BIOAVAILABILITY OF ^{99}Tc IN *FUCUS VESICULOSUS*: MECHANISMS AND PATTERNS OF RELEASE

A thesis submitted towards the degree of

Doctor of Philosophy

Shona Webster

School of Biological and Environmental Sciences

University of Stirling

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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PREFACE

This thesis contains no material that has been accepted or previously submitted for any other degree or diploma and no material previously published or written by another person except where due reference is made in the text.

ABSTRACT

About 1665 TBq of the highly mobile radionuclide, technetium-99 (^{99}Tc), has been discharged into the Irish Sea by BNFL, Sellafield since 1994. This long-lived radionuclide with a half-life of 213,000 years has the potential to contaminate the marine environment for many generations. As ^{99}Tc is highly soluble in the aerobic surface waters, it remains in the water column making it available for uptake by marine organisms. High ^{99}Tc activity concentrations up to of 270 Bq kg^{-1} (dry weight) in lobsters and 60 kBq kg^{-1} (wet weight) in brown seaweed species have been reported in the literature.

The use of contaminated seaweed as a soil conditioner in coastal areas, including Cumbria, creates a potentially important pathway for the transfer of ^{99}Tc from marine to terrestrial ecosystems and thence to man. While there is some evidence that this practice can lead to contamination of some crops, the mechanisms and dynamics of the seaweed-to-soil-to-plant pathway have so far been neglected. The key objectives for this research programme were to determine the rate of release of ^{99}Tc from environmentally contaminated brown seaweed (*Fucus vesiculosus*) into soil and soil solution over time, to identify the mechanisms involved and to quantify the availability of the released ^{99}Tc to crop plants.

The results from a pot experiment, using seaweed collected from the shore close to Sellafield incorporated into a sandy coastal soil, established that between 54% and 71% of the ^{99}Tc within the seaweed had accumulated in the soil 15 weeks after application, despite low

temperature conditions. Concurrent CO_2 monitoring (used as a measure of microbial decomposition) suggested the initial ^{99}Tc release (up to 40% in the first eight weeks) was due to leaching and that microbial decomposition was responsible for the release of the remainder in the latter phase.

A second, larger scale experiment confirmed the release of ^{99}Tc from *Fucus vesiculosus* into soil solution with around 3% of the ^{99}Tc added within the seaweed present in the soil solution after 18 weeks. In the initial six weeks the rate of release of ^{99}Tc was faster from seaweed cut into 4-6 cm pieces that from those cut into 20-25 cm pieces. Monitoring of the CO_2 production indicated that there was no significant difference between the two treatments suggesting that a factor other than microbial decomposition was influencing the rate of ^{99}Tc release. The predominant mechanism appeared to be leaching. Around 46% of the ^{99}Tc contained within the seaweed was released into the soil over 21 months, of which around 11% was present in the soil solution. A proportion of ^{99}Tc released into the soil was readily taken up by spinach plants with an average concentration ratio calculated as 120 (Range 10 – 414). This suggested that some ^{99}Tc was present in the soil solution as pertechnetate.

Around 36% of ^{99}Tc could be leached out of the seaweed when shaken in rain water over a six day period, the majority being lost between days two and four. This was similar to the time period when soluble sugars were lost. While this suggested a considerable proportion of ^{99}Tc was present in a soluble form the percentage was markedly lower than was expected

from the results of published experiments carried out with artificially contaminated seaweed.

Investigation of the chemical forms of ^{99}Tc present in the soil solution using gel chromatography revealed that one month after seaweed addition to the soil between 36% and 50% of the ^{99}Tc in the soil solution was present as pertechnetate. This confirmed that a substantial amount of ^{99}Tc released from *Fucus vesiculosus* into soil would be available for plant uptake.

The high rate of ^{99}Tc release from *Fucus vesiculosus* into the soil and the subsequent presence of bioavailable pertechnetate in the soil solution shown by these experiments confirm that the practice of using contaminated seaweed as a soil conditioner is a potentially important pathway for the sea-to-land-to-plant transfer of ^{99}Tc. There is the potential, therefore, for human exposure to ^{99}Tc on consumption of vegetables grown on plots conditioned with contaminated seaweed.

CHAPTER 1 - INTRODUCTION

In the latter decades of the twentieth century, contamination of the environment by anthropogenic radionuclides became a major issue in scientific, political and public circles. This contamination, and its possible health threats to humans, led to much research into the behaviour of these radionuclides in the environment in the 1960s to 1980s (Mackenzie, 2000). One important field of study has been the environmental impact of liquid discharges from reprocessing plants associated with the nuclear fuel industry into the marine environment and the associated risk to human health due to radionuclides passing through food chains. The main source of anthropogenic radionuclides in the sea around the UK is the reprocessing plant at Sellafield in Cumbria, owned by British Nuclear Fuels Ltd (BNFL). The company has made advances in reducing the quantity of most radionuclides discharged into the marine environment but one exception to this trend was technetium-99 (^{99}Tc) which showed a dramatic increase during the mid 1990s.

1.1 LIQUID DISCHARGES FROM BNFL SELLAFIELD

Used fuel from nuclear power stations has been recycled in the UK for almost 40 years on a single site at BNFL Sellafield in Cumbria (BNFL, 2001). This process separates reusable uranium and plutonium in the spent fuel rods from waste products that are then stored as high-level waste with each tonne of fuel producing approximately 0.1 m^3 of waste (BNFL, 2003a). These discharges from Sellafield are the main source of anthropogenic radionuclides in the marine environment around the UK (DEFRA, 2003). The radionuclides present in the discharge include the beta emitters, tritium, plutonium-241, caesium-137, ruthenium-106 (the latter two also emit gamma radiation), technetium-99 and

alpha emitters, plutonium and americium-241 (DEFRA, 2003). The activity concentrations of radionuclides in the discharge were highest in the 1970s with 4000-9000 Terabequerels (TBq) of beta-emitting radionuclides (not including tritium) and 40-160 TBq of alpha-emitting radionuclides discharged (BNFL, 2001).

The 1970s saw an increase in awareness of the impact of such practices on the environment by scientists, governments and the public and the need to reduce the activity concentrations in these discharges was recognised. The UK adopted the Convention for the Prevention of Marine Pollution from Land-Based Sources in 1974 which, amongst other things, aimed to address the issue of marine pollution from the discharge of dangerous substances via pipelines (JNCC, 2004). This was superseded by The Convention for the Protection of the Marine Environment of the North East Atlantic (OSPAR) which was adopted in 1992 and ratified by the UK in 1998 (JNCC, 2004). This committed the Government to "ensure that discharges, emissions and losses of radioactive substances are reduced by the year 2020 to levels where the additional concentrations in the marine environment above historic levels, resulting from such discharges, emissions, losses, are close to zero" (MAFF & SEPA, 1999). In response to legislation, BNFL Sellafield has reduced the total activity concentration of the radionuclides discharged into the Irish Sea over 100-fold since the 1970s (BNFL, 2001).

1.2 TECHNETIUM-99

While the activity concentration of most radionuclides in the discharge from BNFL Sellafield has decreased, that of technetium-99 (^{99}Tc) has increased since 1994. This is due to the operation of the Enhanced Actinide Removal

Plant, which although it successfully removed most of the alpha and some of the beta emitting radionuclides from the waste stream, does not remove ^{99}Tc (Busby *et al.*, 1997; Leonard *et al.*, 1997: Brown *et al.*, 1999). The consequence was that ^{99}Tc, which before 1994 constituted around 5% of the total beta activity (ignoring tritium) in the discharge, became the major constituent making up 57% to almost 100% of the total in 1994 to 2001 (DEFRA, 2003). The increase in ^{99}Tc discharged into the Irish Sea resulted in a series of studies to monitor and evaluate its impact on the marine environment, which will be discussed in detail in Chapter 2. Some of the major findings were that ^{99}Tc was bioaccumulated readily by brown seaweed species and some crustaceans and that it was transported by ocean currents to contaminate the coastal waters of Norway and Ireland (Leonard *et al.*, 1997; Brown *et al.*, 1998; Kershaw *et al.*, 1999). These findings raised the profile of ^{99}Tc within government and environmental bodies and many concerns, which are still ongoing, have been aired. In the late 1990s the use of ^{99}Tc contaminated seaweed as a soil conditioner in coastal areas, including Cumbria, was recognised as creating a new pathway for the transfer of ^{99} Tc from marine to terrestrial ecosystems and onto man (Camplin *et al.,* .1999)

1.3 THE SEA-TO-LAND-TO-PLANT PATHWAY

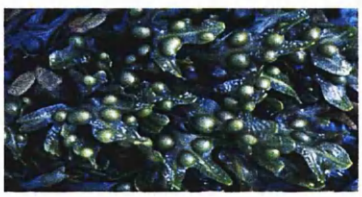
The potential for the sea-to-land-to-plant pathway for ^{99}Tc was first recognised in 1984 but it was not until ^{99}Tc activity concentration in the Sellafield discharge rose in 1994 that concerns grew about the practice (Camplin *et al.,* .1999). A simplified diagram of the proposed pathway is shown Figure 1:1.

BNFL Sellafield

^{99}Tc discharged via pipeline

^{99}Tc remains in water column

Bioaccumulation by brown seaweed



High activity concentration of ^{99}Tc in brown seaweed

? ^{99}Tc released as seaweed added to soil decomposes

^{99}Tc accumulation in soil

Plant uptake of ^{99}Tc

^{99}Tc accumulation in crop plants

Figure 1:1: The proposed sea-to-land-to plant pathway for ^{99}Tc

When seaweed, which has bioaccumulated ^{99}Tc, is used as a soil conditioner on vegetable plots it is thought that the ^{99}Tc may be released as the seaweed

decomposes and could then be available for plant uptake. The produce of individuals who apply seaweed on their vegetable plots is being routinely monitored as part of the Terrestrial Monitoring Programme which is published annually in the Radioactivity in Food and the Environment reports (FSA & SEPA, 2000). While this monitoring has shown that ^{99}Tc is present in vegetables, knowledge of the transfer mechanisms and dynamics is incomplete. As the pathway is considered to be the most important pathway for the sea-to-land-to-plant transfer of ^{99}Tc (Camplin *et al.,* 1999), these mechanisms merit further research.

1.4 OUTLINE OF THESIS

Before undertaking any research programme a thorough investigation of previous research carried out on the subject is necessary. Chapter 2 contains an overview of the historical and present day use of seaweed as a soil conditioner and an outline of decomposition processes in soil, likely to be relevant to the release of ^{99}Tc to soil. Published research on ^{99}Tc in the environment is reviewed, concluding with a discussion on the gaps in knowledge in the sea-to-land-to-plant pathway that this study aims to address.

Chapter 3 gives a detailed description of the generic experimental methods used throughout the study. The experimental design and results for each experiment will be presented in Chapters 4, 5, 6 and 7. A pot experiment, which aimed to quantify the amount of ^{99}Tc released from the brown seaweed species, *Fucus vesiculosus* into a sandy coastal soil and interpret the temporal release pattern in relation to decomposition processes, is presented in Chapter 4. Chapter 5 contains details of a larger scale pot experiment, which build on the results of the first experiment. This experiment aimed to quantify the

amount of ^{99}Tc released from *Fucus vesiculosus* into soil solution and determine its availability to spinach plants. In addition, the rate of release of ^{99}Tc with size of seaweed pieces was investigated. The role of leaching in the release of ^{99}Tc from *Fucus vesiculosus* is further investigated in Chapter 6. This experiment aimed to quantify the readily leachable fraction of ^{99}Tc contained within the seaweed and compare the rate of leaching of ^{99}Tc with that of other ions. Chapter 7 describes efforts to identify the chemical species of ^{99}Tc present in the soil solution by gel chromatography and contains details of trials of different gelfiltration columns. The results from these trials are discussed. Chapter 8 presents a summary discussion of all results and assesses their implications for practices in the field. A critical appraisal of the methods used and suggestions for further work are also included.

CHAPTER 2 - LITERATURE REVIEW

The starting point for this study was the identification of a potential route of ^{99}Tc transfer from marine to terrestrial environment through the use of seaweed as a soil conditioner in coastal areas (Camplin *et al.,* 1999). To underpin the research programme and provide a secure experimental design the existing literature on ^{99}Tc, seaweed and its use as a soil conditioner was undertaken. This chapter presents the results of this review and is divided into four discrete sections. Section 1 outlines the historical and present day use of seaweed as a soil conditioner and the species of seaweed collected. An overview of decomposition of organic matter in soil is then presented as these processes will influence the transfer of ^{99}Tc from the seaweed into the soil. The third section presents a review of the environmental behaviour of ^{99}Tc. The final section discusses current knowledge of ^{99}Tc with respect to the sea-to-land-plant pathway and exposes the gaps in our knowledge that this research programme aims to fill and introduces the experimental hypotheses to be tested.

2.1 SEAWEED AS A SOIL CONDITIONER

2.1.1 Collection and application

Seaweed has been gathered in coastal areas for use as a soil conditioner and fertiliser for generations in Britain, Ireland, France and other parts of Europe (Stephenson, 1968). The method of collection varied between different regions with hand collection the most usual method (Stephenson, 1968; Chapman & Chapman, 1980). Generally mixed species of seaweed, predominantly brown seaweed, thrown up on shore by storms (drift weed) were collected but in some

regions specific species were collected or cut from rocks (Blunden, 1991), e.g. *Fucus* species in the Channel Islands and Ireland (Stephenson, 1968). The method of seaweed application also varied. In the Scottish Hebrides the seaweed was layered on top of the soil but not ploughed in, on the Isle of Man it was composted with manure and straw from animal stalls and in the Channel Islands it was dug into the soil to about one foot below the surface (Stephenson, 1968).

The use of raw seaweed in this manner has generally ceased on any large scale (Stephenson, 1968; Noble, 1978) but it is still used on a small scale by gardeners, small holders and a number of National Trust for Scotland properties (Royal Botanic Gardens, 2000). Evidence for this type of use can be found in parts of England including Cumbria (Chapman & Chapman, 1980, Camplin *et al.*, 1999; Anonymous, 2001), in Scotland and in Ireland (Stephenson, 1968; Blunden 1991).

2.1.2 Benefits of adding seaweed to soil

The majority of coastal soils are sandy soils that have low organic matter content, high drainage rate and a poor ability to store essential plant nutrients (Brady & Weil, 1999). The addition of seaweed adds essential nutrients and organic matter to these soils. Seaweed has similar nitrogen content to farmyard manure but with higher potassium and lower phosphorus levels (Chapman & Chapman, 1980). It also contains many trace elements e.g. manganese (Stephenson, 1968), that are essential for healthy plant growth. The availability of some of

these nutrients is dependent on the microbial breakdown of complex molecules within the seaweed (Blunden, 1991; Vymazal, 1995). Soluble substances are released first e.g. sugars, amino acids, sodium and potassium (Vymazal, 1995). The loss of nitrogen is dependent on the initial nitrogen content of the seaweed, plants with low nitrogen content and therefore a high C:N ratio can lose nitrogen quickly at the start of the decomposition process but then accumulate nitrogen in the later stages (Vymazal, 1995). Brown seaweed species decompose slowly (Smith & Foreman, 1984) due to their high polyphenol content (Blunden, 1991), which may delay the release of some nutrients into the soil. Manuring sandy soil with seaweed increases the capacity of the soil to grow crops such as oats, brassicas (Royal Botanic Gardens, 2000), and potatoes (Stephenson, 1968; Chapman & Chapman 1980).

The addition of seaweed also has beneficial effects on the physical structure of the soil. The addition of *Laminaria digitata* (kelp) to a sandy loam soil increases the total pore volume and the aggregate stability of the soil resulting in an increase in the water holding capacity of the soil (Haslam & Hopkins, 1996). The release of algal polysaccharides from the decomposing seaweed was a possible reason for the increase in aggregate stability (Haslam & Hopkins, 1996). A number of seaweed derived products have been found to improve aggregate stability e.g. alginates and fucoidan (Blunden , 1991).

2.1.3 Commercial seaweed products

Several species of seaweed are collected around the coast of the UK, Europe and the rest of the World for the production of agricultural and horticultural fertilisers and soil conditioners as well as various products for the food and cosmetic industries (Minch Project, 1995). There is evidence to suggest that seaweed collection and processing could be a sustainable industry which could provide employment in remote areas such as the Western Isles of Scotland (Minch Project, 1995). Brown seaweed species are the ones most commonly collected as these contain biologically active compounds such as cytokinins and auxins, which promote plant growth (Minch Project, 1995; Blunden, 1991). The seaweed products also improve soil stability and increase the resistance of plants to frost and other stress conditions (Blunden, 1991). Seaweed products, both granular and liquids are marketed for use in agriculture, gardens, golf courses and other sports grounds (Royal Botanic Gardens, 2000; Guiry & Nic Dhonncha, 2002). However, little if any of the seaweed collected comes from the Irish Sea and the species collected do not tend to bioaccumulate ^{99}Tc (MAFF & SEPA, 1999).

