Fusarium sp.	0.01	0.84	0.92	0.01	0.98	0.95
Trichoderma sp.	0.03	0.22	0.88	0.12	0.24	0.94
Aspergillus sp.	0.01	1.6	0.99	0.01	2.11	0.99
Strontium						
Fusarium sp.	0.14	0.79	0.93	0.11	1.04	0.93
Trichoderma sp.	0.15	0.23	0.70	0.95	0.25	0.75
Aspergillus sp.	0.40	0.14	0.91	0.55	0.15	0.91

It can be seen in Table 5-5 that the correlation coefficients for fitting of the pseudo second-order equation were generally greater than the r^2 values for fitting of the pseudo first-order equation. Based on these results, it can be concluded that, in contrast to the results for the stable isotopes of Cs and Sr, the kinetic data for their radioactive isotopes under competitive biosorption conditions was best correlated to the pseudo second-order kinetic model.

Note that the rates of biosorption of the radioactive isotopes of Cs and Sr (^{134}Cs and ^{85}Sr, respectively), which are important for designing batch biosorption

experiments, were different than those for the stable isotopes of the two elements, particularly for *Fusarium sp.*, but also for *Trichoderma sp*. It can be clearly seen in Figure 5-5 that the biosorption yields for ^{134}Cs and ^{85}Sr for these two fungi considerably increased and did not reach a plateau, which is in contrast to the results obtained for the stable isotopes.

5.5 Conclusions

Sorption experiments for Cs and Sr were conducted using three genera of fungi (*Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp.) under various conditions. The sorption characteristics were examined at different contact times in order to provide kinetic data, which were fitted with both pseudo first-order and pseudo second-order two kinetic equations. The experimental data for the stable isotopes of Cs and Sr were better described by the pseudo first-order model for both individual and competitive sorption. Nearly the same trends were observed for mixed between Cs and Sr system. The competitive sorption between those element was occurred as those obtained q_1 value were much lower than the individual sorption of Cs and Sr indicated that in mixed condition between 2 elements, the amount of Cs and Sr which were sorbed are decrease from the individual condition and the rate constant (K) was higher in mixed condition indicated that in the competitive sorption, it takes a faster time to reach equilibrium than in the individual condition. Therefore, the sorption equilibrium was rapidly reached within 60 min, and no further sorption was observed, even when each of the solutions with the different elements was mixed for a further 2 h. On the other hand, the experimental data for the radioactive isotopes (^{134}Cs and ^{85}Sr) were better described by the pseudo second-order model, as indicated by the values of the corresponding correlation coefficients.

5.6 Achievement

Parts of this chapter have been presented at the 2014 4th International Conference on Environment and BioScience (ICEBS 2014), Jinju, Republic of Korea and publish in International Journal of Pharma Medicine and Biological Sciences (IJPMBS, ISSN: 2278-5221) Vol.4 No.2 pp.110-114

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Chapter 6

Biosorption Isotherm of Cesium and Strontium

Whereas the previous chapter examined the rate of sorption, this chapter intends to clearly understand how a soil fungus accumulates cesium (Cs) and strontium (Sr) in the form of both stable and radioactive isotopes. The sorption isotherms such as Langmuir and Freundlich have been used to describe the sorption characteristics and to quantify the sorption capacity. Batch experiments described in this chapter have been performed under various conditions.

6.1 Objective:

- To assess the potential of soil fungi for the sorption of Cs and Sr.

6.2 Theory and Significance

Microbiota, including fungi, bacteria, actinomycetes, protozoa, microfauna, and algae, collectively comprise one of the most important components determining radionuclide bioavailability and cycling. Unicellular organisms (e.g., *Euglena* and *Chlorella*) were the first microorganisms to be investigated for their ability to accumulate Cs (Williams, 1960). Research on freshwater algae, cyanobacteria, and bacteria isolated from the natural environment (e.g., *Synechocystis* spp., *Rhodococcus* spp., and *Streptomyces* spp.) has been carried out to investigate their bio-accumulation capacity. Moreover, yeasts (e.g., *Saccharomyces cerevisiae*, *Rhodotorula rubra*, and *Candida albicans*) (Harvey & Patrick, 1967); (Avery, 1995); (Tomika, Uchiyama, & Yagi, 1994); (Kuwahara, et al., 2005) showed greater accumulation in the logarithmic phase than in the stationary phase of kinetic growth (Perkins & Gadd, 1993). Fungi are often the major component of soil microbiota that play a critical role in the soil ecological function. Fungal biomass can function as a bio-accumulator of soluble forms of inorganic pollutants including radionuclides. Higher fungi (e.g., lichens and mushrooms) have been investigated as indicators of Cs contamination in the environment and have demonstrated Cs accumulation in their fruit

bodies or hyphae. The mechanism of Cs accumulation has been explained as Cs being immediately and non-specifically adsorbed onto the negatively charged cell surfaces and then absorbed into the cytoplasm (Kuwahara, Fukumoto, Nishina, Sugiyama, Anzai, & Kato, 2011). (Dighton, Clint, & Poskitt, 1991) reported fungi in organic soil layers have the potential to immobilize radioactive Cs. Thus, the migration of Cs through the soil layers was reduced, which also may alter the availability of Cs to plants. Mycorrhizal fungi are an important group of soil fungi that may have similar effects, thus influencing the uptake of inorganic pollutants including radionuclides (Riesen & Brunner, 1996). Saprotrophic fungi are a group of free-living soil fungi that have the ability to decompose organic residues. In the current study, the fungi were selected within the following genera: 1) *Fusarium*, which is a large genus of filamentus fungi widely distributed in soil and associated with plants; 2) *Trichoderma*, which is a genus of saprotrophic fungi that produces various kinds of secondary metabolites; and 3) *Aspergillus*, which is a genus originally isolated from soil. Radionuclides may attach to these fungal cells extracellularly, or be actively taken up intracellularly.

Radioactive isotopes, particularly Cs and Sr, are present in the soil in their cationic forms, and there are no general differences between the radioactive and stable isotopes with respect to their chemical elements or their behavior in the environment. Cs is a rare type of alkali metal existing in the environment as a monovalent cation (Cs^{+}) and whose fission products include four main Cs isotopes, and its stable isotope is ^{133}Cs. The isotopes of Cs have long half-lives (2 years for ^{134}Cs and 30 years for ^{137}Cs) and, thus, have become the dominant nuclides contaminating the environment. In nature, Sr occurs only in the Sr^{2+} oxidation state, whereas radioactive isotopes are produced by fission reactions. In particular, ^{90}Sr is a problematic environmental contaminant because of its long half-life (29 years), high transferability, and high fission yield. In general, the fate of Cs and Sr in the environment is mostly influenced by the sorption process (Newsome, Morris, & Lloyd, 2014); (Bakken & Olsan, 1990). Thus, in the current chapter, the sorption characteristics graph was plotted and fitted with the sorption isotherms to quantify the ability of soil fungi as through sorption isotherm.

The sorption process considered involves a solid phase, which contains fungal cells and a liquid phase, which contains the Cs and Sr solution to be sorbed. The sorption isotherms are plots between the sorption uptake and the final equilibrium concentration of the residual sorbate in the solution. Correspondingly, the amount of Cs and Sr bound to the cell that disappears from the solution can be calculated on the basis of the mass balance in the system. The relationship between Cs and Sr was sorbed onto the cell (q) and the residue of Cs and Sr in the solution (C_e) can also be mathematically expressed. Firstly, using the Langmuir isotherm, which is the relationship of the hyperbolic form represented by Equation 6-1

$$q_e = \frac{q_m K_L C_e}{1 + C_e} \tag{6-1}$$

Where q_e is the amount of Cs or Sr sorbed, C_e is the amount of Cs or Sr residue in the solution, q_m is the maximum amount of Cs or Sr sorbed, and K_L is an equilibrium constant representing the affinity between the fungi cell and Cs and Sr solution

The Langmuir isotherm considers sorption as a chemical phenomenon. The Langmuir constant K_L, which is related to the energy of adsorption through the Arrhenius equation, can also be interpreted as the total number of binding sites that are in fact occupied by Cs or Sr at the concentration (C_e). The Langmuir model provides information on uptake capabilities and assumes that the forces that are exerted are chemically unsaturated, which do not extend further than the diameter of one sorbed molecule; therefore, sorption is restricted to the monolayer.

The Freundlich isotherm relationship is exponential and is represented by Equation 6-2

$$q_e = K_F C_e^{1/n} \tag{6-2}$$

Where q_e is the amount of Cs or Sr sorbed, C_e is the amount of Cs or Sr residue in the solution, K_f is a constant that is related to sorption capacity, and n is an empirical parameter that varies with the degree of heterogeneity.

The Freundlich relationship is an empirical equation that does not indicate a finite uptake capacity of Cs or Sr and, thus, can only be reasonably applied in low to intermediate concentration ranges. However, the Freundlich relationship is easier to represent mathematically in more complex calculations where it may quite frequently appear. The Freundlich model can be easily linearized by plotting the data in a log format, and it has become the most commonly used empirical model because it contains two useful and easily conceptualized parameters, which are more easily understandable because they reflect the two important characteristics of the sorption system (Holan & Volesky, 1994); (Volesky & Holan, 1995).

Currently, incidents of soil contamination caused by radioactive elements resulting from nuclear accidents are leading to a resurgence of interest in microbe-radionuclide interactions. However, information on the role of fungi as storage organisms has been scarce. The current study intends to determine the mechanism behind Cs and Sr accumulation as both stable and radioactive isotopes in soil saprotrophic fungi within the genera: *Fusarium, Trichoderma*, and *Aspergillus*. The effects of pH on accumulation were also investigated. These results could contribute to a better understanding of the biosorption phenomena to facilitate a high capacity for Cs and Sr retention in the organic soil system.

6.3 Procedure

6.3.1 Material

- Fungi cell culture; *Fusarium sp.*, *Trichoderma sp*., *Aspergillus sp*.
- Cesium Chloride (CsCl)
- Strontium Chloride ($SrCl_2$)
- Radiotracer; ^{134}Cs and ^{85}Sr
- Centrifuge tubes
- Filter paper (Whatman no.1)

- 50 mL Potato dextrose Broth (PDB) per experimental conditions
- Inductively coupled plasma mass spectrometry (ICP-MS; XSeries 2, Thermo Scientific)
- Gamma spectrometer with a HPGe detector (GMX series, EG&G, Ortec)
- Shaker
- Benchtop balance

6.3.2 Method

1. Active fungal cells within *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp. were cultured in potato dextrose broth (PDB) while shaking the culture at 110 rpm at 25 °C until a late exponential growth phase was reached. The fungal cells were then washed three times with sterile distilled water to halt cell growth so as to prepare the resting cells.