2.1.4 Seaweeds

Many species of seaweed are found around the coast of the UK and Europe. They can be divided up into three groups, *Chlorophyta* (Green algae), *Rhodophyta* (Red algae) and *Phaeophyta* (Brown algae) (Lobban & Harrison, 1994). This study will focus on one species of brown algae, *Fucus vesiculosus*, as it is one of the most common species collected for use in agriculture and

industry (Chapman & Chapman 1980) and has been extensively monitored in ^{99}Tc studies in the Irish Sea and beyond (MAFF & SEPA, 1998, 1999; Brown *et al.,* 1999; FSA & SEPA 2000, 2001, 2002, 2003).

2.1.5 Fucus vesiculosus

Fucus vesiculosus (Figure 2:1) (Class: Phaeophyceae, Family: Fucaceae), a brown seaweed known commonly as bladderwrack, is native to Britain and has a wide distribution on rocky coasts (Figure 2:2). It is also found throughout the Atlantic coasts of Europe and North America.

Figure 2:1: *Fucus vesiculosus*

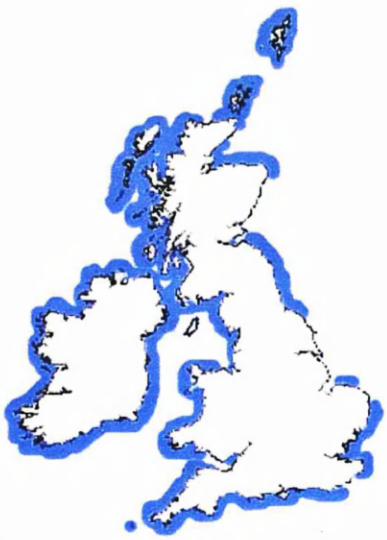


Figure 2:2: Distribution of *Fucus vesiculosus* around the coast of Britain and Ireland (Guiry & Nic Dhonncha, 2002)

2.1.5.1 General biology

Fucus vesiculosus is a mid-intertidal seaweed consisting of a dichotomously branched thallus with paired gas-filled vesicles which help the fronds float when immersed (Guiry & Nic Dhonncha, 2002). It grows permanently anchored to the rocky substrate by a structure known as a holdfast (White, 2000). The plant which has a simple cellular structure with no true leaves, roots, stems or vessels is a photoautotroph and extracts all the nutrients required for growth from the surrounding seawater through its whole surface area (Chapman & Chapman, 1980). The life span of individual plants varies with the growing conditions but on average they live for two to five years, growing up to two metres in length before they are dislodged from their anchor site by rough seas (Chapman & Chapman, 1980; White, 2000).

2.1.5.2 Chemical Composition

Brown seaweed species contain 70-90% water. Analysis of the remaining dry matter content shows that it contains 40 - 70% carbohydrate, 5 - 18% protein, 2 - 7% fat, 1-10% tannins and small amounts of sodium, potassium, iodine and other minerals (Indergaard & Minsaas, 1991). The main fibrillar material in cell walls of all brown seaweed is the polysaccharide, cellulose (Lobban & Harrison, 1994), while carbon is stored within the cell as the polysaccharide laminarin, the polyhydroxyalcohol, mannitol (a soluble storage carbohydrate), and other carbohydrates (Indergaard & Minsaas, 1991). Brown seaweed species, including *Ascophyllum nodosum, Fucus* and *Laminaria* species, contain phenolic and polyphenolic compounds including brominated phenolics, coumarins and halogenated bromines that may act as antibacterial agents (Lobban & Harrison, 1994). These antibacterial compounds may retard the activity of soil microbes that play a role in decomposing seaweed added to the soil.

2.2 DECOMPOSITION IN SOIL

Specific studies of the decomposition of seaweed in soil are lacking, therefore a review of the generic processes of decomposition of terrestrial plant litter is presented.

2.2.1 Introduction

The functioning of all ecosystems is dependent on the recycling of organic material from plants and animals into the soil to maintain further plant growth. Dead plant (litter) or animal material and waste products from animals thus

become a food resource for other plants and animals in the ecosystem. This review will concentrate on the decomposition of plant litter.

The plant litter that is returned to the soil needs to be broken down to simpler compounds by a number of processes, which occur simultaneously, so that the nutrients they contain can become available for plant uptake. Together these processes are known as decomposition and produce changes in the physical and chemical state of the litter. The following sections give a brief overview of decomposition processes, the factors that influence decomposition rates in the soil and methods available for the measurement of the rate.

2.2.2 Decomposition processes

2.2.2.1 Comminution

Comminution is the physical break up of litter that happens as a result of the feeding activities of soil organisms or abiotic factors such as freeze–thaw or wet-dry cycles in the soil (Swift *et al.*, 1979). In agricultural systems the action of ploughing or digging in of plant material results in a breaking up of the litter. As well as resulting in reduction of particle size, comminution can also result in changes in chemical composition when the plant material is passed through the digestive system of soil organisms. This chemically altered material then becomes a food source for other soil organisms. A large variety of soil organisms are involved in this process including earthworms, millipedes, molluscs and various insect larvae, depending on the habitat, resource type and the environmental conditions and usually successions of organisms are seen (Mason, 1977). The fragmentation of the material enables increased leaching of

soluble compounds and creates a larger surface area which catabolic enzymes can attack to further breakdown the material (Swift *et al.*, 1979).

2.2.2.2 Leaching

Leaching is an abiotic process that involves the rapid removal of soluble compounds from both intact and comminuted resource material by water flow, with the most rapid loss occurring in the first month after addition to the soil (Mason, 1977). This results in a loss of weight and change in chemical composition of the litter. The soluble material removed from the litter by leaching is transferred to sites within the soil where further decomposition processes can act upon it. Different compounds leach out of the material at different rates. While the rate and order of compounds leached from plant litter vary according to litter type and environmental conditions a general order can be seen. Initial losses include soluble sugars and amino acids (Wagner & Wolfe, 1998). Sodium and potassium ions are rapidly leached whilst calcium and magnesium, which tend to be bound within complex structural compounds, are lost at a slower rate (Mason, 1977). The slowest ion leaching rates are found with iron, zinc and lead (Laskowski, 1995).

2.2.2.3 Catabolism

Catabolism or microbial decomposition is a chain of enzyme controlled reactions which results in the biochemical transformation of complex molecules in the litter into simpler molecules. This is carried out in the soil by a variety of microbes that produce a range of extracellular enzymes which break down the complex molecules. In the final step of microbial decomposition, the simple compounds

are transported into the microbial cell to provide energy and building blocks for new cell tissue. These reactions can occur under aerobic or anaerobic conditions but in most soils aerobic decomposition is predominant (Wagner & Wolfe, 1998). Figure 2:3 summarises the reactions involved in microbial decomposition. The release of CO_2 occurs in both aerobic and anaerobic decomposition pathways and is accepted as a quantitative expression of active microbial decomposition (Wagner & Wolfe, 1998).

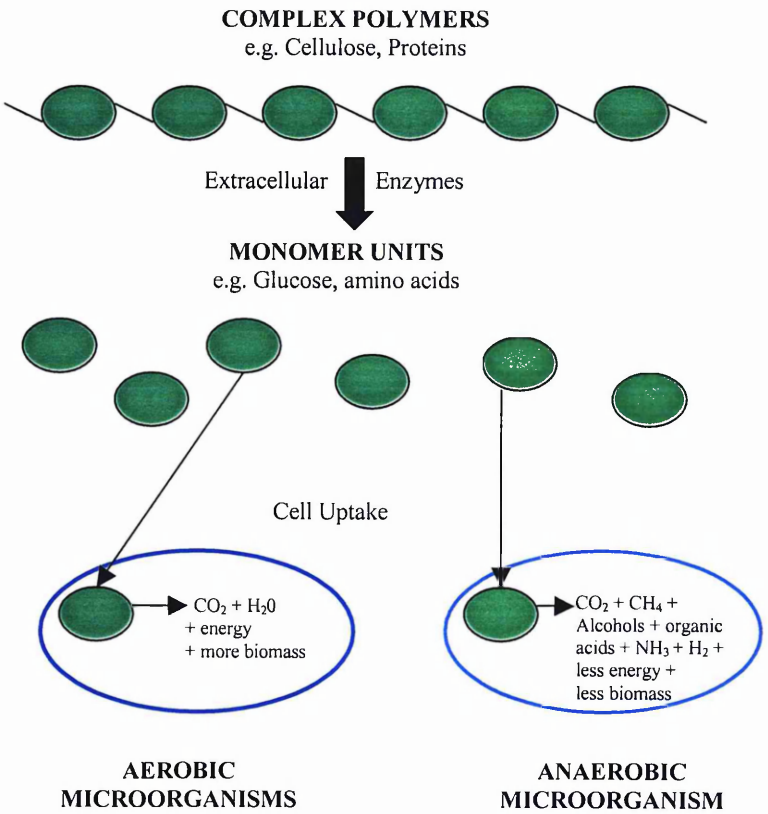


Figure 2:3: Catabolic reactions involved in microbial decomposition (adapted from Wagner & Wolfe, 1998)

Microbes in soil compete for the food resource and can be split into two main groups by their life strategy. R-strategy, (zymogeneous) microbes are opportunists that can readily exploit new organic material added to the soil, they are fast growing and tend to have short life spans. K-strategists, (autochthonous microbes) are slower growing organisms that cannot compete with the r-strategists in the early phases of decomposition but increase in activity in the latter stage when the available resource decreases (Killham, 1994). These organisms tend to be specialists at decomposing the more resistant material in plant litter.

Fresh supplies of litter, containing readily available, simple compounds, added to soil trigger the proliferation of opportunist microbes that rapidly deplete the easily decomposed material (Brady & Weil, 1999). These opportunist microbes die once their food supply is depleted allowing small populations of autochthonous microbes to slowly decompose the more resistant material such as cellulose, lignin and phenolic compounds (Ross, 1999). The ability of microbes to degrade particular compounds depends on their capacity to produce the enzymes required to catalyse the breakdown of the compound (Wagner & Wolfe, 1998).

2.2.3 Factors affecting decomposition in soil

The rate of decomposition of organic material in the soil is dependent on several factors, many of which are inter-related. These can be divided into factors that affect the physico-chemical environment in the soil and those which affect the resource quality (Swift *et al.*, 1979). The main factors that influence the soil

environment are temperature, moisture, pH and aeration (Swift *et al.*, 1979), while those that affect litter quality include the structural and chemical composition of the material (Dickenson & Pugh, 1974). These factors combine to affect the metabolic activity of soil microbes and thus the decomposition of the litter.

2.2.3.1 Temperature

Soil temperature fluctuates diurnally and seasonally. Soil is a poor conductor of heat (Brady & Weil, 1999), consequently there is a lag in response time to surface heat fluctuations with increasing depth. At 10 - 15 cm depth the diurnal temperatures are higher at night than during the day, also the amplitude of the fluctuation decreases with depth (Swift *et al.*, 1979; Hartel, 1998).

Temperature has a large influence on microbial metabolism and thus on decomposition processes. There is a strong positive correlation between CO_2 evolution from clay loam and sandy field soils and air temperature (Kowalenko *et al.*, 1978). Within a certain temperature range (generally 5 - 40°C (Brady & Weil, 1999)), a 10 degree increase in soil temperature roughly doubles microbial activity (MacDonald *et al.*, 1995). This is referred to as the Q_{10} for biological systems, where Q_{10} =2 (Hartel, 1998). Below 5°C microbial activity and decomposition virtually stop and therefore this temperature is often referred to as biological zero (Brady & Weil, 1999). The main groups of soil microbes have optimum temperature ranges of 25 - 30°C and minimum activity temperature

ranges of 5 – 10°C, while few species are able to thrive at lower temperatures (e.g. some *Pseudomonas* species and some fungi) (Dickenson & Pugh, 1974).

2.2.3.2 Moisture

The moisture content of soil is important as water facilitates the movement of mobile micro-organisms, allows gas exchange, is a reactant in many biochemical reactions and also influences other factors such as pH, aeration and temperature (Swift *et al.*, 1979). The water content of a particular soil is dependent on the amount of precipitation it receives balanced against how much is lost through drainage and evapotranspiration (Swift *et al.*, 1979). The structure of the soil greatly influences how quickly water drains away under gravity, with clay soils retaining larger amounts of water than very sandy soils (Hartel, 1998). This is because clay soils have a larger percentage of small micropores to trap water than sandy soils that tend to have large macropores that are easily drained.

Soil water can be expressed in terms of its volumetric (θ_v) or gravimetric (θ_w) water content or as a measure of the potential energy (Ψ) in the soil compared to the potential energy of pure free water ($\Psi = 0$). Potential energy is the most accurate way of measuring soil moisture content and is expressed in units of pressure, kilopascals (kPa) or megaPascals (MPa) (Hartel, 1998).

If water from saturated soil is allowed to drain away freely, the macropores will drain first and when they are empty the soil is said to be at field capacity. The water potential at which this occurs is approximately -33 kPa except for sandy soils where it is approximately -10 kPa (Hartel, 1998). Maximum decomposition

rates occur when the soil water content is around 60% of field capacity as this provides conditions where there is both enough water and oxygen for aerobic decomposition processes (Wagner & Wolfe, 1998). Kowalenko *et al.,* (1978), showed that when water content of a clay loam was below the optimum, increasing the water content increased CO_2 production, while when it was above the optimum increasing water content decreased CO_2 production. This suggests any deviation from the optimum moisture level decreases the rate of microbial decomposition. The pattern was not quite so clear for a sandy soil.

2.2.3.3 pH

The majority of soil micro-organisms have a preference for near neutral pH conditions (pH = 6 to 7), however some are adapted to higher or lower pH conditions and microbes can be found in soils from pH 1 to 13 (Alexander, 1998). The pH of a soil however will vary between microsites (Dickenson & Pugh, 1974), so microbes may move within the soil to more favourable conditions. The pH plays an important role in the availability of essential elements to micro-organisms (Swift *et al.*, 1979), and most of these elements are most available at near neutral pH (Alexander, 1998), e.g. phosphate availability is reduced at high and low pH because it forms calcium, iron and aluminium phosphates (Mason, 1994).

2.2.3.4 Aeration

As can be seen from Figure 2:3, aerobic decomposition is a more efficient process, yielding more energy and biomass, than anaerobic decomposition. The aeration of the soil, which is dependent on soil moisture, texture and porosity, is

therefore important for decomposition processes. Soil aeration can be measured as a redox potential which is the tendency of a compound to accept or donate electrons (measured in millivolts). Aerobic (oxidised) soils have a redox potential of around 600 mV while waterlogged, anaerobic (reduced) soils have lower potentials between 400 and -200mV (Rowelll, 1994).

Soil microbes vary in their requirement for oxygen for metabolic processes. Some are strict aerobes but others, known as facultative anaerobes, can thrive in either aerobic or anaerobic conditions, while obligate anaerobes can grow only when there is no oxygen present (Alexander, 1998). The highest level of microbial decomposition however, is found in aerobic conditions (Swift *et al.*, 1979).

2.2.3.5 Litter or resource quality

The composition of the dry matter content of the litter can determine the rate of decomposition. The major types of organic compounds in green plant material are typically cellulose (45%), hemicellulose (18%), lignin (20%), protein (8%), sugars and starches, (5%), fats and waxes (2%) and polyphenols (2%) (Brady & Weil, 1999). These percentages however can vary widely with plant species. The ease at which soil microbes can decompose these compounds varies. Soluble compounds such as sugars and some amino acids are quickly utilised by soil microbes, however lignins and polyphenols are very slow to decompose (Brady & Weil, 1999). Polyphenols can inhibit the decomposition process as they make the litter less palatable to soil fauna thus repressing the feeding activity that

breaks up the resource into small particles. They can also leach out of the resource to produce tannins which cause proteins (e.g. enzymes) to precipitate, thus slowing down the chemical decomposition of the litter (Mason, 1977).

While soil microbes metabolise carbon compounds to obtain energy they also require a certain balance of nutrients to grow and thrive. One major nutrient is nitrogen found in proteins and amino acids. Micro-organisms require around 1 g of nitrogen for every 24 g of carbon in their food source. If the litter contains a carbon/nitrogen (C/N) ratio greater than 25:1, decomposition will slow down unless the microbes can obtain nitrogen from elsewhere in the soil (Brady & Weil, 1999). Average C/N ratios of some common materials are pine sawdust 225:1, oat straw 80:1, rotted cattle manure 18:1 (Zibilske, 1998) and household compost 7:1 (Brady & Weil, 1999).

The size of the particles making up the litter has an effect on the decomposition rate with the rate increasing as particle size decreases (Brady & Weil, 1999). Small particle size may be achieved by comminution of the litter by abiotic factors or feeding mechanisms of soil fauna (Swift *et al.*, 1979).

2.2.4 Methods of monitoring decomposition in soil

Decomposition in soil can be monitored by direct or indirect methods. Some direct methods involve measurement of weight loss and change in chemical composition of litter in the field using some form of litter bag placed on the soil surface or within the soil profile (Swift *et al.*, 1979). The problems associated with this method include (Swift *et al.*, 1979):

- Determining the size of the mesh of the litter bag to allow soil fauna to enter but small enough to prevent losses of small fragments
- Litter bags easily contaminated by roots, moss etc.
- Difficult to measure losses through leaching in the field
- Mesh size of litter bag can have an impact on moisture content so can influence rate of decomposition

Labelling plant material with a radioisotope, then monitoring the losses of radiolabelled elements through the various decomposition processes can be another direct method of measuring decomposition. This method can be limited by the difficulty of obtaining a suitable radioisotope and the costs involved (Swift *et al.*, 1979).

Indirect methods of monitoring decomposition include the measurement of CO_2 produced by soil microbial metabolism. Carbon dioxide production is widely accepted as a quantitative expression of catabolic processes (Wagner & Wolfe, 1998). This method can be used with or without radiolabelled CO_2. Using radiolabelled CO_2 differentiates between CO_2 produced by decomposition and CO_2 from other sources in the soil, e.g. root respiration (Wagner & Wolfe 1998). This method is difficult to use in the field but it can be used more readily for laboratory experiments (Swift *et al.*, 1979). Another, more complex, indirect method is the measurement of extracellular enzymes e.g. dehydrogenase, produced by micro-organisms during decomposition (Swift et al., 1979).

2.3 TECHNETIUM IN THE ENVIRONMENT

2.3.1 Introduction

Technetium (Tc), a word derived from Greek meaning artificial, is a synthetic radioactive metal of Group VIIb of the periodic table with the atomic number 43 (Encyclopaedia Britannica, 2001). In 1937, it was the first element that does not occur naturally, to be artificially produced when molybdenum was bombarded by deuterons in a particle accelerator (Encyclopaedia Britannica, 2001). It has over forty known isotopes, none of which are stable, with physical half-lives ranging from a few seconds to millions of years, some of which are shown in Table 2:1 (Berkley, 2001).

Table 2:1: Physical half-lives of some technetium isotopes

Isotopes	Half-life
^{99m}Tc	6.02 hours
^{96}Tc	4.3 days
^{99}Tc	213,000 years
^{98}Tc	4,200,000 years

The predominant isotope present in the environment, ^{99}Tc, has a long half-life of 213,000 years and is a low energy beta emitter (E_{max} = 292 keV) (Holm, 1993). This isotope is a fission product that has entered the environment through the testing of nuclear weapons and the discharge of low level radioactive waste from nuclear reprocessing plants (Schulte & Scoppa, 1987; Holm, 1993; Leiser, 1993) with nuclear processing plants being the main contributor (EA, 2000). The fission yield of 6.13% means that approximately one kg of ^{99}Tc is produced from one ton of uranium (Leiser, 1993).

2.3.2 Chemistry

Speciation has been defined as the sum of the chemical species in which an element occurs in a component of the environment (Desmet *et al.*, 1991). Tc-99 can exist in the environment in several oxidation states, primarily (VII), (IV) and (0) (Schulte & Scoppa, 1987; Sparkes & Long, 1988; Leiser, 1993). The pertechnetate ion (TcO_4^-) (VII) is the dominant species in aqueous solution and as it is considered to be stable under a wide range of pH and E_h values (Figure 2.4,(Beasley & Lorz, 1986)), it is probably the most stable form of ^{99}Tc in the oxic environment (Wildung *et al.*, 1977; Beasley & Lorz, 1986; Sparkes & Long 1988).

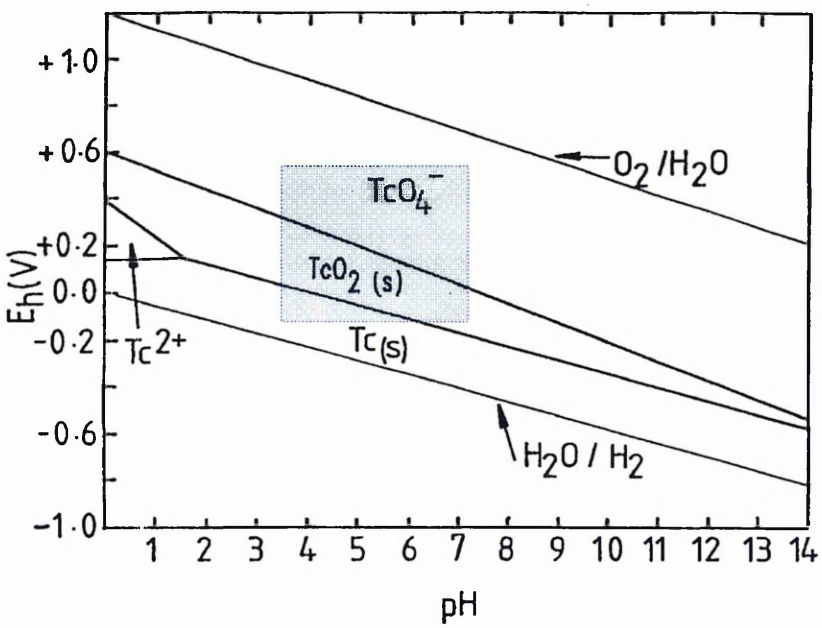


Figure 2:4: Stability diagram for technetium under various environmental conditions of pH and E_h (Beasley & Lorz, 1986). (Shaded area indicates likely area of interest for this study)

Tc(IV) is formed under reducing conditions and other valance states, (III), (V) and (VI) have been reported (Leiser, 1993). In the reduced form (TcIV) technetium can bind to several ligands on soil organic matter (Bishop *et al.*, 1989; Leiser 1993). The oxidation state of ^{99}Tc can have a significant effect on its behaviour in the environment (Leiser, 1993). This will be discussed in more detail in a later section.

2.3.3 Tc-99 and BNFL Sellafield

The discharge of liquid waste from nuclear reprocessing plants is recognised as one of the main ways that ^{99}Tc enters the environment (Schulte & Scoppa, 1987; Holm, 1993; Leiser, 1993). BNFL Sellafield in Cumbria, UK and COGEMA – La Hague in France are the two main sources of ^{99}Tc around the waters of the UK and Northern Europe. However, since 1990, the discharges from La Hague have fallen to almost zero while those from Sellafield have increased (Kershaw *et al.*, 1999). In total, Sellafield has discharged over 10 times the amount of ^{99}Tc into the marine environment than La Hague (Holm, 1993). This study will focus on ^{99}Tc discharges from Sellafield.

2.3.3.1 Tc-99 and reprocessing

British Nuclear Fuels Limited (BNFL) has been reprocessing spent nuclear fuel at their plant at Sellafield, Cumbria since 1952 (Leonard *et al.*, 1997) . A number of different processes are carried out at the plant to treat waste from different sources, but most of the ^{99}Tc is produced from the reprocessing of fuel from Magnox reactors (EA, 2000). Current amounts of ^{99}Tc resulting from the process annually equal 45 – 60 kg or 30 - 40 terabequerels (Tbq = 10^{12} Bq). (EA, 2000).

Reprocessing the Magnox fuel produces a concentrated liquid waste known as Medium Active Concentrate (MAC). Pre-1981 the MAC was stored on site for several years, to allow radioactive decay of short-lived radionuclides, before being discharged into the Irish Sea without any further treatment. In the early 1980s, due to increasing concerns about public health, MAC discharges were halted and the waste stored in tanks until suitable processes could be installed to remove as much of the radioactivity from it as possible (EA, 2000). The waste was stored until the Enhanced Actinide Removal Plant (EARP), which removes radionuclides by precipitation and ultrafiltration, was commissioned in 1994 (EA, 2000). However, ^{99}Tc cannot be removed effectively by these processes, as it is present in the acidic MAC as the highly soluble pertechnetate ion (TcO_4^-) (Busby *et al.*, 1997; Leonard *et al.*, 1997; Brown *et al.*, 1999). At present, discussions are taking place between relevant government departments, the Environment Agency, the Health and Safely Executive and BNFL aimed at finding a suitable technical and economic method to remove ^{99}Tc from MAC (EA, 2000; DEFRA, 2003; BNFL, 2003). The Environment Agency has proposed two solutions,

- MAC diversion in which newly produced MAC will be converted into glass blocks, and
- Treatment of stored waste with tetraphenylphosphonium bromide (TPP) resulting in the solidification of ^{99}Tc which will then be mixed with cement and encased in steel drums (DEFRA, 2003; BNFL, 2003). This has been successful in laboratory trials (BNFL, 2003)

In October 2003 BNFL began larger scale trials using TPP to remove ^{99}Tc from MAC. This trial has been deemed a success and in April 2004, BNFL announced that it was implementing the process and subsequently ^{99}Tc discharges from Sellafield will be reduced by 90% (BNFL, 2004).

2.3.3.2 Tc-99 discharges to the Irish Sea

BNFL have been authorised by the Government to release controlled amounts of radionuclides, including ^{99}Tc, from Sellafield through a pipeline into the Irish Sea since 1952 (Schulte & Scoppa, 1987). The annual discharge limits have varied over that time. A 10 TBq per year limit was set during the period of MAC storage (1981-1994) but this was raised to 200 TBq when EARP began operating to allow the discharge of liquid waste produced from the treatment of the stored waste (DEFRA, 2003). The total amount of ^{99}Tc discharged until 2002 is around 1665 TBq (EA, 2000; FSA & SEPA, 2001, 2002, 2003). Figure 2:5 shows the annual discharges in TBq from 1970–2002.

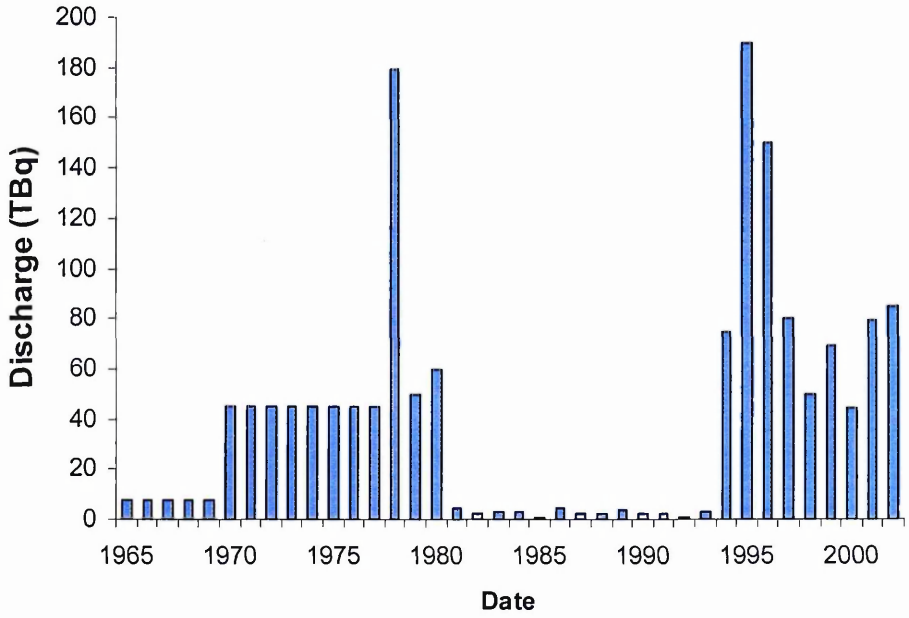


Figure 2:5: Annual discharge of ^{99}Tc from BNFL, Sellafield (1970-2002) (EA, 2000; FSA & SEPA, 2001, 2002, 2003)

Three phases of discharge, reflecting the authorised discharge limits can be seen:

- High levels of ^{99}Tc in the effluent in the1970s,
- Lower levels of ^{99}Tc during the period of MAC storage and
- A marked increase when EARP began operating in 1994 with ^{99}Tc levels similar to those in the 1970s

The lower discharge levels towards the end of the period are due to a reduction in the amount of MAC being treated while techniques to remove ^{99}Tc are being investigated (BNFL, 2001). In 1999, the Government reduced the annual discharge limit from 200 to 90 TBq and with the current discussions on

abatement techniques the Environment Agency is looking to reduce the limit to 10 TBq by 2006 or sooner if possible (EA, 2000; DEFRA, 2003).

2.3.4 Tc-99 in the marine environment

2.3.4.1 Tc-99 in seawater

In well-oxygenated seawater the most stable form of ^{99}Tc is the pertechnetate ion (Beasley & Lorz, 1986; Brown *et al.*, 1999). The ion is highly soluble and is not readily removed from the water column as it has very low (1 - 4) distribution coefficients for sediments low in organic matter(Beasley & Lorz, 1986).

The distribution coefficient (K_d) is defined as:

$$K_d = \frac{A_s}{A_l} \qquad \text{(Equation 1)}$$

Where:

A_s is the activity per unit mass solid

A_l is the activity per unit mass liquid

As ^{99}Tc behaves conservatively in well oxygenated surface waters, ^{99}Tc discharged from Sellafield remains in the water column and can be readily transported by marine currents up the west coast of the UK, around into the North Sea and onwards to the coasts of Norway and Greenland (Aarkrog *et al.*, 1986; Leonard *et al.*, 1997; Kershaw *et al.*, 1999; Brown *et al.,* 1999). Tc-99 discharged from Sellafield reaches the North Sea in about six months (Leonard

et al., 1997) and the Norwegian coast in around two and a half to three and a half years (Brown *et al.*, 1998; Brown *et al.*, 1999).

2.3.4.2 Tc-99 and marine organisms

The tendency for ^{99}Tc to remain in the water column means that is available for uptake by marine organisms. Whilst the concentration ratios for ^{99}Tc are generally low, certain types of brown seaweed and crustaceans concentrate ^{99}Tc to a high degree (Beasley and Lorz, 1986).

The term concentration ratio (Cr) can be defined as:

$$C_r = \frac{Radionuclide\ concentration\ in\ organism\ (fresh\ or\ dry\ wt)}{Radionuclide\ concentration\ in\ medium\ (water\ or\ soil)} \qquad \text{(Equation 2)}$$

(The term concentration ratio will be used in this thesis, as it is the term recommended by the International Commission on Radiation Units and Measurements (ICRU, 2001). In published literature it has also been known as transfer factor and concentration factor).

The main focus of research has been *Fucus vesiculosus*, but other species, including *Fucus serratus* and *Ascophyllum nodosum,* have been included in various studies. Concentration ratios (dry weight) for *F. vesiculosus* range from around 25,000 to 85,000 (Holm *et al.*, 1986). Concentration ratios (dry weight) vary with the species of brown seaweed, with the declining order *Ascophylum nodosum* > *Fucus vesiculosus*> *Fucus serratus* > *Laminaria digitata* reported by Holm *et al.,* (1986) confirming the observations of (Jeanmarie *et al.,* 1983).

Figure 2:6 illustrates the ^{99}Tc activity concentrations in *F. vesiculosus* (fresh weight) in the Irish Sea, close to the Sellafield reprocessing plant, from 1990 to 2002.

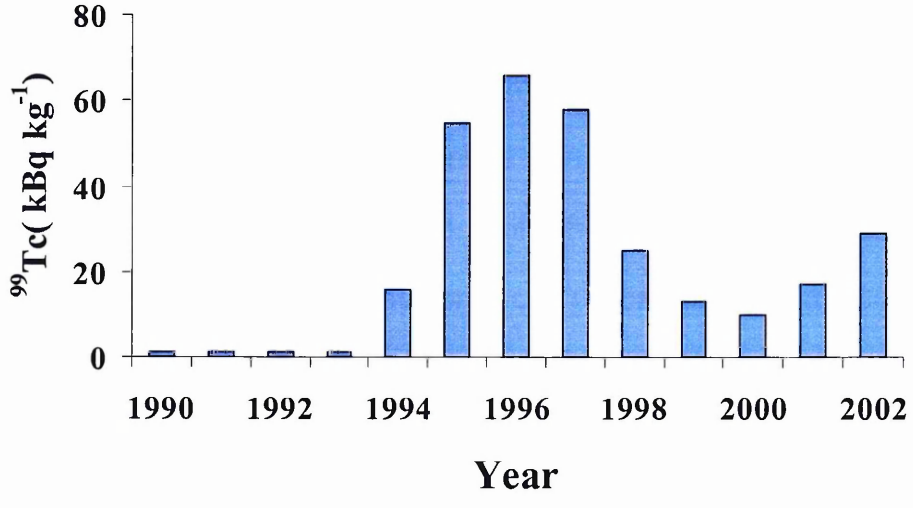


Figure 2:6: Tc-99 in *Fucus vesiculosus* close to Sellafield (1990-2002) (Camplin *et al.*, 1999; FSA & SEPA, 2000, 2001, 2002, 2003)

It shows that since the commissioning of EARP the concentration of ^{99}Tc in the seaweed has risen over 50 fold from around one kBq kg^{-1} to over 50 kBq kg^{-1} (wet weight) (Camplin *et al.*, 1999). *Homarus gammarus* (common lobster) and *Nephrops norvegicus* (norwegian lobster) also concentrate ^{99}Tc to a significant degree, with concentration ratios varying between species and between different types of tissue within each species (Busby *et al.*, 1997). The muscle generally contains the lowest activity concentration of ^{99}Tc (CF = 720 - 970) whilst the highest concentrations were in the green gland and hepatopancreas (CF = 2300) (Brown *et al.*, 1999).