2. To investigate the effect of Cs and Sr, in both stable and radioactive isotopic forms, on sorption, the resting fungal cells were re-suspended in 25 mL sterile purified water. The experimental conditions used are shown in Table 6-1. The cells were incubated by shaking at 110 rpm at 25 °C until time equilibrium (3 hours). The cells were then filtrated through a dry filter paper (Whatman no.1). The remaining Cs and Sr in the aliquot were measured. In this experiment, the pH was adjusted, whereas the initial pH of each experimental condition was approximately 4.

3. The stable isotopes Cs^{+} and Sr^{2+} were measured by inductively coupled plasma mass spectrometry (XSeries 2, Thermo Scientific). Sample preparation was performed as per the standard methods ISO17294-2 and EPA 6020a (Henk, 2003). To measure the radioactive isotopes ^{134}Cs and ^{85}Sr, the γ-ray spectrum was measured for 30–60min using a gamma spectrometer with a HPGe detector (GMX series, EG&G, Ortec), and a channel analyzer was used for the detection of gamma radiation from the samples.

Table 6-1 Experimental Conditions

No.	Elements	Concentration	pH
1	Cs^{+}	1, 10, 20, 25, and 50 μg/L	n.d.[1/]
2	Sr^{2+}	1, 10, 20, 25, and 50 μg/L	n.d.[1/]
3	Cs^{+}/ Sr^{2+}	2.5,5,10, 25, and 50 μg/L	n.d.[1/]
4	^{134}Cs/ ^{85}Sr	^{85}Sr; 1, 2, 4, 10, and 50 kBq/L[2/] ^{134}Cs; 5, 10, 20, 50, and 100 Bq/L[3/]	n.d.[1/]
5	Cs^{+}/ Sr^{2+}	10 μg/L	3,5, 7 and 9
6	^{134}Cs/ ^{85}Sr	^{85}Sr; 4 kBq/L[2/]; and ^{134}Cs; 20 kBq/L[3/]	3,5, 7 and 9

[1/] pH was not adjusted. The initial pH was approximately 5

[2/] The activity of ^{85}Sr are approximately 1.1 $x10^{-6}$ to 5.7$x10^{-5}$

[2/] The activity of ^{134}Cs are approximately 1.1 $x10^{-4}$ to 2.1$x10^{-3}$

6.4 Results and Discussion

To determine the mechanistic parameters associated with Cs and Sr sorption, data were gathered using several sorption isotherms. The best fitting model was indicated with high values of the correlation coefficient of determination. The results obtained by the Langmuir and Freundlich models are depicted in Figures 6-1 to 6-3. The results mostly indicated that Cs and Sr were sorbed under all conditions in agreement with the Langmuir isotherm (Table 6-2), which was theoretically derived assuming that no interaction between the elements and the cell surface took place, and that adsorption occurred on fixed homogenous adsorption sites forming a monolayer surface coverage (Equation 6-1). Within the model, q_e is the amount of a particular element adsorbed ($\mu g\ g^{-1}$), C_e is the concentration of the element in the solution ($\mu g\ L^{-1}$), q_m is the maximum amount of Cs or Sr sorbed ($\mu g\ g^{-1}$), and K_L is an equilibrium constant representing the affinity between the Cs^{+} or Sr^{2+} ions and fungi cell.

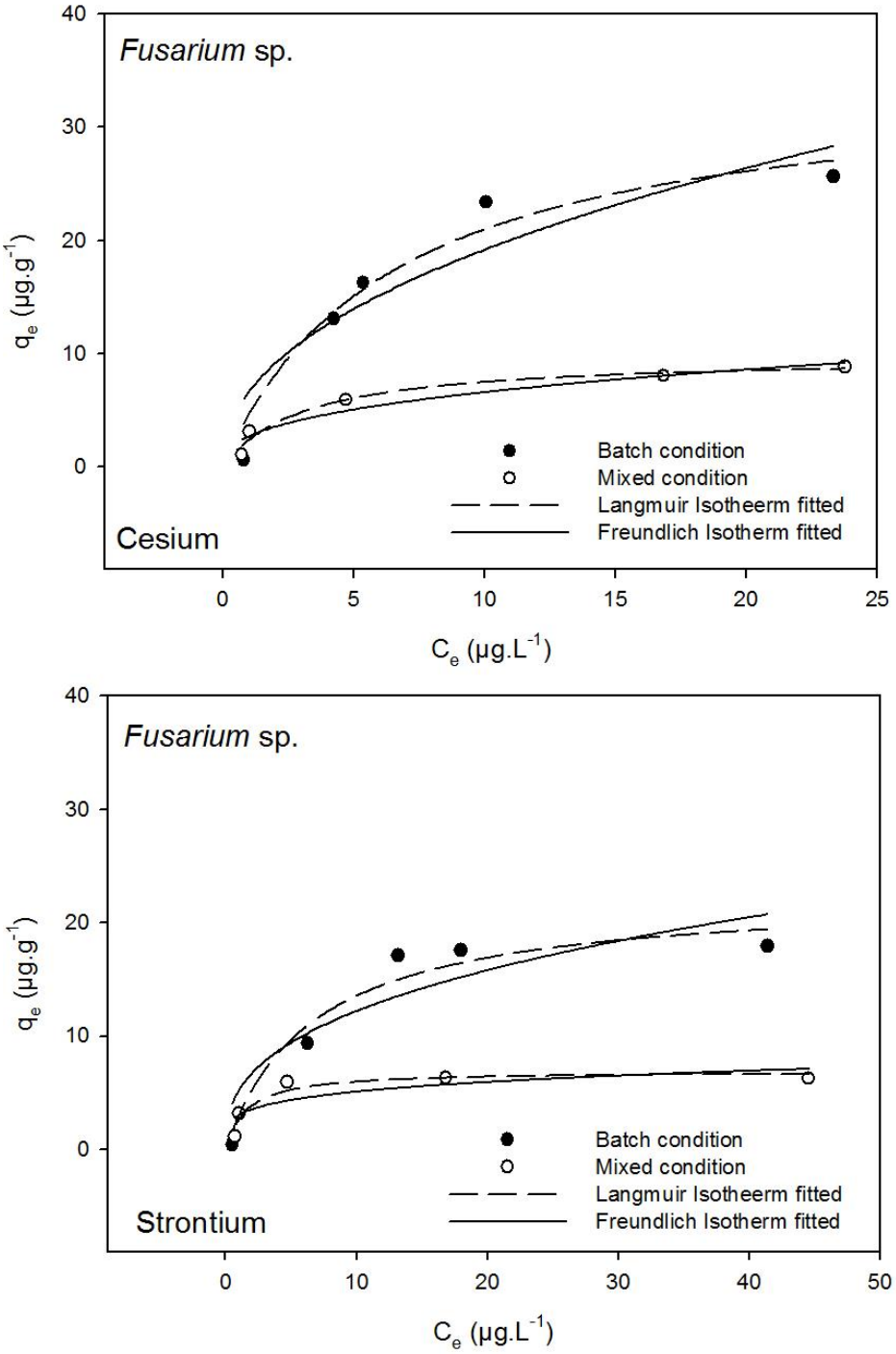


Figure 6-1 The fitting of *Fusarium* sp. uptake data using the Langmuir and Freundlich isotherms for (a) cesium and (b) strontium

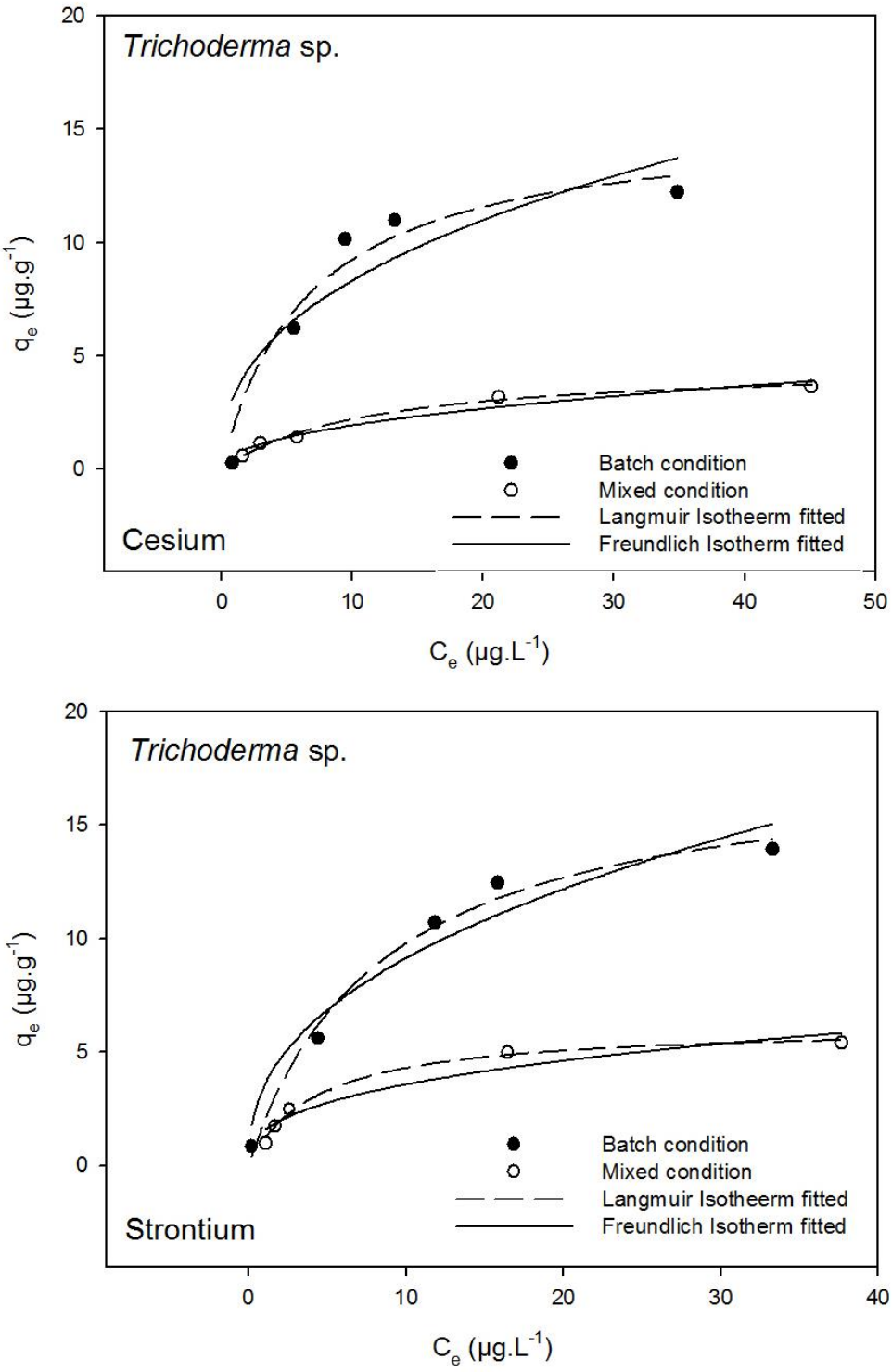


Figure 6-2 The fitting of *Trichoderma* sp. uptake data using the Langmuir and Freundlich isotherms for (a) cesium and (b) strontium