2.3.5 Tc-99 in soil

Technetium, predominantly ^{99}Tc, can enter the soil from fallout from nuclear weapons testing (Schulte & Scoppa, 1987), accidental releases from radioactive waste storage and disposal facilities (Holm, 1993), the deposition of remobilized marine sediments (Morris *et al.*, 2000) and possibly the use of contaminated seaweed as a soil conditioner (Camplin *et al.*, 1999). The main forms of technetium in fallout are pertechnetic acid ($HTcO_4$) and ditechnetium heptoxide (Tc_2O_7) (Schulte & Scoppa, 1987), whilst the predominant form in waste stored or discharged is the pertechnetate ion (TcO_4^-) (Sparkes & Long, 1988).

Once in the soil, the behaviour and speciation of technetium is governed by a combination of chemical, physiochemical and biological factors (Stalmens *et al.*, 1986) and these factors have been studied using both laboratory and field methods. The solubility and mobility of ^{99}Tc in the soil depends on the chemical form present, which in turn is governed by factors such as the redox conditions, organic matter content and microbial conditions (Cataldo *et al.*, 1986; Schulte & Scopppa, 1987; Sparkes & Long, 1988; Nicholson *et al.*, 1990; Desmet *et al.*, 1991) all of which are inter-linked.

2.3.5.1 Tc-99 and redox conditions

Reduction and oxidation states of chemical elements in soil are influenced by the aeration conditions present (Brady & Weil, 1999). In well-drained soil and under aerobic conditions ^{99}Tc is predominantly present as the highly soluble and mobile pertechnetate ion (Vandecasteele *et a*l., 1986; Schulte & Scoppa, 1987). Pertechnetate is poorly sorbed to mineral soil particles under aerobic conditions,

even when it has been present in the soil for extended time periods. Distribution coefficients (K_d) vary from 0.007 to 2.8 for a range of soils (Wildung *et al.*, 1974) and values are near zero for sandy soils (Routsen *et al.*, 1977; Sheppard et al., 1983). Sorption percentages of less than 5.7% after 24 hours, to around 12% over a period of six weeks in semi-arid grasslands have been observed (Wildung *et al.*, 1986). The poor sorption of pertechnetate ions in aerobic soils means that they are present in the soil water surrounding the soil particles and are therefore highly mobile. Sheppard *et al.,* (1991) investigated the sorption of ^{99}Tc in a number of soil types with variable organic matter content and found no indication of sorption. The high mobility of the pertechnetate form of ^{99}Tc has been confirmed by soil chromatographic movement studies (Balough & Grigal, 1980). Furthermore, studies of vertical migration of several radionuclides, including caesium, iodine, and cadmium, in silty sand found that ^{99}Tc had the fastest migration rate as it had the lowest K_d value (Bunzl & Schimmack, 1989). Table 2:2 compares the K_d values for some radionuclides in that study.

Table 2:2: K_d Values for radionuclides in silty sand (Bunzl & Schimmack, 1989)

Radionuclide	K_d (median value)
Technetium	14
Iodine	29
Cadmium	87
Caesium	2300

Under anaerobic conditions, pertechnetate is reduced to the Tc(IV) form and as a result its sorption to the soil matrix increases. In a study of various groundwater/

sediment systems, a four orders of magnitude increase in ^{99}Tc sorption was found under reducing conditions (E_h= -200 to 100 mV) compared to oxidising conditions (E_h = 200 - 600 mV) (Leiser, 1993). Sorption values including 90% sorption in peat (Sheppard *et al.*, 1983) and 5 – 31% sorption after 24 hours, rising up to 46.6% over a period of six weeks in forest soils (Wildung *et al.*, 1986) have been reported. Tc(IV) produced under reducing conditions can precipitate or form complexes with organic matter (Bishop et al., 1989). Tc-99 added to soil as soluble pertechnetate converts over time to insoluble Tc(IV) through what is known as the 'ageing effect' (Wildung *et al.*, 1896; Vandecasteele *et al.*, 1989; Nicholson *et al.*, 1990; Tagami & Uchida, 1998). This is thought to be the result of the creation of anaerobic conditions within the soil either through waterlogging (Tagami & Uchida, 1999) or through localised anaerobic pockets produced by bacterial metabolism (Henrot, 1989). Stalmans *et al.,* (1986) suggested that continual altering of redox conditions in the soil leads to a re-oxidation of readily oxidisable forms of ^{99}Tc which could then be removed by plants. As the readily removable ^{99}Tc is removed, the remaining ^{99}Tc would increasingly occur in less oxidisable forms, thus causing the observed ageing effect.

2.3.5.2 Tc-99 and organic matter

Tc(IV) can form complexes with several ligands including insoluble sulphur compounds and organic matter (Schulte & Scoppa, 1987; Sparkes & Long, 1988; Bishop *et al.*, 1989; Desmet *et al.*, 1991; Leiser, 1993). Organic matter plays an important role in binding ^{99}Tc in the reduced form (Van Loon *et al.*, 1986; Stalmans *et al.*, 1986; Bondietti & Garten 1986). Sorption of ^{99}Tc is higher in

soils with high organic carbon, amorphous iron and aluminium content and lower pH, suggesting that sorption is related to pH dependent sites on oxidic iron and aluminium and organic matter fractions of the soil (Cataldo *et al.*, 1986). Sorption to the organic matter fraction was considered the most important process after ^{99}Tc had been in the soil for over 48 hours. Leiser (1993) states that ^{99}Tc has a tendency to react with organic sulphur compounds. A variety of co-ordinating groups, e.g. amines, carboxyl, hydroxyl and sulphydryl can stabilise reduced ^{99}Tc in chelating complexes (Van Loon *et al.*, 1986) and hydroxyl and carboxylic groups within humic acids can form stable complexes with reduced ^{99}Tc (Stalmans *et al.*, 1986).

The formation of complexes with soil organic matter generally reduces the solubility and mobility of ^{99}Tc in the soil (Wildung *et al.*, 1986; Stalmans *et al.*, 1986; Nicholson *et al.,* 1990). Re-oxidation of bound ^{99}Tc is possible when conditions are favourable (Nicholson *et a*l., 1990). Anion exchange experiments showed that 20% of bound ^{99}Tc could be readily oxidised at a rate of 0.015 d^{-1} (Bondietti & Garten 1986). This was measured over a fifteen day period therefore probably relates to the most readily oxidised Tc-organic matter fraction (Van Loon, 1986). The kinetics of this re-oxidation cannot be described by a single first order rate constant. It is better described by a range of rate constants (varying by an order of magnitude) which can be attributed to the oxidation of different functional groups within the organic matter involved in the binding of ^{99}Tc (Van Loon *et al.*, 1986). In contrast to Bondietti & Garten's, *(1986)* findings, Stalmans *et al.,*(1986) reported humic acids, especially those containing hydroxyl

and carboxylic acid groups formed complexes with ^{99}Tc that were characterised by slow rates of ligand exchange and were therefore relatively stable.

2.3.5.3 Tc-99 and soil microbes

Soil microbes may play an important role in the mobility and solubility of ^{99}Tc in the soil but little research has been undertaken in this area. The mechanisms may include uptake of ^{99}Tc by soil microbes and modification of the redox condition in the soil (Henrot, 1989). Sterilising sediments greatly reduces the sorption of ^{99}Tc to soil particles with 90 to 99% remaining in the soluble phase after a 73 hour period compared to 2.4 - 3 % in untreated sediment (Mania *et al.*, 1986). Marine microorganisms can take up ^{99}Tc from the water column but uptake by microorganisms in the sediment was not measured. Aerobic soil bacteria do not take up ^{99}Tc to any high degree (Concentration ratio (C_r) 15). Anaerobic bacteria can bioaccumulate ^{99}Tc with C_r over 200 hours ranging from 50 – 600. Sulphate reducing bacteria bioaccumulate ^{99}Tc to a very high extent with mean C_r values ranging from 6974 to 9311 (Henrot, 1989). After calculation of the amount of ^{99}Tc associated with anaerobic soil bacterial biomass, it was concluded that the high concentration ratios did not necessarily mean that bioaccumulation was an important mechanism for the immobilisation of ^{99}Tc in soil (Henrot, 1989).

Through the process of respiration, microrganisms can modify soil redox conditions by creating reduced microniches in otherwise oxic soils, thus promoting the reduction of Tc(VII) to Tc(IV) and allowing its absorption onto soil

mineral and organic matter fragments (Wildung *et al.*, 1986; Stalmans *et al.*, 1986; Nicholson *et al.*, 1990). Some soil bacteria such as *Escherichia coli, Clostridia* species and sulphate reducing bacteria can reduce Tc(VII) to Tc(IV) directly rather than just alter the conditions in the soil (Lloyd *et al.*, 1999; Francis *et al.*, 2002).

2.3.6 Tc-99 and plants

2.3.6.1 Plant uptake of ^{99}Tc

The speciation and therefore the solubility and mobility of ^{99}Tc in the soil, as influenced by redox potential, soil organic matter content and microbial activity, will affect its bioavailability to plants. Several studies, using both nutrient solutions and soils, have shown that ^{99}Tc, present as pertechnetate (TcO_4^-), is readily taken up by (Wildung *et al.*, 1977; Routsen & Cataldo, 1978; Cataldo *et al.,* 1983; Garten *et al.*, 1984; Van Loon *et al.*, 1986b; Van Loon 1986, Vandecasteele *et al.*, 1986; Lembrechts & Desmet, 1986; Cataldo *et al.*, 1986; Echevarria *et al.*, 1997). Plants can remove up to 90% of the ^{99}Tc added to soil as pertechnetate (Wildung *et al.*, 1977). Tumbleweed and cheatgrass removed 23 - 28% and 10 - 69% respectively after 3 months growth (Routsen & Cataldo, 1978) and rye grass removed 62 - 78% of ^{99}Tc applied to soil over a 22-week growth period (Echevarria *et al.*, 1997).

The mechanisms by which pertechnetate is taken up by plants have been the subject of debate. Uptake may be an active process involving mechanisms present in plants for the uptake of sulphate, phosphate and selenate as some studies indicated that these compounds act as competitive inhibitors of ^{99}Tc

(Wildung *et al.*, 1977; Cataldo *et al.*, 1983; Bennet & Wiley, 2003). However, Sheppard *et al.,* (1983) proposed that there was no plant mediation of ^{99}Tc uptake, with the controlling factor being the extent of soil mineral or organic matter sorption of ^{99}Tc. The uptake of ^{99}Tc from soils with a high organic matter content (a peat soil) was four times lower than uptake from a sandy soil. It was suggested that this was due to reduction of Tc(VII) to Tc(IV) followed by complexation with organic ligands in the peat soil (Sheppard *et al.*, 1983). Plant uptake of ^{99}Tc was reduced in organic rich and poorly drained soils (Mousney & Myttenaere, 1981). The uptake of reduced ^{99}Tc-organic complexes has been reported, but the rate of uptake was two orders of magnitude lower than that of pertechnetate and therefore was considered irrelevant (Van Loon, 1986; Van Loon *et al.*, 1989). A few studies have reported that the uptake of pertechnetate is directly proportional to its concentration in a nutrient solution (Wildung *et al.*, 1977; Sheppard *et al.*, 1983; Van Loon *et al.*, 1989; Masson *et al.*, 1989; Echevarria *et al.*, 1997).

Wide ranges of concentration ratios have been reported. These vary within and between species. Concentration ratios of 17 – 390 (Wildung *et al.*, 1977), 183 (mean value) (Murphy & Johnston, 1993), 64 – 443 (over successive harvests) (Echevarria *et al.*, 1997) have been reported for wheat. Values in order of 10^2 for the leaves of potatoes, barley and tomatoes (Dehut *et al.*, 1989) and in the order of 10^3 for swiss chard (Sheppard *et al.*, 1983) have been reported. Concentration ratios are generally higher for green leaf plants than for the edible parts of root vegetables (Yanagisawa & Muramatsu, 1997). The IAEA has produced a

handbook that gives recommended values for the transfer of radionuclides, including ^{99}Tc, to various plant species. The concentration ratios (based on dry weight data) for some radionuclides originating from nuclear fuel reprocessing are shown in Table 2:3 (IAEA, 1994)

Table 2:3: Concentration ratios for leafy vegetables (dry weight) (IAEA, 1994).

Radionuclide	Concentration ratio
Strontium-90	3.0
Uranium-238	0.46
Plutonium-239 - 240	0.0083
Americium-241	0.00006
Technetium-99	210

This shows that the concentration ratio for ^{99}Tc is several orders of magnitudes higher than for other radionuclides arising from the reprocessing process. Many of the studies reporting concentration ratios have relied on laboratory experiments that tend to give higher values than field studies. Experiments carried out in the field have shown significantly lower concentration ratios (C_r less than 2 for grasses, leafy vegetables and other plants) (Green *et al.*, 1995; Uchida *et al.*, 2000). The differences may be due to greater sorption of ^{99}Tc to soil and organic matter particles under field conditions (Bennet & Wiley, 2003).

It has been emphasised that the uptake of ^{99}Tc and therefore the transfer factors are dependent not on the total concentration of ^{99}Tc in the soil but on the concentration of the bioavailable pertechnetate in the soil solution (Van Loon, 1986; Van Loon *et al.*, 1989; Desmet *et al.*, 1991). A model was developed by to

describe the soil-to-plant transfer of ^{99}Tc in a soil based system mathematically (Van Loon, 1986),

$$TF(transfer\,factor) = f(RGR, k_o)b, K, t) \qquad \text{(Equation 3)}$$

Where *RGR* is the relative growth rate of the plant,

k_0 and **b** are parameters for plant metabolism, which increase the TF with time due to the concentration of ^{99}Tc in the leaves,

K is the equilibrium constant (equilibrium exists between the concentration of ^{99}Tc in the soil solution and the concentration in the plant shoots)

t is time.

Observed concentrations of ^{99}Tc in spinach plants matched values predicted by the model well (Van Loon *et al.*, 1986b).

Nitrate may affect the adsorption of pertechnetate. (Van Loon *et al.*, 1989). The addition of nitrate to soil reduced the uptake of ^{99}Tc by rye grass (Echevarria *et al.*, 1998) and uptake into tomato plants was also reduced when nitrate was added to the soil (Krijger *et al.*, 2000). The authors of these studies suggest that pertechnetate may be taken up into plants via one or more of the nitrate transport mechanisms.

2.3.6.2 Tc-99 within plants.

It is thought that ^{99}Tc is transported in plants as pertechnetate within the xylem (Lembrechts & Desmet, 1986; Cataldo *et al.*, 1986). Research into the chemical form of ^{99}Tc within plants indicates that a large percentage remains in soluble forms. Cataldo *et al.,* (1986) found that 50-70% was associated with soluble plant macromolecules and Garten *et al.,* (1986) concluded that 50% of the ^{99}Tc in corn (*Zea Mays*) was either in an ionic form or associated with soluble proteins or small organic molecules. Storage within plants is predominantly within the vegetative parts rather than seeds (Sheppard *et al.*, 1983; Murphy & Johnston, 1993; Echevarria *et al.*, 1997). In soybean seedlings found that ^{99}Tc was localised in membranous structures, particularly chloroplasts (Woodard-Blankenship *et al.*, 1995). The association of ^{99}Tc with chloroplasts confirmed earlier studies, which showed that reduction of pertechnetate within plant cells is inhibited by increasing $NADP^+$ concentration in the growth medium. As $NADP^+$ is an electron acceptor molecule in the electron transport chain of photosynthesis which occurs within the chloroplast, this suggests that ^{90}Tc is competing with $NADP^+$ in that chain (Lembrechts, 1986).