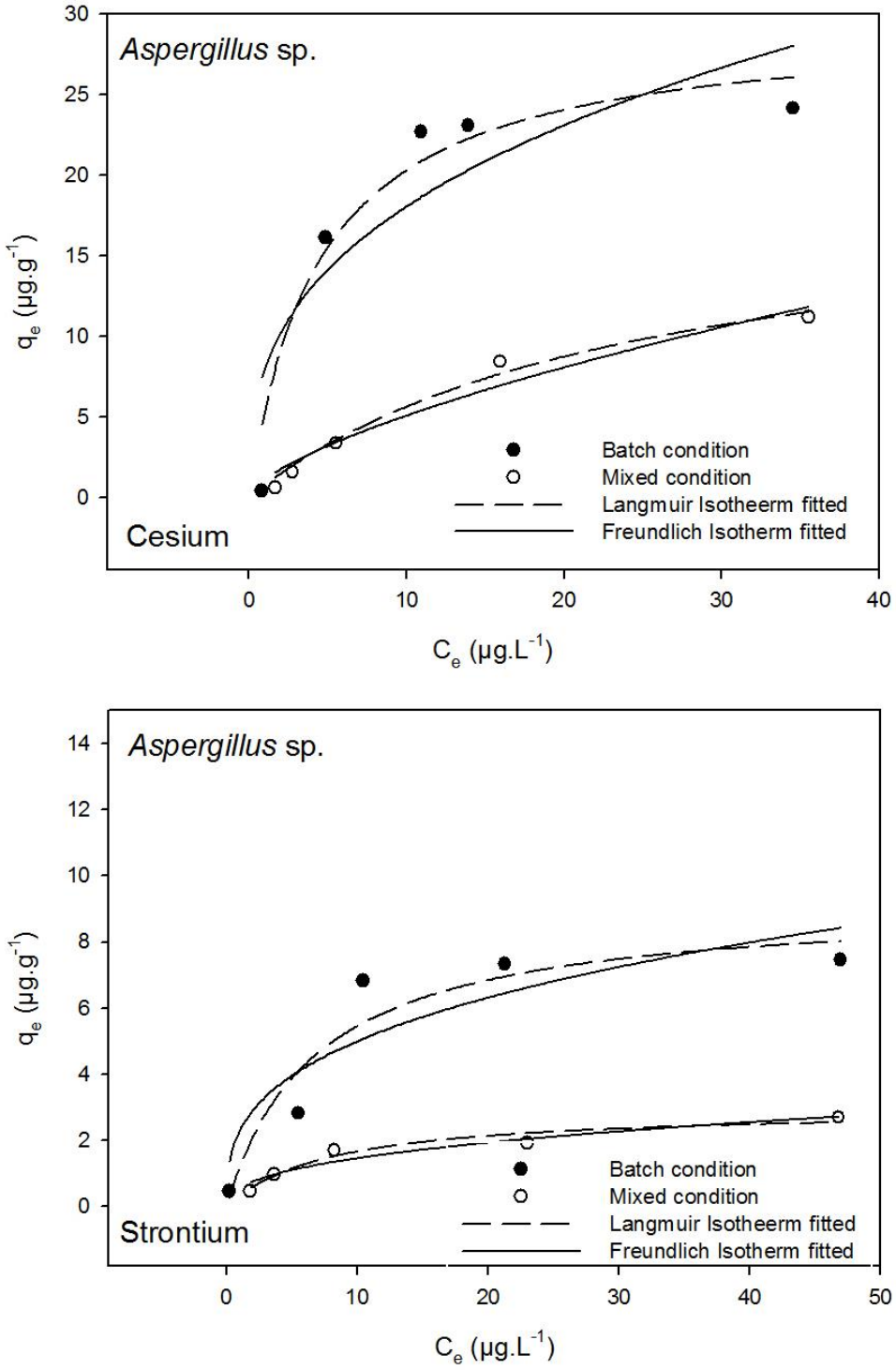


Figure 6-3 The fitting of *Aspergillus* sp. uptake data using the Langmuir and Freundlich isotherms for (a) cesium and (b) strontium

The results showed the same trend for the both elements. For the stable isotopes of Cs and Sr, the sorption capacity increased when the initial concentrations of the elements were increased and showed a specific trend pertaining to approaching a plateau corresponding with the previous reported which is a similar result that assumed that the bio-sorption of Cs onto *Rhodosporidium fluviale* (initial Cs concentration varied from 0.01 to 2.0 mg/L) was a monolayer adsorption through electrostatic attraction (Lan, et al., 2014).

However, in this study the treatments which mixed between Cs and Sr to provide the competitive conditions some of the result showed it was more strong agreement with the Freundlich isotherm (Table 6-2), which is an empirical equation based on adsorption on the heterogeneous surface. It can be assume that under the competitive between two elements, the highly associate with the heterogeneity occurred and that full adsorption capacity was attained.

The linear form of the Freundlich sorption isotherm can be defined using Equation 6-2, which K_f is related to sorption capacity. All the experiments showing 1/n values <1 represent a favorable sorption. The values of n, which reflect the intensity of sorption, also reflect the same trend. The n values obtained for the sorption process represented a beneficial sorption.

The effect of the presence of competitive cations occurred under the mixed conditions, whereby each element was less adsorbed than it would under the single element condition. This suggests that Sr^{2+} and Cs^{+} are adsorbed onto similar sites. A similar result was observed by (Ofomaaj, Pholosi, & Naidoo, 2013), who studied the effect of Cs sorption in the presence of Na^{+} and Ca^{2+} (Tomika, Uchiyama, & Yagi, 1994), which have an ionic radius of 0.99 Å similar to that of 1.12 Å for Sr^{2+}. To determine the mechanistic parameters associated with stable isotope sorption, results were obtained from sorption experiments for both single element and combined elemental conditions.

Table 6-2 Isotherm parameters for cesium and strontium sorption

Condition	Langmuir constants			Freundlich constants		
	q_m	K_L	R^2	K_f	1/n	R^2
Fusarium sp.						
Cs (single)	34.47	6.42	0.95	6.66	2.17	0.86
Cs (combined)	9.79	3.05	0.97	2.74	2.63	0.93
Sr (single)	22.48	6.58	0.95	5.2	2.7	0.83
Sr (combined)	6.9	1.57	0.91	3.04	4.55	0.69
Trichoderma sp.						
Cs (single)	15.39	6.62	0.95	3.33	2.56	0.82
Cs (combined)	4.6	10.82	0.99	0.67	2.17	0.95
Sr (single)	17.99	8.39	0.99	3.52	2.44	0.95
Sr (combined)	6.14	4.32	0.99	1.52	2.7	0.93
Aspergillus sp.						
Cs (single)	29.46	4.49	0.94	8	2.86	0.77
Cs (combined)	19.36	24.29	0.98	1.1	1.52	0.95
Sr (single)	9.18	6.78	0.91	2.31	2.94	0.82
Sr (combined)	2.96	7.72	0.95	0.58	2.5	0.93

In the other hands for radioactive isotopes, the effect of the initial activity of Cs and Sr, which was derived from the experiments under the combined elemental conditions between ^{134}Cs and ^{85}Sr have been demonstrate in Figure 6-4. The experiment was performed under the combined elemental condition to investigate the sorption capacity (Q), which was calculated on the basis of Equation 5-3. The percent biosorption was then calculated as per Equation 5-4, which was compared with that of a blank solution without fungal cells.

$$\mathrm{Q}\ (Bq/mg) = \frac{(A_i - A_f)}{M} \qquad (5\text{-}3)$$

$$\text{Percent biosorption } (\%) = \frac{(\mathrm{A_i} - \mathrm{A_f})}{\mathrm{A}_i} \times 100 \qquad (5\text{-}4)$$

Where A_i and A_f are the initial and final activities in the solution (A), respectively, and M is the mass of fungal cells (mg).

The ability to accumulate Cs and Sr was dependent on the species of microorganisms, as reported in previous studies. For example *Pseudomonas fluorescens* and *Rhodococcus* spp. isolated from the soil were grown in the presence of Cs, and no detectable Cs accumulation was observed for *P. fluorescens* (Avery, 1995).

Table 6-3 Comparison of the percent biosorption of fungal cells for Cs and Sr

Cell	**^{134}Cs**	**^{85}Sr**
Fusarium sp.	27.54 ± 2.85	60.88 ± 2.77
Trichoderma sp.	21.02 ± 1.24	52.00 ± 1.94
Aspergillus sp.	32.21 ± 2.51	44.05 ± 3.26
Control	1.28 ± 0.61	4.79 ± 8.4

Table 6-3 demonstrated that the ability of cell fungi to adsorb radioactive isotopes of radioactive ^{85}Sr was higher than ^{134}Cs.

The results indicated the same trend for both stable and radioactive isotopes, among the representative soil fungi for cesium, the ability of biosorption was follow by *Aspergillus* sp. >*Fusarium* sp.>*Trichoderma* sp., respectively. For Strontium *Fusarium* sp. is greater adsorb follow by *Trichoderma* sp. and *Aspergillus* sp., respectively.