Highest activity concentrations of ^{99}Tc are recorded in young, rapidly growing tissues of brown seaweed and algae (Beasley & Lorz, 1986; Benco *et al.*, 1986). This is supported by autoradiography that showing the highest concentrations of ^{99}Tc in young tissues and reproductive cells of brown seaweed (Bonotto *et al.,* 1988). A study of the subcellular distribution of ^{95m}Tc in unicellular algae showed

that 68.8% was present in the cell cytosol, 26.6% in the chloroplasts, 3.6% in the cell wall and 0.6% in the mitochondria (Bonotto *et al.*, 1988).

Toxic effects of ^{99}Tc have been seen in soybeans and wheat grown on soil with ^{99}Tc concentrations above 0.1μg g^{-1} (approximately 63 kBq kg^{-1}) (Wildung *et al.*, 1977) and this effect was confirmed by Echevarria *et al.,* (1997) but no toxic effects were reported for ryegrass at higher soil concentrations. Toxicity effects that have been reported include reduction in growth and concentration of chloroplasts (Bennassar *et al.*, 1991), reduced photosynthetic rate and increases concentration of carbon dioxide within cells (Degenkolb *et al.*, 1994) and decrease in biomass (Wildung *et al.*, 1977; Echevarria *et al.*, 1997). Bennassar et al., (1991) and Degenkolb *et al.,* (1994) found that these effects were only seen under low light conditions. A single study has suggested that chemical toxicity arises from ^{99}Tc being substituted for sulphur in some metabolic processes resulting in dysfunction of some enzymes and proteins (Cataldo *et al.*, 1986).

2.3.6.3 Tc-99 release from plant material

There has been little published research found by the author on the release of ^{99}Tc from plant material. An experimental study of ^{99}Tc incorporated into pasture grass used water extraction to estimate that around 55% of the ^{99}Tc was readily soluble and therefore available for plant uptake (Dehut *et al.*, 1989). When the technetium spiked grass was incorporated into the soil, the transfer of ^{99}Tc into crop plants was of the same magnitude as for pertechnetate added to the soil in

solution. Examination of the change in transfer factors over time suggested that a proportion of the ^{99}Tc is rapidly released (over about 30 days) after which the rate slowed down before increasing again about ten weeks later. The rate returned to the initial high value and remained relatively constant over the following year. A later study confirmed that ^{99}Tc incorporated into plant material (wheat leaves) was readily extractable, mobile in the soil and as bioavailable as pertechnetate added in solution (Echevarria *et al.*, 1997). The two-compartment kinetics for the release of ^{99}Tc from plant material reported by Dehut *et al.,* (1989) has also been seen for its release from brown seaweed (artificially contaminated with ^{95m}Tc) into uncontaminated water (Benco *et al.*, 1986). Beasley & Lorz (1986), also reported this pattern and gave biological half-lives of one to three days for the rapid loss and 16 - 196 days for the slower loss fraction (seaweed also artificially contaminated with ^{95m}Tc). In another study, three successive overnight soakings of chopped *Fucus vesiculosus* in water at 4°C was found to remove 60% of the incorporated ^{99}Tc (Vandecasteele *et al.*, 1986). All these studies suggest that around half of the ^{99}Tc present in artificially contaminated plant material is in a soluble form that is readily leachable.

2.4 SUMMARY

2.4.1 Evidence for the sea-to-land pathway

In order to verify the existence of the sea-to-land pathway for ^{99}Tc through the use of contaminated seaweed as a soil conditioner, it is necessary to examine the published research to find evidence for each step of the pathway. In addition,

accurate figures are required for each step, to calculate the dose received by the exposed group e.g. ^{99}Tc concentration in seaweed, amount released in to soil and transfer factors for plant uptake etc, as inputs to the calculation.

The literature review shows that the processes involving the first two steps in the pathway, the discharge of effluent containing ^{99}Tc into the Irish Sea and its subsequent uptake by *Fucus vesiculosus,* have been extensively monitored. It is evident that *Fucus vesiculosus* collected from the shore close to Sellafield bioaccumulates ^{99}Tc and accurate figures are available for imputing to dose calculations. Information from literature provides some estimation of the amount and type of seaweed added to soil and the timing of the addition. At the other end of the pathway, a great deal of research has been carried out on plant uptake of ^{99}Tc from artificially contaminated soil. It has been shown that, under aerobic conditions, ^{99}Tc can be taken up from soil solution readily by some plant species, primarily green leafy plants, and a range of transfer factors have been calculated.

It has, therefore, been shown that contaminated seaweed is added to the soil by some growers and that once in aerobic soil, ^{99}Tc is bioavailable to plants, but there is crucial information missing. There is little published research on the release of ^{99}Tc from plant material and to date no research on the rate or mechanics of ^{99}Tc release from seaweed into soil. Until this information is available the existence of the pathway cannot be confirmed or the dose and therefore risk to humans and the environment assessed. This research

programme, therefore, aims to quantify the amount of ^{99}Tc released from *Fucus vesiculosus* into soil over time, investigate the mechanisms involved in that release and determine the bioavailability of the released ^{99}Tc. This information could provide data for model development.

2.4.2 *Research programme*

The investigation of ^{99}Tc release from seaweed involves studying a complex natural system and its response to agricultural management. Taking into account the information gained from the literature review, a simplified system diagram was constructed (Figure 2:7). The complexity of the system made it necessary to focus the investigation on a key aspect: the mechanisms and dynamics of ^{99}Tc release from the seaweed into soil.

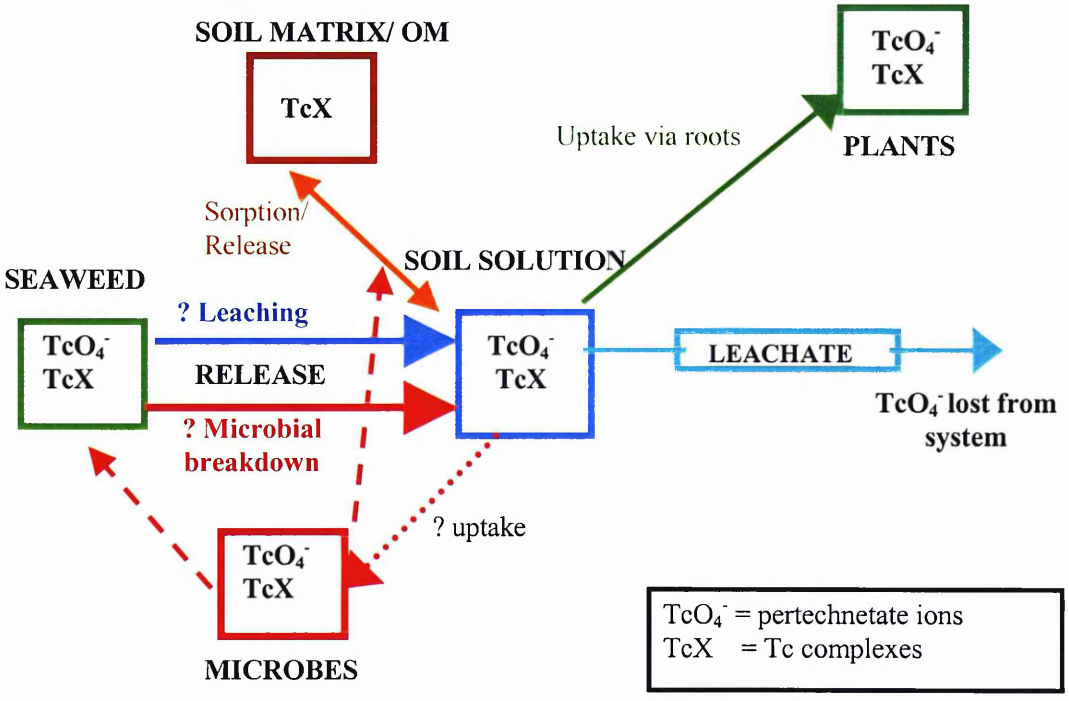


Figure 2:7: Diagram of natural system under investigation

Based on the literature review four initial hypotheses were formed:

1. Tc-99 within *Fucus vesiculosus* will be released into soil over time

2. The temporal release pattern will be dependent on decomposition processes, leaching and microbial decomposition

If hypothesis 1 proves true then:

3. In aerobic sandy soils, around half of the ^{99}Tc within *Fucus vesiculosus* will be released in a soluble form and remain within the soil solution

4. The majority of ^{99}Tc released into the soil solution will be available for plant uptake

Pot experiments were designed to provide controlled conditions to fully test the above hypotheses. They were designed to mimic field conditions as closely as was practically possible. This included using environmentally contaminated seaweed collected close to BNFL Sellafield, rather than artificially contaminated seaweed, using similar application rates and techniques for the seaweed and using a soil representative of those to which seaweed is added in Cumbria and elsewhere. The details of the pot experiments will be given in Chapter 4 and 5 but first Chapter 3 details the general laboratory methods used throughout the study.

CHAPTER 3 - MATERIALS AND METHODS

3.1 RESEARCH PROGRAMME

A series of experiments were designed to investigate the release of ^{99}Tc from the brown seaweed species, *Fucus vesiculosus,* into a sandy coastal soil. Experiment 1 was a small scale pot experiment designed to measure the extent to which ^{99}Tc was released into soil from the seaweed and investigate the mechanisms involved in its release. Experiment 2 was a larger scale pot experiment to investigate the release of ^{99}Tc into soil solution, identify any differences in release rates with size of seaweed pieces and determine if the released ^{99}Tc was available for plant uptake from the soil solution. The third experiment was designed to investigate the fraction of ^{99}Tc that could be readily leached from *Fucus vesiculosus* by shaking the seaweed in water. The fourth investigation aimed to determine the chemical forms of ^{99}Tc in the soil solution by gelfiltration chromatography. Investigations three and four were carried out on soil solution obtained from Experiment 2. The details of the set up and sampling regime of each experiment will be given in chapters 3, 4 5 and 6. This chapter will detail the more generic materials and general laboratory methods used during the research programme.

3.2 INFORMATION GATHERING

3.2.1 Literature review

A literature review was carried out to investigate the behaviour of ^{99}Tc in the environment, particularly the soil-plant system (see Chapter 1). The information gained was used to produce a system diagram of the

seaweed-soil-plant system, determine the focus of this study and shape the design of the experiments.

3.2.2 Interviews with growers

In January 2001, personal interviews were carried out with two individuals who have been using seaweed on garden plots in Cumbria for many years. These individuals were members of a study currently being undertaken by CEFAS (Centre for Environment, Fisheries and Aquaculture Sciences) to monitor intake doses of ^{99}Tc through the consumption of vegetables from garden plots where contaminated seaweed is used as a soil conditioner. The individuals were asked questions on seaweed collection practices, application methods and types of crops grown (For questionnaire see Appendix 1). This information was used to design the experiments so they could reflect the actual use of seaweed as a soil conditioner.

3.3 MATERIALS

3.3.1 Seaweed collection

The seaweed chosen for this study was *Fucus vesiculosus* because it has been identified in previous studies as a species that bioaccumulates ^{99}Tc to a high degree (Brown *et al.*, 1998) and it is a common species gathered from the Cumbrian coast for use as a soil conditioner(Anonymous, 2001). Seaweed collection took place on the shore close to the Sellafield reprocessing plant (Grid reference NY 018034) in January 2001, July 2001 and July 2003. This shore is indicated by the arrow in the top right hand corner of Figure 3:1.

Figure 3:1: BNFL Sellafield and seaweed collection site

Figure 3:2: *Fucus vesiculosus* showing paired vesicles (Guiry & Nic Dhonncha, 2002)

Fucus vesiculosus, identified by the presence of pairs of air filled vesicles on the fronds (Figure 3:2), was freshly cut from rocky outcrops on the shore. Fronds were cut as close to the base as possible and were taken

randomly from plants along the shore. The seaweed was stored at 5°C prior to its preparation and addition to the soil.

3.3.2 Soil collection

The soil used in this study was collected in an area of unimproved grassland on the east coast of Scotland (Grid reference NO 470237). The material is classified as a freely draining, stabilised, wind blown sand (Macauley, 1980) and is representative of the type of soil to which seaweed is added in Cumbria and other coastal areas. Vegetation was removed from the soil surface then the top 10 - 15 cm of soil was collected, including the soil around the roots of the plants, which contains the greatest concentration of the microbes present in soil (Kilham, 1994). The field moist soil was passed through a coarse sieve to remove most root material before use.

3.3.3 Spinach plants

Spinach plants were used in this study to investigate plant uptake of the ^{99}Tc released from the seaweed. Spinach was chosen because it had been widely used in previous ^{99}Tc uptake studies and it was one of the crops grown on the seaweed-conditioned plots in Cumbria. The spinach plants used in the second experiment were grown from seed. Two sets of plants were grown, a summer cropping and winter cropping variety. The seeds were planted in compost in a blocked tray, two to three seeds per block. The summer cropping spinach seeds (variety: Dominant, Johnston Seeds) were planted at the beginning of July 2001 and the winter cropping spinach seeds (variety: Medania, Unwins Seeds Ltd) at the beginning of

October 2001. After germination, when the seedlings had two leaves, they were thinned out to leave one seedling per block. The seedlings were left to grow in the blocks until they had four to six leaves (three to four weeks), before transplanting into the experimental pots.

3.3.4 Water collection

Rain water was collected from the roof of a garden shelter in the grounds of Stirling University (Grid reference NS 817969) via a pipe into a plastic water butt. This was used to water the pots from experiments 1 and 2.

3.4 GENERAL LABORATORY METHODS

3.4.1 Soil analyses

The soil was air-dried and sieved using a 2 mm sieve to remove roots and stones. The remaining fine earth fraction was used for all analyses.

3.4.1.1 Soil moisture content

A 50 g sub-sample of field moist soil was placed overnight in an oven at 105°C, and then reweighed. The percentage soil moisture content was calculated as the difference between the field moist and the oven dried weights multiplied by 100. This procedure was carried out in triplicate.

3.4.1.2 Field Capacity

Field capacity is defined as the water content of a soil that has been waterlogged then allowed to drain freely, with most of the water lost being from macropores (Hartel, 1998). It can also be expressed in terms of water potential which is a measure of the force or suction that is required to extract water from the soil(Hartel, 1998). A tensiometer was used to apply a measured hydraulic head to the soil sample, thus creating the

suction necessary to drain the macropores. This allowed the calculatation the field capacity of the sandy soil used in this study. The tensiometer consisted of a Buchner funnel attached to a length of rubber tubing bent in a U-shape to form a manometer. The other end of the tube was attached to a 50 ml glass burette. It was primed with water and the funnel and burette were clamped to two retort stands. Fifty grams of air-dry soil was placed into the funnel and saturated with water. The burette was lowered until a hydraulic head of one metre was created which gave an equivalent suction of 10 kPa (Rowell, 1994).

The system was allowed to return to equilibrium for 24 hours and then the soil re-weighed to give the gravimetric water content. The soil samples were then oven-dried at 105°C and after cooling in a dessicator the samples were re-weighed. This procedure was carried out in triplicate.

The gravimetric water content of the soil at field capacity was calculated as the difference between the weight of saturated soil samples after their return to equilibrium and the oven-dried samples. This value was used to calculate the amount of water added to the pots to maintain the required water content.

3.4.1.3 pH measurement

A Griffin pH meter (Model 81) was calibrated using pH 7 and pH 4 buffers at room temperature. Ten grams of air-dried soil was suspended in 25 ml of distilled water at room temperature. Three replicates were set up. After standing for 30 minutes, the suspensions were stirred and the pH of each read to one decimal place using the calibrated meter.

3.4.1.4 Organic matter content

Organic matter content of the soil was calculated by an adapted 'Loss on Ignition' method. Three samples, each containing approximately ten grams of air-dried soil, were further dried at 105°C for four hours. The air-dried samples were weighed into crucibles and placed in a furnace at 425°C overnight. After cooling in a dessicator the samples were re-weighed. The amount of organic matter in the soil was calculated as the difference between the ashed and oven-dry sample weights. This method gives only a rough estimate of organic matter content as some of the weight loss can be due to water loss from clay minerals in the soil (Rowell, 1994) but since the soil used was a wind-blown sand with negligible clay content the effect of clay minerals should be small.

3.4.2 Soil solution collection

Soil solution was collected using rhizon soil moisture samplers supplied by Eijkelkamp. These consist of 10 cm by 2.5 mm diameter hydrophyllic porous polymer tubes connected to a 10 cm PVC tube fitted with a luer lock (Figure 3:3).

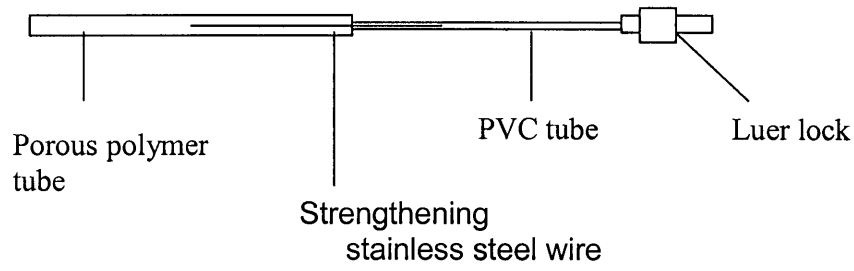


Figure 3:3: Rhizon soil solution sampler

Pots were prepared by drilling two 3.8 mm holes, 6 cm apart, into opposite sides of the pots containing soil-seaweed mixture. A fine steel rod was inserted horizontally through these holes into the moist soil to make a guide hole in the soil, then removed. The sampler was gently inserted into the soil up to the beginning of the PVC tube keeping it as horizontal as possible. Plasticine was used to seal around the edges of the hole. The PVC tube was then covered with black plastic tubing to prevent algal growth and a 0.6 x 25 mm hypodermic needle was attached to the luer lock, and sealed on using plasticine.

Before extraction of the soil solution the pots were watered to bring them to 75% field capacity. The samples were extracted by attaching the hypodermic needle to a 10 ml vacutainer, and opening the luer lock thus creating sufficient suction to extract soil moisture (see Figure 3:4).

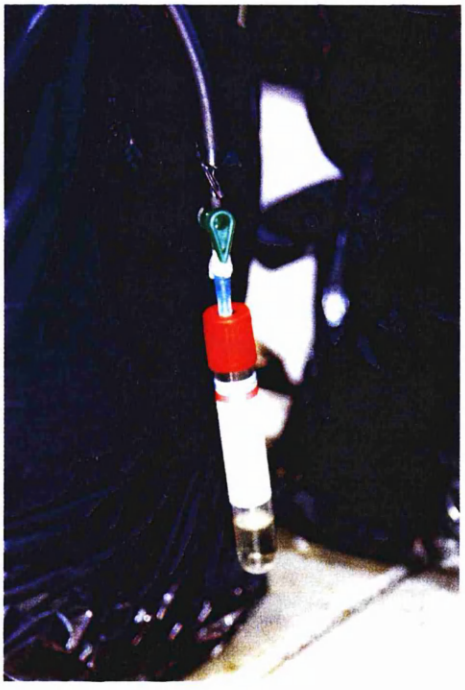


Figure 3:4: Soil solution collection

The manufacturer's literature stated that a 10-ml vacutainer should produce around 7 ml of soil solution in 1–16 hours (Eijkelkamp, 1998) so the vacutainers were left attached to the samplers overnight.

3.4.3 Carbon dioxide production

The carbon dioxide (CO_2) produced by soil microorganisms was monitored by measuring the change in CO_2 concentration in air trapped in the headspace of the experimental pots over a 24-hour period. Each pot was capped with an airtight lid which had a rubber septum inserted in the centre. Immediately after the pot was sealed, an air sample was drawn out through the rubber septum using a syringe. Air was drawn out and returned to the pot several times to ensure that the air trapped in the headspace was mixed thoroughly before the final sample was extracted. The pots were left capped for 24-hours and a further air sample was extracted as before. In the initial experiment, the small pots used were taken to the laboratory for sampling and a final air sample of one ml was taken from the pots and injected directly into the gas chromatograph. The larger pots from the second experiment could not be carried to the laboratory for sampling, therefore 10 ml vacutainers were filled with the air samples for transport to the laboratory. In the laboratory, one ml air samples were taken from the vacutainers and injected into the gas chromatograph.

The air samples were analyzed using a Varian 90-P gas chromatograph with a 1.32 m long by 3 mm internal diameter stainless steel column packed with 80/100 mesh Poropak Q and a thermal couple detector. The

carrier gas was helium at a pressure of 160 kPa (1.6 bar). To calculate the concentration of CO_2 in the samples, a standard containing 1% CO_2 (with the balance being nitrogen) supplied by Bedfont Gases was also analysed.

The size of the peaks on a chromatogram is related to the amount of a particular substance in the sample (McCarthy, 1997), so the CO_2 peaks from the chromatograms were measured and those from the samples compared with those from the standard. The carbon dioxide produced per gram of soil (dry weight) in 24-hours was calculated using the equations:

$$CO_2 \text{ in 1 ml sample (moles)} = \frac{\text{Peak measurement sample}}{\text{Peak measurement standard}} \times 4.46 \times 10^{-7}$$

(4.46 x 10-7 = moles CO_2 in standard) (Equation 4)

$$CO_2 \text{ produced in 24 hrs (g soil dry wt) (moles)} = \frac{CO_2 \text{ in 1 ml sample} \times \text{headspace volume}}{\text{dry weight soil (g)}}$$

(Equation 5)

3.4.4 ^{99}Tc analysis

For this study, ^{99}Tc analysis was carried out using a method devised for the analysis of ^{99}Tc in environmental samples (Wigley *et al.*, 1999) but modified according to procedures used by CEH, Merlewood (Singleton, 2000). This method comprises a number of steps to preconcentrate the ^{99}Tc and remove contaminants, so it is necessary to use a yield monitor to determine how much of the ^{99}Tc in the original sample is lost during the process. In this study, ^{99m}Tc, a γ-emitter with a half-life of 6.06 hours was used as the yield monitor (Wigley *et al.*, 1999). This isotope, commonly

used in nuclear medicine, was supplied in a liquid form as pertechnetate in normal saline. The activity of the isotope varied between batches, but was generally between 2-3 MBq ml^{-1}. The procedure summarised below allows eight samples (including a blank and a standard) per week to be prepared for counting. For full details see the original paper by Wigley *et al.*, (1999). The method was validated using five *Fucus* species samples provided by the Scottish Universities Research and reactor Centre, East Kilbride, UK, that had been used in an international intercomparison exercise (McCartney *et al.*, 1999).

3.4.4.1 Analytical reagents

The ^{99m}Tc was supplied by the Nuclear Medicine Department of Stirling Royal Infirmary. Eichrom anion exchange resin was supplied by Hichrom Chromatography, Reading, UK, Gold Star scintillation cocktail by Meridian, Epsom, UK and the remaining chemicals by Fisher Scientific, Loughbrough, UK. All chemicals used were analytical grade.

3.4.4.2 Preparation of samples

Seaweed, soil and spinach samples were dried overnight at 60°C, ground to a fine powder using a steel grinding dish in a gyro-mill then stored at room temperature prior to analysis. Soil solution samples were frozen at –18°C then defrosted in the fridge overnight prior to analysis. Sample sizes for analysis varied but generally a 2-5 g sample of plant and soil material was analysed. An internal seaweed standard and a blank were included with all analytical batches.

3.4.4.3 Ashing.

The method required plant and soil material to be ashed, but there are some concerns about loss of ^{99}Tc from the samples through volatilisation (Wigley *et al.*, 1999). To prevent this loss, the samples are wetted with 0.88 S G ammonia solution and dried at 100°C on a hotplate prior to ashing. The samples were ashed at stepped temperatures, starting at 250°C for one hour and rising by 50°C increments hourly until 550°C, then after a further hour the furnace was switched off and the samples allowed to cool overnight. Wigley and co-workers found that by following this procedure the loss of ^{99}Tc from the samples was negligible.

3.4.4.4 Leaching.

Wigley *et al.*, (1999), reported that nitric acid was the most effective agent for leaching ^{99}Tc from seaweed. In this study, all samples, including the soil solution samples, were treated with nitric acid. Fifty ml of 8 M nitric acid was added to the samples in tall form beakers. This solution was spiked with 0.5 ml of ^{99m}Tc pertechnetate solution and heated at 100°C for two hours. Watchglasses were placed over the beakers to prevent loss through evaporation. At the same time, a ^{99m}Tc standard was prepared by pipetting 0.5 ml of the pertechnetate solution into a plastic scintillation vial and adding 5 ml of 5% tri-n-octylamine in xylene and 15 ml of Gold Star scintillation cocktail. This was used at the end of the procedure to calculate the recovery percentage of ^{99m}Tc. The samples were left to cool then filtered through Whatman No. 42 filter papers into tall form beakers to remove any solid material. The beakers were rinsed with 10 ml 8 M nitric

acid, followed by 10 ml of distilled water and these washings were passed through the filter.

3.4.4.5 Removal of contaminants and concentration of ^{99}Tc

The seaweed in this study, because it came from a natural source, contained other contaminants, such as transition metals, caesium–137, cobalt–60 and ruthenium-106, which could produce interference in the final step of the procedure i.e. liquid scintillation counting of ^{99}Tc in the sample.

The first step in the decontamination procedure was to remove contaminants by iron 'hydroxide' precipitation. Two ml of iron (III) chloride was added to the filtrate as a Fe^{+++} source, then 0.88 S G ammonium solution was added slowly until a pH of 8 was reached and a reddish-brown iron hydroxide precipitate had formed. This step removed transition metals, actinides, lanthanides, cobalt and nickel from solution (Wigley *et al.,* 1999). The solution was left to stand for 30 minutes to allow the precipitate to settle then it was passed through a Whatmans No. 41 filter and the filtrate collected.

The next step in the procedure was to pass the filtrate through an anion exchange column, which removed many of the remaining contaminants including caesium-137, and cobalt-60 and concentrated the ^{99}Tc (Wigley *et al.*, 1999). The columns were set up as shown in Figure 3:5.

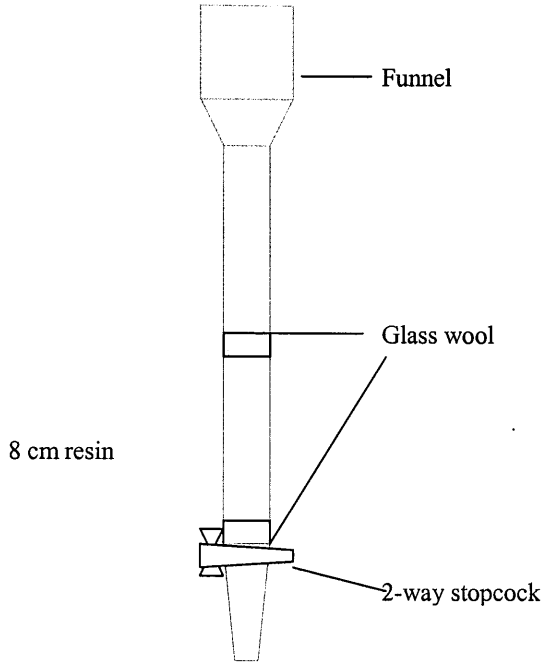


Figure 3:5: Anion exchange column

Before the filtrate was passed through the resin the column was flushed with very dilute ammonia solution (< 0.5%) to make the pH in the column the same as that in the filtrate. The flow rate in the columns was set to one drop every five to ten seconds. The sample was passed through the column, followed by rinses of 15 ml distilled water, 10 ml 1 M HCl and a further 15 ml distilled water. All rinses were discarded. The final step in the anion exchange chromatography was to elute the ^{99}Tc into a 20 ml glass scintillation vial with 20 ml of concentrated nitric acid (Figure 3:6).

Figure 3:6: Anion exchange columns in use

The vials were placed in an aluminium heating block at 85 - 90°C overnight. The temperature was raised to 115°C the following morning and the vials left *in-situ* until the solutions were almost dry. The aim was not to let the solution dry out completely but if this did happen the residue was redissolved in 2 ml concentrated nitric acid and the vial returned to the heating block until almost dry.

The remaining sample was dissolved in 10 ml of 2 M sulphuric acid for the final step in the procedure which was solvent extraction. The sample was placed into a 50 ml extraction funnel and 3 ml of 5% tri-n-octylamine in xylene added. The solution was shaken vigorously for 30 seconds and the aqueous and solvent layers allowed to separate. The aqueous layer was run off back into the glass scintillation vial and the solvent phase into a

plastic scintillation vial. The sample was placed once again into the separation funnel and a further 2 ml of tri-n-octylamine in xylene added and shaken for another 30 seconds. The aqueous layer was run off into the glass vial and discarded, whilst the solvent layer was added to that already in the plastic vial. Any aqueous layer remaining in the plastic vial was removed carefully using a plastic dropping pipette as this could interfere with the quench level in the β counting of the sample as it would result in a cloudy emulsion once the scintillant had been added. This step removed ruthenium-106 from the solution as it remained in the aqueous layer (Wigley *et al.*, 1999). The solvent layer was mixed with 15 ml of Gold Star scintillation cocktail.

3.4.4.6 Gamma spectrometry

The samples were counted for 30 minutes on either an Ortec 35% relative efficiency n-type HPGe detector or a LO-AX™ Series HPGe detector to measure the activity of the ^{99m}Tc tracer. The relative activity concentration was calculated using a computer package Gamma vision 32 $^{(TM)}$, which also decay corrected the activity to the date of collection. The standard prepared at the beginning of the procedure was also counted and used to calculate the recovery rate for each sample. This was calculated by the equation:

$$Recovery\ rate\ (\%) = \frac{^{99m}Tc\ in\ sample}{^{99m}Tc\ in\ standard}\ x\ 100 \qquad \text{(Equation 6)}$$

The samples were then set aside for at least two weeks before β counting to allow the total decay of the ^{99m}Tc tracer.

3.4.4.7 Recovery rates

The mean recovery rate for the ^{99m}Tc tracer in all samples analysed was 65% ±15%.

3.4.4.8 β counting

The activity of ^{99}Tc in the samples was measured by liquid scintillation counting on a Wallac 1220 Quantalus Ultra Low Level scintillation counter at CEH Merlewood in Cumbria. Counts were acquired in the 125 – 647 window for 120 minutes. Counting efficiency was determined by counting a ^{99}Tc standard. As the decay of ^{99m}Tc produces ^{99}Tc (^{99m}Tc → ^{99}Tc + γ), the amount of ^{99}Tc in the sample added with the tracer needs to be determined. This was done by counting the ^{99}Tc activity in ^{99m}Tc standard set up for calculating the recovery rate for each batch of samples. The ^{99}Tc activity in the sample was calculated using the equations:

$$Sample\ corrected\ counts\ counts\ per\ minute\ (Sc) = \quad S - (B + ((T - B) / 100xR))$$

(Equation 7)

Where:

S = Sample cpm

B = Scintillant background cpm

T = ^{99m}Tc standard cpm after decay

R = recovery (Calculated by γ counting)

$$^{99}Tc\ in\ sample\ (Bq\ kg^{-1}) = (Sc/60)x(100/E)x(1000/M) \quad \text{(Equation 8)}$$

Where:

M= Sample size

E = Counting efficiency

3.4.5 Soluble sugar analysis

The level of soluble carbohydrates in the soil solution samples was determined by a colorimetric assay using anthrone reagent. This is a long established method of measuring carbohydrates (Morris 1948; Brink *et al.*, 1980), however there has been some concern about nitrate and other ion interference with the results obtained from soil solution samples (Brink *et al.,*1980; Katz *et al.*, 1983). However Katz *et al.*, (1983) found that instant cooling of samples and use of blanks decreased the interference and (Grandy *et al.*, 2000), concluded that the anthrone method was a suitable method of quantifying soil carbohydrates.

3.4.5.1 Analytical reagents

The anthrone ($C_{14}H_{10}O$) was supplied by Sigma Chemicals LTD, St Louis, USA and the sulphuric acid by Fisher Scientific, Loughbrough, UK.

3.4.5.2 Method

The anthrone solution was prepared by dissolving 0.2 g of anthrone in 200 ml of 76% sulphuric acid. This solution was placed into a dark coloured

bottle that was covered with aluminium foil, as the solution was light sensitive. A series of standard solutions were prepared from a glucose stock solution of 1000 mg l^{-1}, to give a working range of 0 – 400 mg l^{-1}. Each solution sample and standard solution was placed into a large test tube and 10 ml of the anthrone solution added. The tubes were placed into a rack in a water bath at 100°C for 12 minutes, removed and cooled quickly in a tray of cold water. The absorbance was measured at 620 nm. The standards were used to draw a standard curve and this used to calculate the concentration of soluble sugars in the soil solution samples. Some samples that had absorbance readings higher than that of the highest standard had to be repeated with the samples being diluted 1:2 to 1:8.

3.4.6 Amino acid analysis

The measurement of amino acids in soil solution samples was performed by a colorimetric assay using ninhydrin. Molecules containing free α-amino groups (e.g. amino acids, peptides, proteins) react with ninhydrin to form a purple complex (Joergensen, 1996).

3.4.6.1 Analytical reagents

The ninhydrin reagent and glycine were supplied by Sigma Chemicals LTD, St. Louis, USA and the ethanol by Fisher Scientific, Loughbrough, UK.