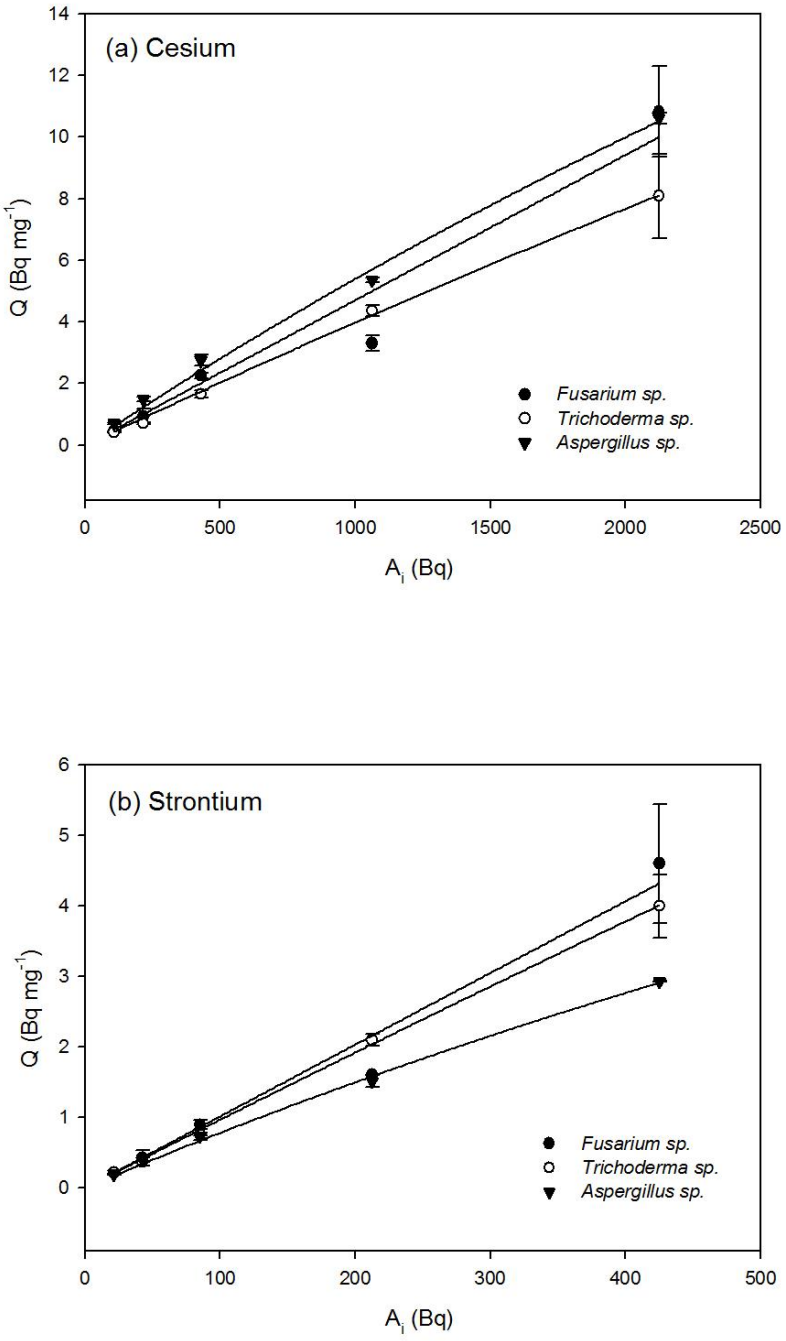
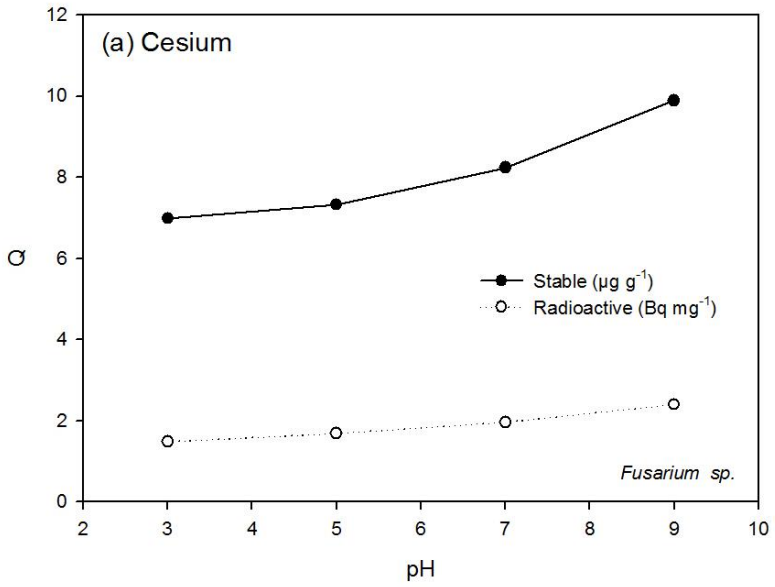


Figure 6-4 Effect of the initial activity of (a) cesium (^{134}Cs) and (b) strontium (^{85}Sr) on the sorption capacity of fungal cells.



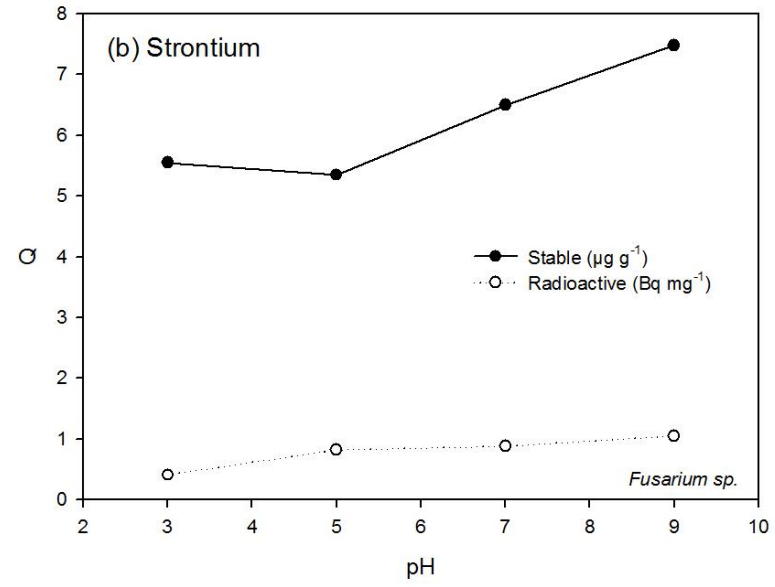
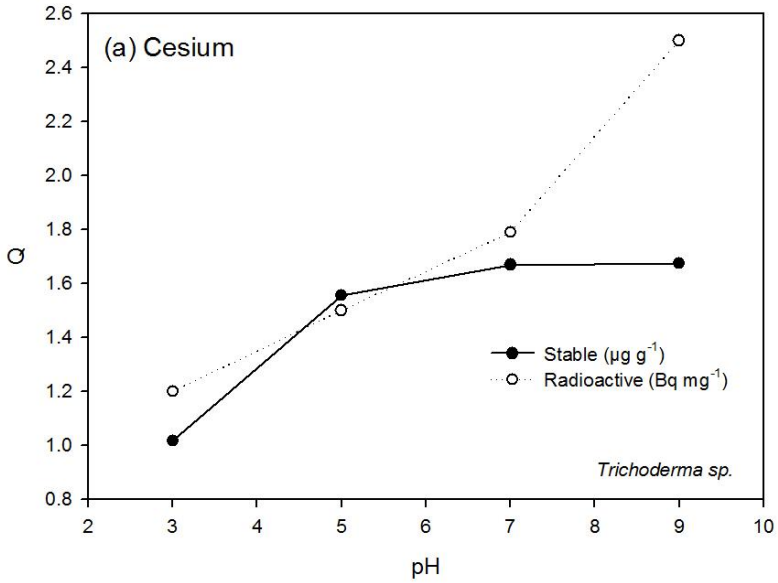


Figure 6-5 Effect of pH on the sorption of (a) cesium and (b) strontium for fungi cell genus *Fusarium* sp.



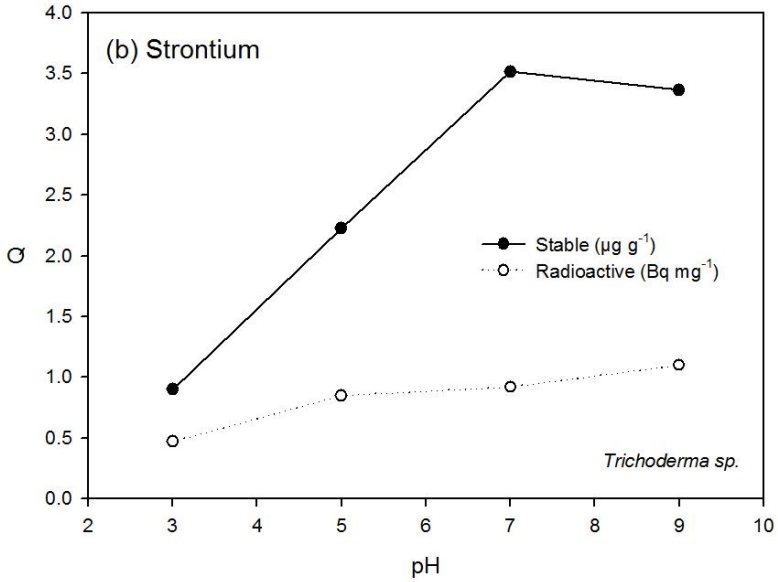
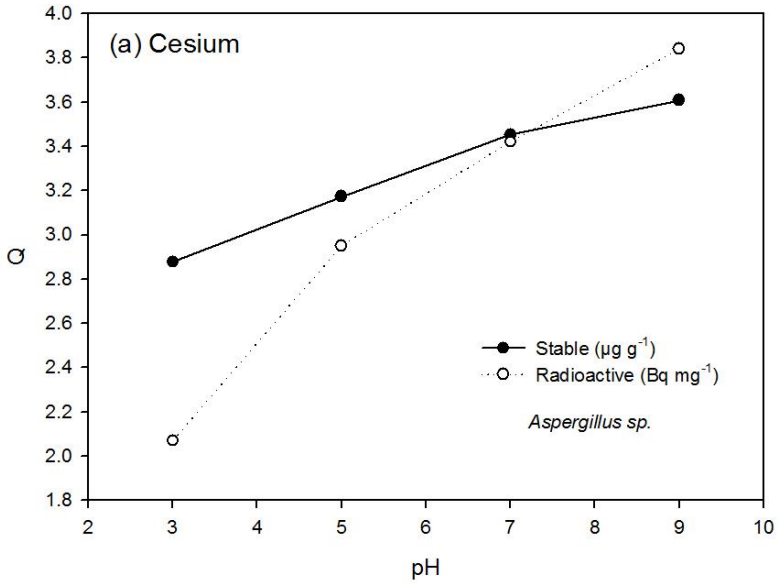


Figure 6-6 Effect of pH on the sorption of (a) cesium and (b) strontium for fungi cell genus *Trichoderma* sp.