3.4.6.2 Method

A series of glycine standards were prepared by dissolving glycine powder in distilled water and diluting the stock solution containing 50 μg N ml^{-1} to

give a working range of 0 – 15 μg N ml $^{-1}$. One ml of each standard solution and sample were placed in glass test tubes (one tube contained 1 ml distilled water to act as a blank). One ml citric acid buffer and 1 ml of ninhydrin reagent were added slowly to each test tube and the tubes placed in a rack in a boiling water bath for 25 minutes. After they had been cooled to room temperature, 4 ml of ethanol water (prepared by diluting ethanol with an equal volume of water) was added to each tube and the contents mixed thoroughly. The absorbance at 570 nm was measured in a spectophotmeter using 1 cm cells. The standards were used to draw a standard curve and this used to calculate the concentration of molecules with free amino groups in the soil solution samples.

3.4.7 Metal analysis of plant material

This procedure involves the digestion of finely ground plant material with a mixed digestion reagent containing concentrated sulphuric acid, hydrogen peroxide, selenium powder and lithium sulphate monohydrate. The method is taken from Allen, (1989).

3.4.7.1 Analytical reagents

All chemicals were supplies by Fisher Scientific, Loughbrough, UK

The reference material used to check the accuracy of the method was Standard Reference Material 1515 (apple leaves).

The composition of the mixed digestion reagent was 350 ml of hydrogen peroxide, 0.42 g selenium, 14 g lithium sulphate monohydrate and 420 ml of concentrated sulphuric acid.

3.4.7.2 Method

The stored dry seaweed samples were dried at 80°C for 4 hours to remove any moisture from the material. The samples were ground with a pestle and mortar and approximately 150 mg of the finely ground material was weighed directly into each digestion tube. Two replicates of each reference material were set up as well as 2 blank tubes. Using an automatic dispenser, 4.4 ml of the mixed digestion reagent was added to each tube. The tubes were left overnight to settle before being transferred to the aluminium heating block in a fume cupboard with an acid scrubber. The tubes were heated at 30°C for 15 minutes and the temperature was gradually increased to 80°C over the following 30 minutes. The temperature was kept at 80°C until any frothing in the tubes had subsided, then it was increased in stages to 330°C over the next hour. The tubes continued to be heated at 330°C until the solution became clear, then for a further 30 minutes. They were removed from the heating block and allowed to cool for 30 minutes before distilled water was added to bring the level of the solution to about 1/3 of the volume of the tube. Each digest was filtered through a No 44 filter paper into a 100ml volumetric flask and the tubes were rinsed twice with 10 ml of distilled water. The filter papers were then rinsed 3 times with small amounts of distilled water and the volume in each flask made up to 100 ml with distilled water. The digests were transferred into clean plastic bottles and stored for analysis using flame atomic absorption spectrophotometry.

3.4.8 Metal analysis of water

3.4.8.1 Method

The water samples were filtered through Whatmans No 1 filter paper to remove any particulate material. The samples were analysed using flame atomic absorption spectrophotometry as for the plant digest samples.

3.5 SUMMARY

This chapter has detailed the generic methods used throughout the research programme. The following chapters, 3-6, will detail the set up, sampling regime and results of four experiments designed to test the hypotheses formed at the end of Chapter 2.

CHAPTER 4 - EXPERIMENT 1: POT EXPERIMENT TO EVALUATE THE RELEASE OF ^{99}TC FROM *FUCUS VESICULOSUS* INTO SOIL OVER TIME

4.1 INTRODUCTION

The results from this experiment have been published (Webster *et al.*, 2003).

4.1.1 Rationale

The information gained from the user interviews was used to design a pot experiment in which the conditions mimicked, as closely as possible, the actual use of seaweed as a soil conditioner in the field. The individuals interviewed collected drift seaweed from the strandline mainly in the late autumn through to early spring, after stormy seas had cast the seaweed onto the beach (Anonymous, 2001). Several species are collected but the bulk of the collection is *Fucus vesiculosus*, which is a common species along the Cumbrian coast. The seaweed may be added directly to the soil in early spring or composted with other plant material before addition to the soil. The soil in the area has a sandy texture with little organic matter. Seaweed added directly to the soil was placed in a layer about 5 cm thick on the surface, and then forked into the top 12-15 cm of the soil.

4.1.2 Aim

The aim of this small-scale pot experiment was to monitor the release over time of ^{99}Tc from the brown seaweed species, *Fucus vesiculosus,* into a sandy coastal soil and investigate the mechanisms involved.

4.1.3 Hypotheses

The hypotheses this experiment was designed to test were:

1. Tc-99 within *Fucus vesiculosus* will be released into soil over time.

2. The temporal release pattern will be dependent on decomposition processes, leaching and microbial decomposition

4.1.4 Design choice

A pot experiment was used in this investigation because it permitted control of certain variables such as moisture regime and allowed easy measurement of ^{99}Tc activity concentration in the soil over time and measurement of CO_2 as an indication of soil microbial activity. To mimic field conditions it was deemed important to use environmentally contaminated seaweed rather than artificially contaminated plants, as that may affect how ^{99}Tc is stored within the seaweed. The soil was selected to be representative of soils to which seaweed is added in coastal areas and the ratio of soil to seaweed in the pots was calculated to be representative of application rates in the field.

4.2 POT EXPERIMENT

4.2.1 Materials

Fucus vesiculosus was freshly cut from rocks on the shore close to Sellafield (Grid reference NY 018034) in January 2001 and a sandy, coastal soil was collected from an area on the east coast of Scotland (Grid reference NO 470237), where it would be unlikely to be already contaminated with ^{99}Tc. (See Sections 3.3.1 and 3.3.2 for details).

4.2.2 Method

A series of one litre plastic pots with removable airtight lids (Nalgene) were prepared by drilling drainage holes in the base and drilling a hole in the centre of the lid and fitting a rubber septum. The pots were then set up as shown in Figure 4:1.

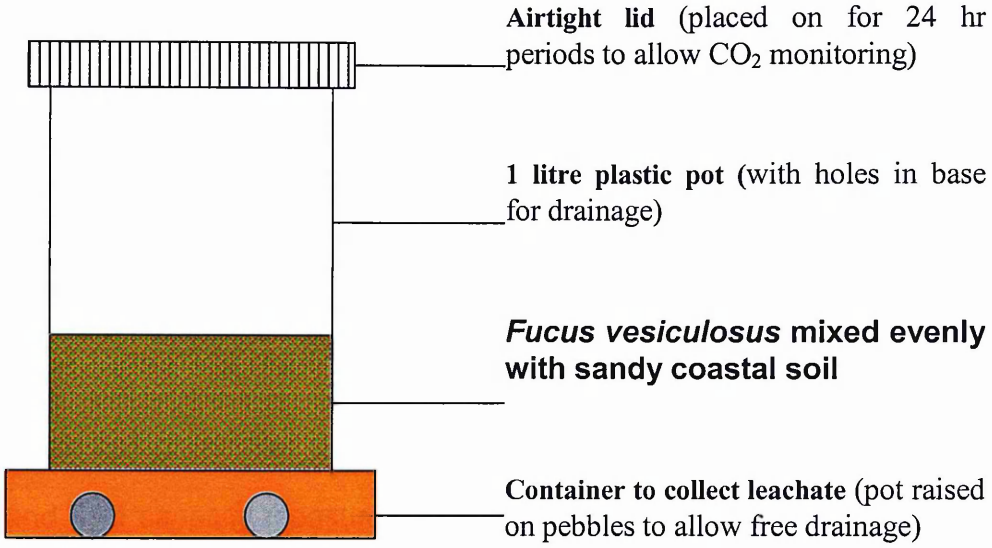


Figure 4:1: Test pots

A thin layer of pebbles was placed in the bottom of each pot and covered with a permeable membrane to allow free drainage of water. Eighteen test pots were prepared with 50 g (wet weight) *Fucus vesiculosus*, chopped into 4 - 6 cm pieces, mixed evenly with 250 g (field moist) ^{99}Tc-free (confirmed by analysis) sandy soil. This represented a 5:1 mix of soil to seaweed (w/w). This amount of material approximately filled one third of the pot volume, allowing a large air space for collecting the CO_2 produced by microbial decomposition. To estimate the amount of ^{99}Tc added to each pot, a 100 g sample of seaweed was divided into equal halves by

cutting individual fronds in two. One half of the sample was added to the pot and the other retained for ^{99}Tc analysis. Eighteen control pots were also prepared containing only 250 g of sandy soil.

The pots were set up at the beginning of February 2001 and kept at ambient temperature in an open-sided shelter at Stirling University (Grid reference NS817969), and maintained at 65% field capacity (measured gravimetrically) by watering regularly with collected rainwater. The ambient temperature on campus was recorded at the University's weather station. The experiment ran for 15 weeks until May 2001.

4.2.3 Sampling regime and sample analyses

Basic soil parameter tests (pH, organic matter content, moisture content and field capacity measurements) were performed on soil samples at the beginning of the experiment (See Section 3.4.1 for details). A bulk sample of *Fucus vesiculosus* was dried at 60°C and ground to a fine powder to be used as the internal control.

On six occasions, three test and three control pots were removed from the experiment for sampling. Sampling was carried out at 5 and12 days and then 3, 8, 12 and 15 weeks. At each of the sampling dates, CO_2 production by soil microbes over a 24-hr period was measured using the method described in Section 3.4.3. The seaweed pieces, which were still largely intact, were removed manually from the pots and the soil dried at 60°C. The soil was thoroughly mixed and analysed for ^{99}Tc using the method detailed in Section 3.4.4. In addition, ^{99}Tc analysis was performed on the retained seaweed samples, three control soil samples and five

seaweed samples provided by the Scottish Universities Research and Reactor Centre at East Kilbride which were used to validate the method.

4.2.4 Statistical analysis

Statistical analysis was carried out using MINITAB (Minitab, 2000). A two-way ANOVA was performed on square root transformed CO_2 production data, using a General Linear Model (GLM) with 'sampling date' (i.e. time) and 'treatment' (i.e. with or without seaweed) as factors. A one-way ANOVA was performed on square root transformed ^{99}Tc in soil (% of that added with seaweed) using 'sampling date' as the factor. The assumptions for the GLM and ANOVA were checked drawing a normality plot of the residuals and plotting the residuals against the fitted values. The data was further analysed using a Tukey's pairwise comparison (family error rate 0.05) between sampling dates.

4.3 RESULTS

4.3.1 Basic soil parameters

The < 2 mm fraction of the soil had a mean pH of 4.1 (SD ± 0.49) and an organic matter content of 2.64% (SD ± 0.43) (n=5). The mean soil moisture content (n=3) of the field moist soil on collection was 2.6% (SD ± 1.3). At field capacity the soil held an average (n=3) of 9.68 g (SD ± 3.38) of water per 50 g of soil (dry weight).

4.3.2 Carbon dioxide production

Carbon dioxide production was calculated as the number of moles of CO_2 per gram of soil (dry weight) produced in a 24-hour period (Table 4:1).

Table 4:1 : CO_2 production in 24-hr period (moles CO_2 per gram dry weight soil) (n = 3)

Week	Treatment pots		Control pots	
	Mean	1 SD	Mean	1 SD
0.7	1.76×10^{-7}	6.89×10^{-8}	1.13×10^{-8}	9.47×10^{-9}
1.7	1.75×10^{-7}	5.42×10^{-8}	3.91×10^{-9}	1.11×10^{-9}
3	1.71×10^{-7}	5.47×10^{-8}	-6.50×10^{-8}	1.70×10^{-9}
8	1.44×10^{-6}	2.29×10^{-7}	7.85×10^{-9}	1.41×10^{-9}
12	5.29×10^{-7}	1.51×10^{-7}	1.84×10^{-8}	3.19×10^{-9}
15	1.68×10^{-6}	3.59×10^{-7}	2.20×10^{-8}	4.17×10^{-8}

The results were plotted (Figure 4.2) to show the change in CO_2 production rate over time for the treatment (with seaweed) and control (without seaweed) pots. The graph also shows the ambient temperature over the period of the experiment.

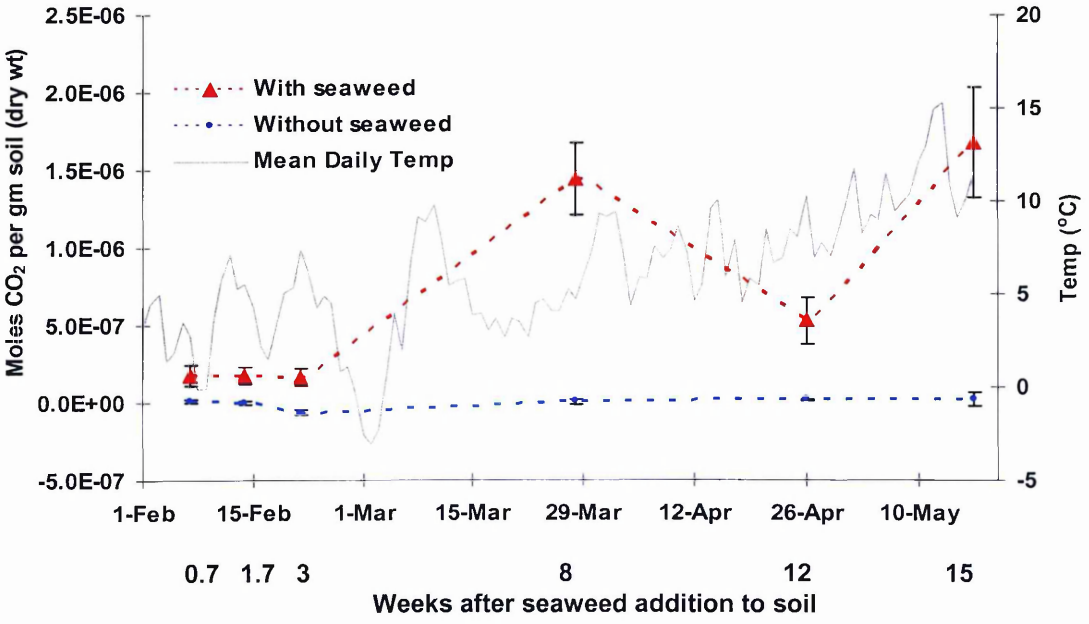


Figure 4:2: Carbon dioxide production (moles per gram, dry weight) in treatment and control pots over time (n=3) (Error bars = 1 SD) and daily mean temperature

The rate of CO_2 production in the control pots (without seaweed) remained low and fairly constant throughout the 15 weeks of the experiment. Over the first three sampling dates (5, 12 days and 3 weeks, on 1-20 Feb) the CO_2 concentration in the treatment pots (with seaweed) remained low and there was little difference in the CO_2 production between the treatment and control pots. CO_2 production had risen 10-fold by the sampling point at the end of March (8 weeks). The rate fell substantially at the 12 week sampling point at the end of April before rising again at the final sampling point in May (15 weeks) to a level approximately equal to that found at the week 8 sampling point. Statistical analysis confirmed that there was a significant interaction of treatment and time ($\rho < 0.01$). The results of the Tukey's pairwise comparison with a family error rate of 0.05 between sampling dates are given in Table 4:2. The blue cells indicate that the value for the sampling date in the left hand column is significantly higher than that for the date in the top row while yellow cells indicate significantly lower values.

Table 4:2: P values for Tukey's pairwise comparison between sampling dates for CO_2 production (Figures in bold = significant difference, For confidence intervals see Appendix 2)

Week	0.7	1.7	3	8	12
0.7					
1.7	1.000				
3	0.928	0.963			
8	**0.000**	**0.000**	**0.000**		
12	**0.001**	**0.001**	**0.000**	**0.000**	
15	**0.000**	**0.000**	**0.000**	0.569	**0.000**

The results show that while there was no significant difference between CO_2 production over the first three sampling dates, there was a significant

increase between those and the later dates. In addition, there was a significant decrease in CO_2 production between week 8 and week 12 followed by a significant rise between week 12 and 15.

The total amount of CO_2 released over the 15-week period by the soil microbes in each treatment pot was calculated to be 2.1 x 10^{-2} moles compared to 2.8 x 10^{-4} moles in the control pots. This is equivalent to 6.9% of the total carbon content of the seaweed.

4.3.3 Tc-99 analysis

4.3.3.1 Validation samples

The results from the analysis of the five seaweed samples from SURRC, East Kilbride are shown in Table 4:3.

Table 4:3: Results from validation exercise

Sample	SURRC study mean (Bq kg^{-1})	1 SD	This study (Bq kg^{-1})
A	5.86	3.53	7.18
B	58.27	16.57	66.99
C	2,900	500	2,699
D	17,910	2,700	16,680
E	133,100	18,000	147,371

All results from the analysis for this study fell within one standard deviation of the mean results from other laboratories in the exercise. Analysis of the internal seaweed standard prepared for this study gave a mean result of 5.93 x 10^4 Bq kg^{-1} (dry weight) (n = 8, SD ± 0.594 x 10^4).