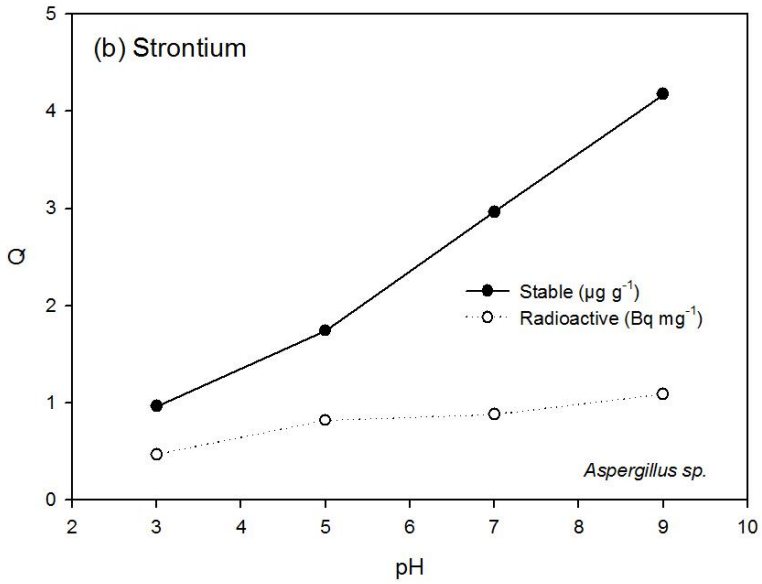


Figure 6-7 Effect of pH on the sorption of (a) cesium and (b) strontium for fungi cell genus *Aspergillus* sp.

In this study, the external physico-chemical factors, such as pH which can be the major factors influencing sorption of Cs and Sr. Figures 6-5 to 6-7 show the effect of

pH on the sorption of Cs and Sr into the fungal genera *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp., where pH was plotted against the sorption capacity in the unit of μg/g for stable isotopes and Bq/mg for radioactive isotopes.

The results demonstrate that pH had a greater effect on the sorption of both the elements, with the same trend evident for stable and radioactive isotopes. When pH was increased from 3 to either 7 or 9, the sorption capacity of fungal cells greatly increased for both Cs and Sr. Meanwhile, at a lower pH (pH 3) the sorption capacity decreased to its lowest capacity probably because H^+ also interacts with sites mediating the monovalent and divalent cation transport system. Therefore, at a lower pH, the binding of H^+ inhibits the sorption of other cations such as Cs^+ and Sr^{2+} (Avery, 1995); (Jones & Gadd, 1990) Thus, during the experiment, the sorption ratio of Cs and Sr of fungal cells increased when pH was increased from 3 to 9. In addition, other species, for example the marine bacteria *Vibrio alginolyticus* and the yeast *Saccharomyces cerevisiae*, have demonstrated the same mechanism (Perkins & Gadd, 1993); (Avery, 1995); (Nakamura, Tokuda, & Unemoto, 1982). In can be assume that when assess the ability of soil fungi in the natural soil the sorption capacity will by decrease because of the present of cation in the soil solution such as Ca^{2+}, K^+, Mg^{2+}, Ba^{2+}, Na^+ will be compete to the same site of the cell.

4.5 Conclusions

Fungal cells of species within several genera were isolated from the soil, including *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp. Experiments were conducted to assess the potential of soil fungi base on the sorption mechanism of Cs and Sr. The biosorption of Cs and Sr was assessed under various conditions using both stable and radioactive isotopes. The results indicated the same trend for both stable and radioactive isotopes, among the representative soil fungi for cesium, the ability of biosorption was follow by *Aspergillus* sp. >*Fusarium* sp.>*Trichoderma* sp., respectively. For Strontium *Fusarium* sp. is greater adsorb follow by *Trichoderma* sp. and *Aspergillus* sp., respectively.

Not only the individual condition, but also, the Cs and Sr ion was examined in the same treatment to perform the competitive condition. The results show the monolayer sorption capacity was decreased. The same trend was observed for Sr in which the monolayer sorption for Sr ions. For the stable isotopes, when the concentration of both Cs and Sr elements was increased, the percent biosorption was decreased, as indicated by the increase in the amount of each element in the solution, until a plateau was reached.

In contrast, for the radioactive isotopes, the percent sorption was not changed by the initial activity and not reached to the saturation. After the, the effect of pH showed a decrease in sorption capacity at lower pH because H^+ also interacts with sites mediating the monovalent and divalent cation transport system. However, this work is not intended to provide a comprehensive result of the biological processes involved but rather to study the possible potential of soil saprotrophic fungi to accumulate radioactive elements. The experimental results indicated that fungal cells effectively and substantially contribute to the long-term retention of Cs and Sr in an organic layer, which is essential to reduce the migration of radioactive elements in soil layers. The mechanism of uptake is thought to be by direct extracellular binding to fungal cells or active intracellular uptake, although further studies analyzing the underlying mechanisms are required.

4.6 Achievement

Parts of this chapter have been published as Tolerance and accumulation of cesium and strontium in saprothophic fungi, P Seeprasert *et al* 2015 *J. Phys.: Conf. Ser.* 611 012022

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Chapter 7

Application within organic soil systems

The precise influence of soil organic matter and microbiological processes in the bioavailability of radioactive elements needs to be further elucidated in detail to obtain the parameters necessary to study the fate and transport of elements in soils and soil–plant systems. Most of the previous chapters have studied the microbial retention of Cs and Sr by measuring the amounts of these elements stored in fungal cells. This chapter addresses the contribution of microbial activity especially the saprotrophic fungi to interact with Cs and Sr into organic material, which is necessary to compare non-sterile with sterile systems. This chapter highlights to characterize the full potential of soil fungi to accumulate Cs and Sr in a soil through the experimental approached.

7.1 Objective:

- To determine the influence of microbial activity to restrict the mobility of Cs and Sr in organic material.

7.2 Theory and Significance

Large quantities of cesium and strontium released from the Fukushima accident in 2011 are still present in terrestrial ecosystems. The fractions of Cs and Sr linked to organic matter, and microorganisms in soil may be relatively small compared with the amount bound to inorganic constituents; however, the organic layers in the soil system are more sensitive to the presence of humic acid, which can induce Cs and Sr to change into their soluble forms and be persistently available for uptake by plants (Howard, Beresford, Burraw, Shaw, & Curtis, 1987); (Absalom, Young, & Crout, 1995); (Sanchez, Parekh, Dodd, & Ineson, 2000). Previous studies demonstrated that the behavior (e.g., distribution and mobility in soil) of Cs and Sr is significantly influenced by organic matter (Brookshaw, Patrick, Lloyd, & Vaughan, 2012); (Ruhm, Yoshida, Marumatsu, Steiner, & Wirth, 1999). Thus, it can be assumed that Cs and Sr are strongly retained in the top layer of organic soil (Yoshida, Marumatsu, Dvornik, & Linkov,

2004). For example, 40 years after the atomic bomb in Nagasaki, Japan, Cs monitoring showed that 95% of the fallout was still present in the uppermost 10 cm of local soil (Mahara, 1993) and the rate of vertical migration was low (Rosen, Oborn, & Lonsjo, 1999) (Newsome, Morris, & Lloyd, 2014). It can be assumed that the strong retention of Cs and Sr in the organic layer is influenced by fungal and microbial activities.

The fungal and microbial activities in the organic soil layer are likely to contribute substantially to the long-term retention of radionuclide in organic layer (Dighton, Clint, & Poskitt, 1991); (Steiner, Linkov, & Yoshida, 2002). In the forest soil, fungi are the greatest living biomass in the decomposing organic layers. They are the primary source of enzymes necessary to degrade the litter. According to (Griffin, 1981) which shows the ability of fungi to takenup the nutrients from soil solution. Using the fungi enzymes to break down the macromolecular complex, then most substances is easily to move into the cell or being bound to specific carrier molecular. Nowadays, the ability of microorganisms that includes bacteria, actinomycetes and fungi to accumulated Cs and Sr from their external environment have been largely studied. However, the mechanisms involved in the uptake and retention of Cs and Sr which is contribution of microbial activity to the sorption of Cs and Sr in organic material by fungi has been scarce, in comparison to plant. Then, the role of soil microbiological process in the bioavailability Cs and Sr need to be more fully elucidated in order to predicting the fate and transfer of Cs and Sr in the soil.

The microorganisms can affect to Cs and Sr in the soil solution by a variety of mechanisms both direct and indirect mechanisms. For example, they can alter the soil pH, they can act as a potential ligand in the form of low molecular weight organic compound with their exudates. Cs and Sr may also become attached directly to the cell-extracellular or actively taken up from the soil solution (Tamponnet, et al., 2008); (Tamponnet, Plassard, Parekh, & Sanchez, 2001); (Lloyd & Renshaw, 2005). A conceptual schematic summarizing Cs and Sr transfer from soil to microorganisms and plants is presented in Figure 7-1.

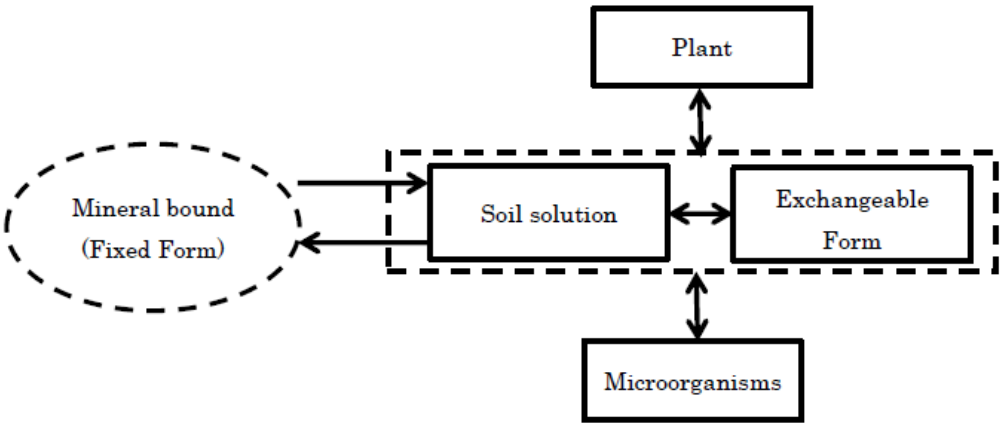

Figure 7-1 A conceptual schematic summarizing Cs and Sr transfer into microorganisms and plants from the soil

The source of Cs and Sr in Figure 7-1 is anthropogenic activities, which may be nuclear weapons testing, discharge of nuclear waste effluent, or accidental release that is deposited in the soil via rainfall. Cs and Sr in the soil are present in various solid components, because of their binding capacity, and in soil solution. Thus, a wide range of soil properties such as pH, the presence of natural cations (K^+, Ca^{2+}), clay content, and the amount of organic matter including microbial or fungi activity are all known to affect the solid-solution exchange that controls the soil solution composition. In general, the organic fraction of soils has a larger cation exchange capacity than the inorganic soil content. It can be assumed that Cs and Sr can be taken up from soil solution into microorganisms.

In the previous chapters, studies were performed on the fungal retention of Cs and Sr by measuring the amounts of Cs and Sr stored in fungal cells. However, it is necessary to compare non-sterile with sterile systems in order to assess the full potential of fungi to cycle Cs and Sr in soil; this is described in this chapter. In this study, organic material was prepared from washed leaf litter to minimize interference from inorganic components and determine the true potential of litter and fungi to accumulate both Cs and Sr in situ.

4.7 Procedure

4.7.1 Material

- Leaf litter from four sites (Figure 7-3)
- Fungal cell cultures: *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp.
- Cesium chloride (CsCl)
- Strontium chloride ($SrCl_2$)
- Centrifuge tubes
- Inductively coupled plasma mass spectrometer (ICP-MS; XSeries 2, Thermo Scientific)
- Distilled water
- Mixed cation solution 0.1 mg/l
- Benchtop balance

7.3.2 Method

1. The organic materials used in the experiment were prepared from leaf litter collected from four areas in Takizawa Research Forest, Iwate University, Japan, during fall 2014 (Figure 7-2). The basic characteristics of leaf litter such as moisture content and pH were analyzed (Table 7-1). The material was sorted to remove trash and washed with deionized water to reduce the interference from inorganic material. Subsequently, the leaf litter was air dried and shredded. After that, deionized water was added to adjust the moisture content to 60% and the preparation was incubated at a temperature of 25°C–30°C in the incubator for 1 month.

2. To prepare the soil solution for inhabitant microbial inoculation, the soil from the O layer from each area was used to provide for all experiments system. A sample of soil approximately 5 g dry wt. equivalent was added to 50 ml of sterile distilled water (SDW) in a sterile centrifuge tube. The tube was shaken for 1 h and left to stand for 30 min. The supernatant was used as inoculum for biotic system.

Table 7-1 Basic characteristics of the leaf litter used in this study

Site Name	Moisture content (%)	pH	Forest Type
IWT 1	65.65±1.9	5.11±0.02	coniferous
IWT 2	62.16±0.59	5.39±0.0.8	deciduous
IWT 3	46.05±2.37	5.73±0.02	deciduous
IWT 4	76.48±2.68	5.48±0.03	coniferous

Figure7-2 Locations of the leaves litter sampling sites in Takizawa Research Forest, Iwate University, Japan

3. A representative of fungal single cultures of *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp. were obtained from Chapter 3. After incubation for 7 days, the active cell was individual inoculated into each organic system.

4. Samples of organic material measuring 5 g dry wt. equivalent were set up as organic system and sterilized by autoclaving. Then, separately systems for abiotic and biotic processes were performed. The biotic samples were

re-inoculated with 5 ml of soil solution and others were set up for the three fungal genera used in this study: *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp. For the abiotic setup, the organic system was amended with an equal quantity of SDW.

5. Cs^{+} and Sr^{2+} 8 μg was spiked to each organic system mention above. SDW was added to adjust the moisture to 70%–80% moisture content before sealing with a cap to avoid moisture loss and prevent contamination. Two replicates of each organic system were prepared.

Figure 7-3 Leaf litter obtained from four areas of Morioka forest, Japan. (a) IWT1, (b) IWT2, (c) IWT3, and (d) IWT4

6. After 30 days, the subsamples of the system were collected for the measurement of Cs and Sr bioavailability. Approximately 1 g dry wt. equivalent of the organic material was removed from each system. Then, the samples were extracted firstly with 20 ml SDW (pH 6.3 – 7.3) for 24 h in the shaking condition. After that, cation mix solution which contains 0.1 mg/l of K^+, Ca^{2+} and Mg^{2+} was used as and extractant to determine the more tightly bound fractions of Cs and Sr.

7. The amounts of Cs and Sr in the solution were measured by inductively coupled plasma mass spectrometry (ICP-MS; XSeries 2, Thermo Scientific). Sample preparation followed the standard methods ISO17294-2 and EPA 6020a (Henk, 2003) and were calculated the percentage of element extraction. The total amount of extractable are represents the exchangeable and the potentially bioavailable fraction of Cs and Sr spiked.

7.4 Results and Discussion

7.4.1 Effects of soil fungi

To interpret the result from the experiment, two sets of observations to infer weather soil microorganisms play an influence to restrict the Cs and Sr in the organic system via the sorption process. The experimental data were consisted of a comparison between the amounts of each elements: Cs and Sr was firstly extracted by distilled water following the incubation a period (1 month). Figure 7-4 demonstrated that the distilled water extracted removed 50 to 27% of the Cs which was spiked to abiotic system. Whereas, only 24 to 8% was extracted from the biotic systems which was re-inoculated with soil extract solution (SES). For Sr, the amount which was extracted was less than Cs, 20 to 10% for the abiotic systems, and compared to only 10 to 5 % from biotic system. The results show the same trend for all location which is ITW1 show the great amount of Cs and Sr extracted compared to IWT3. The results also indicated that the water able to extract the significantly more Cs than Sr. According to the previous study, which were examined the extraction of Cs and Sr in the radioactive isotope: ^{137}Cs and ^{85}Sr also showed the same trend of result in both abiotic system follow by autoclaved

and using the fungicide such as streptomycin (Parekh, Poskitt, Dodd, Potter, & Sanchez, 2008).

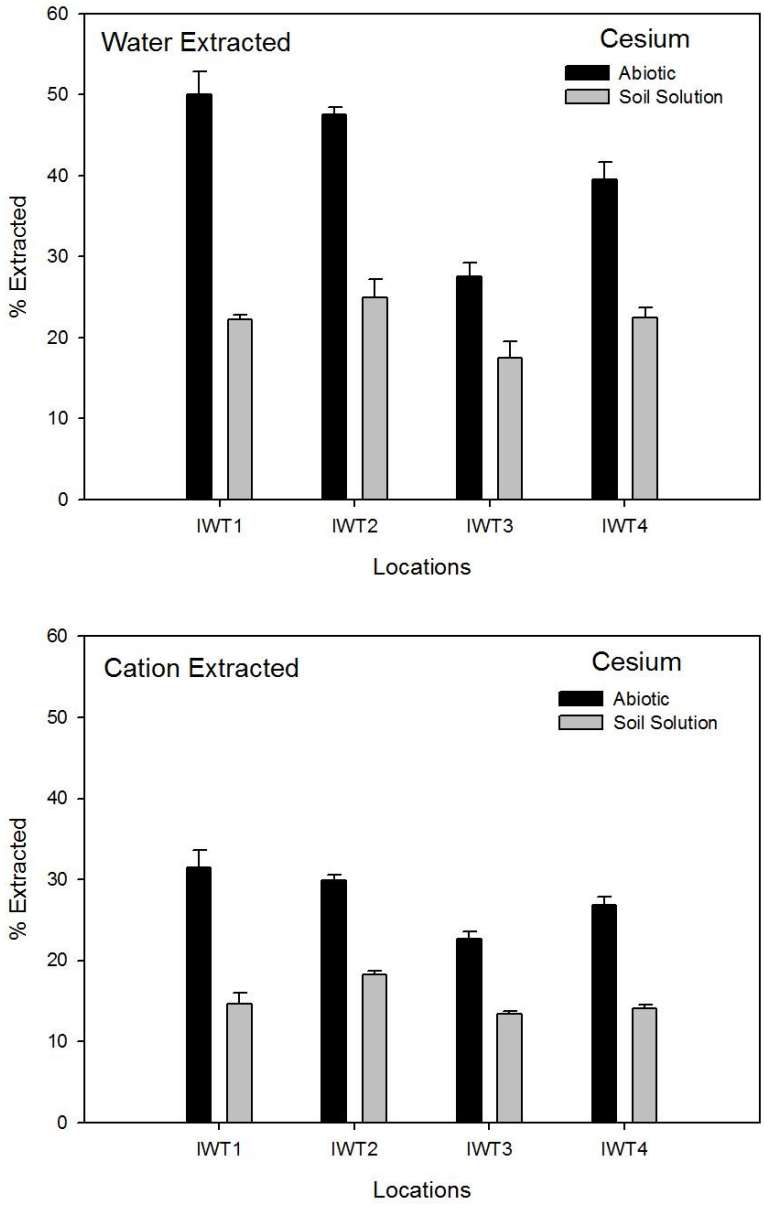

Figure 7-4 Percentages of Cs extracted using (a) distilled water and (b) cation solution from organic material in abiotic and biotic system which is inoculated with soil solution for four different soil locations

In this study we also used a different extractant, a mixed cation solution containing 1 mg/l of K^+, Ca^{2+}, and Mg^{2+} to determine the levels of exchangeable Cs^+ and Sr^{2+} on the organic material for the biotic and abiotic treatments. The results are shown in Figure 7-5 to 7-6.

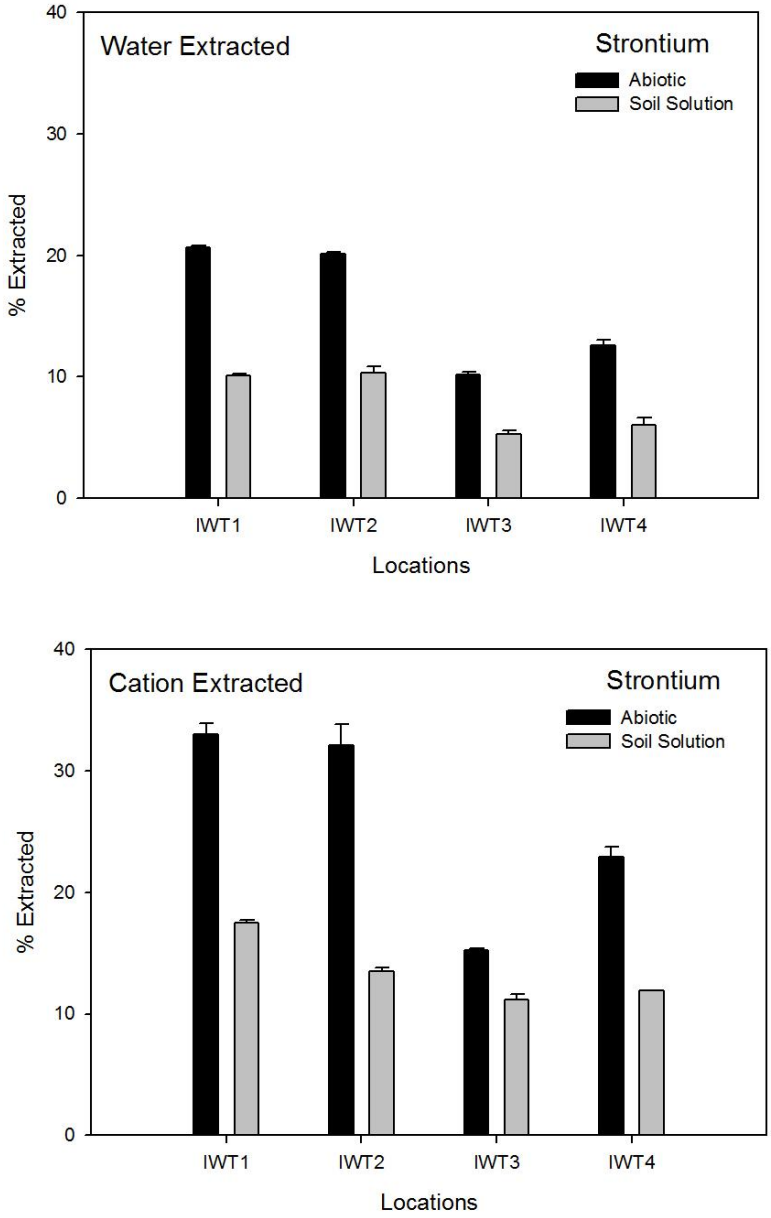

Figure 7-5 Percentages of Sr extracted using (a) distilled water and (b) cation solution from organic material in abiotic and biotic system which is inoculated with soil solution for four different soil locations

The amounts of Cs bound to organic material extracted by distilled water from the abiotic treatment were 50%, 48%, 28%, and 40% of the initial Cs concentration. These values are higher than those extracted by mixed cation solution, which were 31%, 30%, 23%, and 27% of the initial Cs concentration for localities IWT1–IWT4, respectively. The results for the system that inoculated with soil solution to provide the biotic treatment show the percentage of Cs extracted by the mixed cation solution was lower than abiotic system, 15%, 18%, 13%, and 18% of the initial Cs concentration for localities IWT1–IWT4.

This finding indicates that the active inhabitant microbiota in the soil associated with Cs accounted for a significant proportion of Cs in the contaminated soil. This is thought to be because of microbial activity, which can uptake Cs through the active transport of monovalent ions such as K^+ and NH_4^+ (Dighton & Terry, Fungi and Environment Change, 1996); (Avery, 1996). However for Sr, the percentage of Sr extracted by distilled water was less than cation solution extraction due to the strength of ionic bound. Fungi cell surface sorption reactions are influenced by the electric field effects caused by changes in ionic bound strength then, Sr^{2+} was more strongly bound than Cs^+. Also due to the behavior of those elements, Cs is susceptible bound with organic material including fungi cell wall, whereas the sorption of Sr was much tightly bound to organic material. However, those results showed the same trend that both Cs and Sr are more strongly bound in the system that inoculated with soil solution to provide the presence of soil microorganisms than in the abiotic system. This finding constitutes evidence that the microorganisms not only saprotrophic fungi that were re-inoculated from the soil solution can enhance the retention of Cs and Sr, particularly in the organic layer.

7.4.2 Effects of single fungal culture

To determine the influence of saprotrophic fungal cultures, these experiments were performed for the sorption of Cs and Sr in the organic soil system. The results are shown in Figure 7-6. As in the previous chapter, three saprotrophic fungal strains were isolated from soil samples, confirming that these organisms are present within the biotic organic layer. Inoculation of the organic material with each of the three soil fungal

strains increased the sorption of both Cs and Sr, as demonstrated by the amount of element remaining after extraction compared with the amount for the abiotic system.

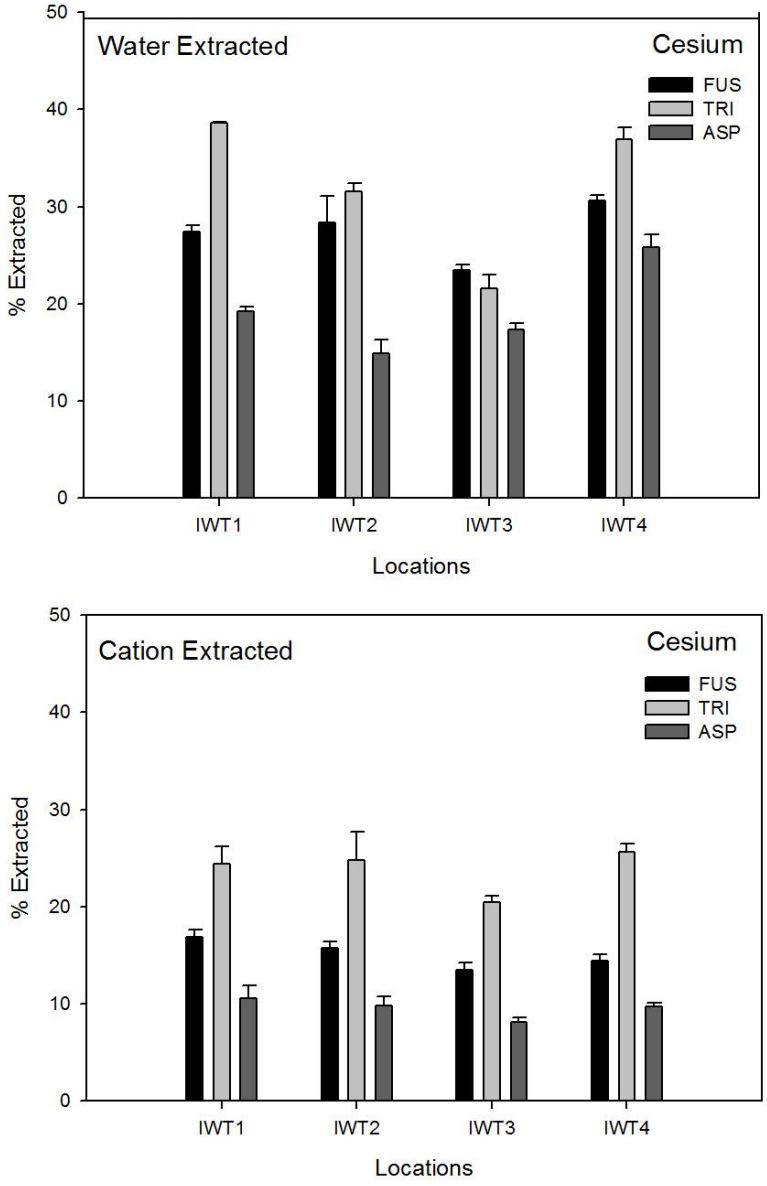

Figure 7-6 Percentages of Cs extracted by (a) distilled water and (b) cation solution from organic material in biotic systems inoculated by single fungal cultures for four different soil locations.

In the system inoculated with single fungal cultures, approximately 18%–39% of the initial Cs was extracted compare to 28%–50% of the initial Cs was extracted from the abiotic system. The treatment inoculated with *Aspergillus* sp. showed a slightly

higher ability to retain Cs than those inoculated with *Fusarium* sp. and *Trichoderma* sp. correspond with the result from the previous chapter which is show *Aspergillus* sp. has more ability to adsorb Cs than other genera.

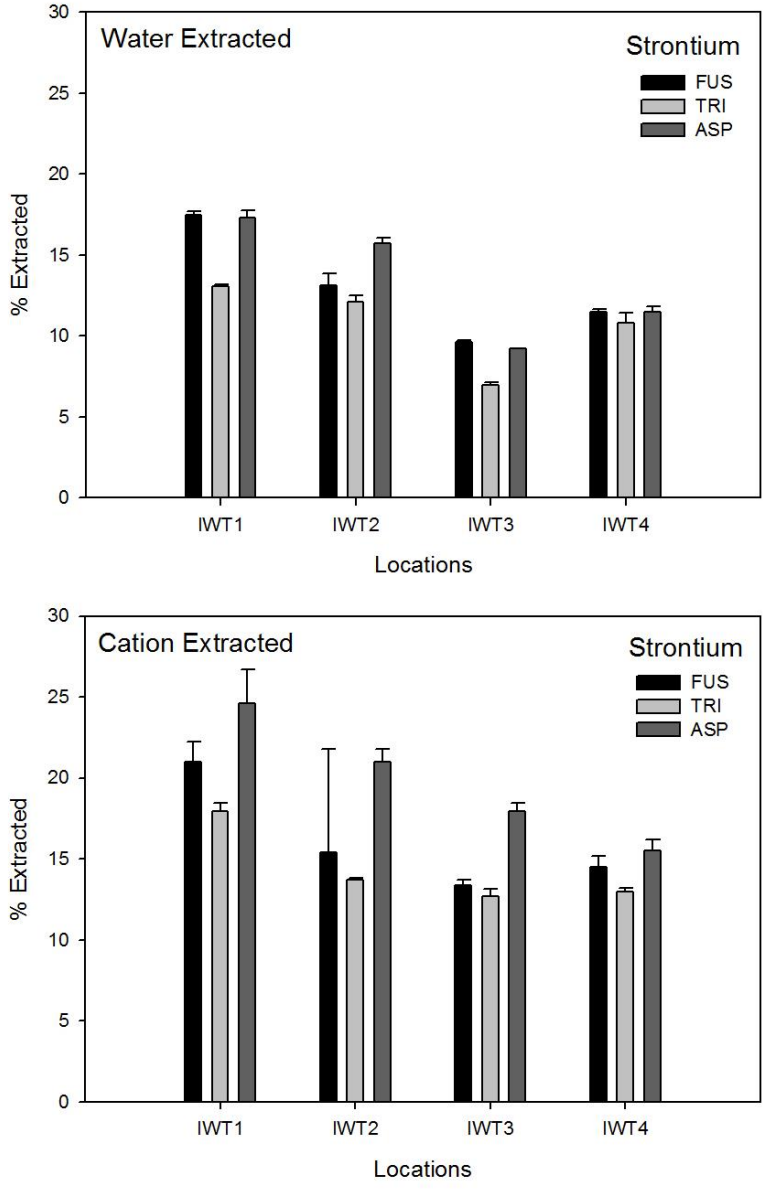

Figure 7-7 Percentages of Sr extracted by (a) distilled water and (b) cation solution from organic material in biotic systems inoculated by single fungal cultures for four different soil locations.

However, for Sr in biotic systems only 7%–17% of the initial Sr was extracted, compared to 10%–20% of the initial Sr was extracted for the abiotic system.

In contrast with Cs, the treatment inoculated with Fusarium sp. and Trichoderma sp. showed a slightly higher ability to retain Sr than those inoculated with Aspergillus sp. which corresponds with the result from the previous chapter. From this limited analysis, the ability of Cs and Sr restriction influence by fungal cells appears to be species-specific, depending on the components of the fungal cell walls that provide binding sites for elements (Tobin, White, & Gadd, 1994). According to the Figure 7-6 to Figure 7-7, the results of extraction using the mixed cation solution showed that biotic systems initially retained a higher proportion of each element. Dighton et al. (1991) estimated the accumulation of cesium by pure cultures of soil fungi and demonstrated that all the studied strains were able to immobilize Cs. Efflux studies showed that more than 40% of the Cs was bound within the hyphae (Dighton, Clint, & Poskitt, Uptake and accumulation of Cs-137 by upland grassland soil fungi: a potential pool of Cs immobilization, 1991) The Fusarium sp. culture of this experiment is similar to that used in previous studies (Parekh, Poskitt, Dodd, Potter, & Sanchez, 2008). In this study, fungal retention increased the amount of non-extractable Cs and Sr compared with the abiotic system. (Clint, Harrison, & Howard, 1991) demonstrated that the pure cultures of various saprotrophic and mycorrhizal fungi can take up Cs; in that study, there was no indication of the permanent binding of elements. The experimental resulting from two fungal cultures, *Aspergillus* sp. and *Fusarium* sp., demonstrated that the majority of sorbed Cs and Sr within organic material remained in the exchangeable form. It is assumed that during Cs and Sr was retained in the organic layer; they are mostly available for uptake by plants. Hence, when inoculating the fungi into the contaminated sites; it can be induced the potential of phytoremediation.

7.5 Conclusions

This chapter was to determine the contribution of microbial activity to the sorption of Cs and Sr into organic material, which is necessary to compare non-sterile systems with sterile systems. This chapter highlights to to characterize the full potential

of soil fungi to accumulate Cs and Sr in soil. The results for an experimental system comparing biotic and abiotic systems conclusively demonstrate that soil fungi play a role to restrict the mobility of Cs and Sr. In all experiments, the retention of both elements was greater in biotic systems than in abiotic systems. Soil microorganisms especially the saprotrophic fungi make a contribution to influence the retention of Cs and Sr in organic systems and may account in part for the strong, irreversible binding observed in biotic systems. This finding may account for the high level of radioactive Cs and Sr retention in the in situ contaminated site, which cannot be satisfactorily accounted for by physicochemical processes. It can be induced the potential of phytoremediation while inoculated with the soil fungi.

7.6 Achievements

Parts of this chapter have been present at the 2015, 13th International Conference on the Biogeochemistry of Trace elements, Fukuoka, Japan and 2015, 5th International Conference on Biotechnology and Environmental Management, Milan, Italy.

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Chapter 8

Conclusion

The increase in soil contamination by cesium (Cs) and strontium (Sr) resulting from anthropogenic activity has caused a resurgence of interest in the role of soil element mobility. The interactions between microbes and Cs and Sr play a role in the study on the fate and transport of radioactive isotopes. Soil fungi are the greatest living microorganism in the decomposing organic layers of forest soil. To a large extent, they determine the fate and transport processes of radionuclides in the soil. Various mechanisms for interactions of soil fungi with radionuclides have been extensively documented. Microbiological activity, in particular fungal activity, is likely to be responsible for the long-term retention of radioactive elements in the organic layer of the soil. It has been hypothesized that this may alter the availability of radionuclides to plants along with their movement in particular areas. The main purpose of this study was to describe the influence of soil fungi as a storage organism and the potential of soil fungi to affect the mobility of the radioactive elements Cs and Sr in the organic soil system.

The study provides some evidence that the different fungal communities found in forest soils may be related to the environmental factors such as soil characteristic and the tree species present. The specialization of microbial communities under different tree species may be important for physiologically restricted ecosystem processes performed by a limited suite of organisms. Thus, it is important to understand the mechanisms that underlie the effects of forest types and different tree species on soil microorganisms. Molecular techniques are facilitating this type of research. In the present study, representative fungi were selected from two forest types, coniferous and deciduous forest using the hyphal tip isolation technique and assigned to three genera: *Fusarium*, *Trichoderma*, and *Aspergillus*. These can all be assumed to be saprotrophic fungi and free living in the soil, where they have the ability to decompose dead organisms and organic residues. Under the plating technique condition, showed some fungal genus was occasionally missing from one of the forest soil types.

However, the soil fungi deal with a wide variety of potentially toxic environmental challenges. Then, not all microorganisms and soil fungi can survive within organic layers containing highly Cs and Sr contamination. Thus, it is necessary to measure growth curves in order to use models to describe the growth of fungi in environments affected by high concentrations of certain toxic elements. The research conducted in the current study mainly relates to the study of the effect of Cs and Sr on the three evaluated fungi genera. Recently, more attention has been focused on this field; as a result, some achievements have been made, including evidence for the direct inhibitory effects of Cs and Sr on these three genera of fungi. The present study further indicated that Cs has a significant direct inhibition effect on fungi, with an EC_{50} of 80–160 mM, whereas Sr has a less significant direct inhibition effect on fungi, with an EC_{50} of 171–222 mM. The severity of the toxicity of Cs to the three fungi genera decreases in the following order: *Trichoderma* sp. > *Aspergillus* sp. > *Fusarium* sp. In contrast, the severity of the toxicity of Sr followed the opposite order: *Fusarium* sp. >*Aspergillus* sp. >*Trichoderma* sp. However, the natural levels of Cs and Sr in soil were lower than EC_{50} values 600 to 650 times. Hence, all representative fungi being adapt or evolutionarily to survive and reproduction even in the environment extremely contaminated by Cs and Sr. As a result, it induced an opportunity to interact with Cs and Sr in the soil solution.

The experiments were conducted base on the biosorption mechanism using the three fungi genera (*Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp.) under various conditions. Because of the fate of Cs in the environment is mostly influenced by that process, the biosorption characteristics were examined for different contact times to provide kinetic data, which were fitted with both pseudo-first-order and pseudo-second-order kinetic equations. The experimental data for the stable isotopes of Cs and Sr were better described by the pseudo-first-order model for both separate and competitive adsorption. Conversely, the experimental data for the radioactive isotopes (^{134}Cs and ^{85}Sr) were better described by the pseudo-second-order model, as indicated by the values of the corresponding correlation coefficients. To clearly understand how soil fungi accumulate Cs and Sr in the form of both stable and radioactive isotopes, Langmuir and Freundlich isotherms were used to describe the sorption characteristics

and quantify the sorption capacity. The effects of pH were also investigated. The results contribute to a better understanding of biosorption phenomena, under single-element conditions; the monolayer sorption capacities for Cs ions were 35, 15, and 29 μg/g cell. The same trend was observed for Sr; the monolayer sorption capacities of Sr ions were 23, 18, and 20 μg/g cell for *Fusarium* sp., *Trichoderma* sp., and *Aspergillus* sp., respectively. Under the competitive conditions of both Cs and Sr, whereby each element was less adsorbed than it would under the single element condition. The biosorption percentages of the stable isotopes were decreased, as indicated by the increase in the amount of each element until a plateau value was reached. In contrast, for the radioactive isotopes, the percent sorption was not changed by the initial activity. The sorption capacity decreased with pH because of the interaction of H^+ with sorption sites, which mediates the monovalent and divalent cation transport system. In can be assume that when assess the ability of soil fungi in the natural soil the sorption capacity will by decrease because of the present of cation in the soil solution such as Ca^{2+}, K^+, Mg^{2+}, Ba^{2+}, Na^+ will be compete to the same site of the cell.

Lastly, the experiment which intends to determine the contribution of soil fungi activity to the sorption of Cs and Sr in the organic material was performed by the comparison within biotic and abiotic systems. The results conclusively show that soil microorganism especially the representative soil fungi play an influence in the retention of Cs and Sr in an organic system. The present of soil microorganism and saprotrophic fungi in the organic system contribute importantly to the sorption of Cs and Sr and may partly account for the strong, irreversible binding observed in biotic systems. Moreover, this finding may account for the high level of in situ Cs and Sr remediation in the upland soil organic layer, which cannot be satisfactorily explained by physicochemical processes. The experimental resulting from two fungal cultures, *Aspergillus* sp. and *Fusarium* sp., demonstrated that the majority of sorbed Cs and Sr within organic material remained in the exchangeable form. It is assumed that during Cs and Sr was retained in the organic layer; they are mostly available for uptake by plants. Hence, when inoculating the fungi into the contaminated sites; it can be induced the potential of phytoremediation.

This work does not intend to provide a comprehensive description of the biological processes involved, but rather to study the potential of soil saprotrophic fungi to accumulate radioactive elements such as Cs and Sr. The experimental results indicated that fungal cells effectively and substantially contribute to the long-term to restrict the mobility of Cs and Sr in an organic layer, which is essential to reduce the migration of that elements in the soil which is important for the remediation techniques. However, the mechanism of uptake is thought to be direct extracellular binding to fungal cells or active intracellular uptake, although further studies analyzing the underlying mechanisms are required.