4.3.3.2 Seaweed and soil samples

Fucus vesiculosus samples (retained when pots were set up) had a mean ^{99}Tc activity concentration of 5.95 x 10^4 Bq kg^{-1} (dry weight) (1.19 x 10^4 Bq

kg^{-1}) (wet weight) (n=18, SD ± 0.149 x 10^4). The measured values have been compared with those for *Fucus vesiculosus* collected from the shore close to BNFL, Sellafield in previous years in Table 4:4.

Table 4:4: Tc-99 concentration (Bq kg^{-1}, wet weight) in *Fucus vesiculosus* close to BNFL Sellafield, Cumbria, UK

Year	^{99}Tc (Bq kg^{-1})	Reference
1997	60,000	MAFF & SEPA (1998)
1998	20,000	MAFF & SEPA (1999)
1999	13,000	FSA & SEPA (2000)
2000	10,000	FSA & SEPA (2001)
2001	17,000	FSA & SEPA (2002)
2001	11,800	THIS STUDY

Analysis of untreated soil confirmed that it did not contain ^{99}Tc. Analyses of the soil samples collected from the treatment pots between 5 and 105 days (15 weeks) after the start of the experiment showed that ^{99}Tc is released from the seaweed into the soil in measurable quantities. Changes in ^{99}Tc activity concentration in the soil over time (assuming even distribution) are given in Table 4:5.

Table 4:5: Tc-99 activity concentration in soil (Bq kg^{-1}, dry weight) over time (n=3 except for week 15 where n=2)

Week of experiment	^{99}Tc (Bq kg^{-1}) (dry wt)	1 SD
0.7	147	57
1.7	297	153
3	423	131
8	1153	421
12	932	225
15	1245 - 2224	

The underlying assumption that all the seaweed pieces had been removed from the soil prior to ^{99}Tc analysis is based on the observation that the frond

pieces appeared intact. Very small fragments potentially not removed, would have no significant effect on the results. The ^{99}Tc released into the soil in each pot was calculated as the percentage of that originally added with the seaweed. The results were plotted as the cumulative percentage of the original ^{99}Tc added to show the pattern of accumulation over time (Figure 4:3).

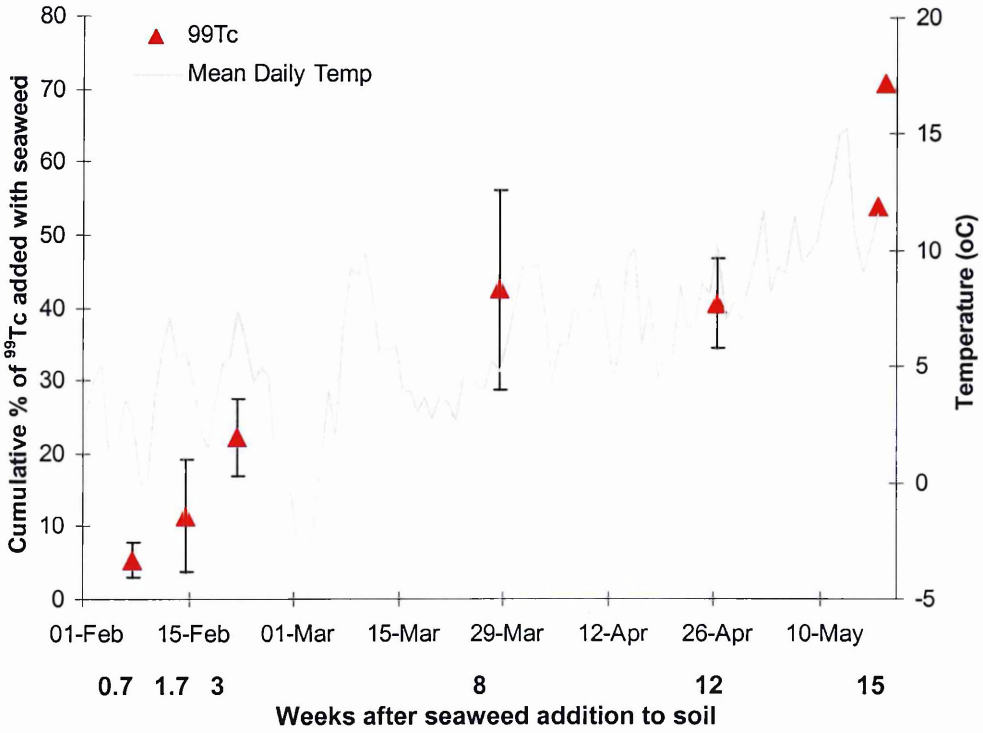


Figure 4:3: Tc-99 accumulation in soil (cumulative percentage of that added with seaweed) over time (n=3, except for week 15 where only 2 replicates were available) (Error bars = 1 SD) and daily mean temperature

About 20% of the ^{99}Tc added to the pot with the seaweed had accumulated in the soil over the first three weeks of the experiment. By the 8-week sampling point this had increased to around 40% and there

was no further increase in this value by the 12-week sampling point. By the end of the experiment (15 weeks) over 60% of the ^{99}Tc originally added with the seaweed had been transferred to the soil. There was a statistically significant overall effect of time on ^{99}Tc levels in soil ($p<0.05$). The results from the Tukey's pairwise comparison of ^{99}Tc activity concentration against sampling date are shown in Table 4:6. As before, the blue cells indicate that the value for the sampling date in the left hand column is significantly higher than that for the date in the top row.

Table 4:6: P-values for Tukey's pairwise comparison between sampling dates for ^{99}Tc activity concentration (Figures in bold = significant difference, For confidence intervals see Appendix 2)

Week	0.7	1.7	3	8	12
0.7					
1.7	0.788				
3	0.311	0.935			
8	**0.002**	**0.011**	**0.050**		
12	**0.006**	**0.043**	0.178	0.957	
15	**0.000**	**0.002**	**0.006**	0.566	0.229

The results show that there was no significant difference in the ^{99}Tc activity concentrations between the first three sampling dates but concentrations from the later dates were significantly higher than those from the first three dates. Whilst Figure 4:3 shows an increase in ^{99}Tc release between weeks 12 and 15, the Tukeys pairwise comparison shows that this increase in not statistically significant. This may be the result of a large variation in the values obtained for the two replicates analysed at week 15.

4.4 DISCUSSION

This experiment attempted to mimic the use of seaweed on garden plots in Cumbria through the use of a sandy coastal soil and representative application rates and techniques for the seaweed. However, because of the small size of the pots, the application rate of the seaweed had to be reduced (by about 50%) and the seaweed cut up to allow its incorporation into the soil. The seaweed also had to be freshly cut from the rocks as there was none present on the strandline at the time of collection. The timing of the application was realistic as the growers often incorporate the seaweed early in the year before planting their vegetables in early spring.

The ^{99}Tc activity concentration in *Fucus vesiculosus* in the vicinity of BNFL, Sellafield has fallen since 1997 (Table 4:4) reflecting the fall in ^{99}Tc discharges from the reprocessing plant (Brown *et al.*, 1999). (See Figure 2:5). The seaweed collected for this experiment shows a lower ^{99}Tc activity concentration compared to the 2001 RIFE (Radioactivity in Food and the Environment) report value (FSA & SEPA, 2002). This may be due to the different sample collection methods or natural environmental variation. In the RIFE reports, seaweed was collected at four different times throughout the year and the samples bulked for ^{99}Tc analysis as opposed to the single sample collected in winter for this experiment.

The results from this experiment were analysed to determine the relative importance of three processes involved in decomposition; commutation (mechanical reduction of particle size), leaching and catabolism (energy producing, enzymatic transformation of complex organic molecules into

simple ones, e.g. by soil microbes) (Swift *et al.*, 1979), for the release of ^{99}Tc from contaminated seaweed into soil. These processes are regulated by many factors including the type and number of decomposer organisms in the soil, the resource quality and physio-chemical factors such as temperature, soil moisture and soil aeration (Swift *et* al., 1979). In the pot experiment, the soil was kept at 65% field capacity which was optimal for decomposition (Wagner & Wolfe, 1998), providing enough soil moisture and oxygen for microbe activity.

The cutting up of the seaweed prior to its addition to the soil mimics commutation of the resource. This may destroy cell walls allowing the escape of soluble material and give a larger surface area for microbial action. Carbon dioxide production is widely accepted as a quantitative expression of catabolic processes (microbial decomposition) (Wagner & Wolfe, 1998). In the first 3 weeks, CO_2 production in the pots containing seaweed remained low, similar to those in the control pots, suggesting that little, if any, catabolism of the seaweed was taking place, probably due to the low ambient temperature (2 - 6°C) over this period (below 5°C microbial activity virtually stops (Anonymous1999)). Despite this, around 20% of the ^{99}Tc added with the seaweed had accumulated in the soil over this initial period. This may be due to leaching, an abiotic process, of soluble pertechnetate ions and Tc complexed with various low molecular weight ligands within the cell, possibly enhanced due to the cutting up of the seaweed fronds prior to their addition to the pots. About 50 - 70% of ^{99}Tc in plants is in an ionic form or associated with soluble proteins or small organic molecules (Cataldo *et al.*, 1986).

Although the cumulative percentage of ^{99}Tc accumulated in the soil had increased to 40% by week eight the overall rate of release from the seaweed between three and eight weeks was slower than that in the first three weeks. The 10-fold increase in CO_2 production at this point does not seem to be a temperature response (as the temperature over this period generally did not exceed 6°C), so it may represent the microbial decomposition (catabolism) of leached soluble sugars, with the slowing release rate of ^{99}Tc representing the continuing leaching of the remaining soluble forms of the radionuclide. At 12 weeks, the CO_2 production rate fell considerably which may indicate the depletion of the leached, readily decomposable material and the subsequent death of many of the opportunist bacteria (again there is no obvious response to temperature). Between eight and 12 weeks after seaweed addition, no ^{99}Tc appeared to be released suggesting that the remaining ^{99}Tc was bound to cell organelles such as chloroplasts (Woodard-Blankenship *et al.*, 1995) or large insoluble molecules that could not be readily leached out of the cells. At the last sampling point (15 weeks), CO_2 production had risen again. This may partly be a response to a steady increase in ambient temperature or the decomposition of the dead opportunist micro-organisms but also may indicate commencement of catabolism of less readily decomposable material, perhaps some of the insoluble storage molecules within the cells by slower growing autochthonous microbes. This was accompanied by a further increase in ^{99}Tc released into the soil suggesting that some of the bound ^{99}Tc was being released due to the catabolism of large organic molecules with which they had formed

complexes. By the end of the experiment between 54 and 71% of the ^{99}Tc added with the seaweed had accumulated in the soil. This was much higher than expected since the low ambient temperature at the time, the short duration of the experiment and the intact appearance of the seaweed pieces on their removal from the pots at every sampling date, suggested that very little decomposition of the seaweed had taken place. There was a lack of information on the percentage release of ^{99}Tc from seaweed or any other plant material into soil in published literature with which to compare the results from this study.

The amount of carbon released as CO_2 over the period of the experiment equalled approximately 6.9% of the carbon content of the seaweed. This suggests that large-scale decomposition of the structural and storage carbohydrates of the seaweed had not yet taken place.

Two-compartment kinetics for the release of ^{99}Tc into water from plant material (including brown seaweed) have been previously reported (Benco *et al.*, 1986; Dehut *et al.*, 1989) but to date there have been no reports of studies into the kinetics and mechanisms involved in the release of ^{99}Tc from seaweed into soil. The results of this experiment suggest that the ^{99}Tc release from seaweed into soil is the product of two mechanisms, leaching and microbial decomposition (catabolism).

4.5 CONCLUSION.

This experiment has confirmed that ^{99}Tc is released from *Fucus vesiculosus* into soil over time and that the release appears to be dependent on the processes of leaching and microbial decomposition.

Under the winter conditions prevailing in the initial phase of the experiment, leaching appears to be the predominant mechanism for the release of ^{99}Tc from seaweed into soil. Microbial decomposition seems to have become more important as the ambient temperature rose in the spring in the latter stages of the study. Over 60% of ^{99}Tc contained within the seaweed was released into the soil between the beginning of February and mid-May and a similar release can reasonably be expected under field conditions in Cumbria to coincide with the start of the growing season. It therefore seems likely that there will be a high availability of released ^{99}Tc for plant uptake under aerobic conditions. However, plant uptake has been shown to be dependent on the concentration of soluble ^{99}Tc in the soil solution rather than total soil concentration (Van Loon *et al.*, 1986b), so it is important to investigate how much of the released ^{99}Tc is present in the soil solution.

CHAPTER 5 - EXPERIMENT 2: RELEASE OF ^{99}TC INTO SOIL SOLUTION AND UPTAKE INTO SPINACH PLANTS

5.1 INTRODUCTION

5.1.1 Rationale

The results from the small-scale pot experiment confirmed that ^{99}Tc was released from *Fucus vesiculosus* into the soil over time, with around 60% released over a 15-week period. Whilst leaching appeared to be the predominant mechanism for ^{99}Tc release, microbial decomposition may become more important once ambient temperature rises. It was postulated that the size of the seaweed pieces used in the experiment may have influenced leaching and microbial decomposition rates, hence the rate of ^{99}Tc release. The large percentage of ^{99}Tc released into the soil suggests that there is the potential for a considerable amount of ^{99}Tc to be present in the soil solution and therefore available for plant uptake.

Previous research has shown that plant uptake is dependent on the activity concentration of ^{99}Tc in the pertechnetate ion form in soil solution rather than total soil concentration (Van Loon *et al.*, 1986), therefore an experiment was designed to monitor the release of ^{99}Tc from seaweed into soil solution. As some released ^{99}Tc may not be or remain in a soluble form, its concentration in soil solution will be lower than that of the bulk soil. However in aerobic soils, ^{99}Tc is poorly sorbed to soil matrix particles with K_d values near zero for sandy soils (Routsen *et al.*, 1977; Sheppard *et al.*, 1983) so, as the soil used in this investigation is a sandy soil, it seems likely that a large proportion of the ^{99}Tc released in a soluble form should remain in the soil solution. Whilst there has been little research into the release of ^{99}Tc from plant material, around 55% of

^{99}Tc artificially incorporated into grass material (Dehut *et al.*, 1989) and 60% of that incorporated into *Fucus vesiculosus* (Vandecasteele *et al.*, 1986) was readily soluble when the plants were shaken in water. The soluble fraction of ^{99}Tc is likely to be released from the seaweed before bound ^{99}Tc. It was predicted therefore that around 50% of the ^{99}Tc released from the seaweed would be in a soluble form that should remain in the soil solution.

5.1.2 Aim

The aim of this pot experiment was to monitor the release of ^{99}Tc from *Fucus vesiculosus* into soil solution over time, investigate the mechanisms involved and measure plant uptake of the released ^{99}Tc.

5.1.3 Hypotheses

The hypotheses this experiment was designed to test were:

3. In aerobic, sandy soils around half of the ^{99}Tc within *Fucus vesiculosus* will be released in a soluble form and remain within the soil solution

4. The majority of the ^{99}Tc released into soil solution will be in a form that is available for plant uptake

To evaluate the effect of cutting up the seaweed on the rate of ^{99}Tc release a further hypothesis was formed.

5. The rate of ^{99}Tc release from *Fucus vesiculosus* will be faster from small seaweed pieces than larger pieces due to increased leaching and microbial decomposition

5.1.4 Experimental design

As for experiment 1, a key objective was to design an experiment that mimicked field conditions, as closely as possible. This experiment was designed using information gained from both the user interviews and the results of the first experiment.

5.1.4.1 Information from users

This experiment used the same information on seaweed type and application rate that was gathered from users of seaweed for the first experiment. The types of crop grown on seaweed conditioned plots were also recorded and included, beetroot, potatoes and green leafy vegetables such as leaf beet and spinach.

5.1.4.2 Information from Experiment 1

As two mechanisms, leaching and microbial decomposition seem to be involved in the release of ^{99}Tc from seaweed into soil, it was important to continue to monitor CO_2 production to further investigate these mechanisms. Cutting up of the seaweed into small pieces for the first experiment may have influenced the rates of leaching and microbial decomposition and hence the rate of ^{99}Tc release. These differences may be due to the cut surfaces providing more damaged cells allowing greater leaching and a larger surface area for microbes to attack. This question was investigated in this experiment by monitoring CO_2 production and ^{99}Tc release in two sets of treatment pots, each set containing seaweed cut into different sizes.

5.1.5 Design choice

Pots were again chosen for this experiment as it permitted regular collection of soil solution samples and easy measurement of CO_2 production. It also

allowed regulation of soil moisture content, which was essential for a more accurate calculation of ^{99}Tc activity concentration in the soil solution. The experiment was designed to allow the collection of a series of soil solution samples over time with minimal disturbances to the pots. This was achieved through the use of in-situ rhizon soil solution samplers, through which soil solution could be drawn through a vacuum (See section 3.4.2).

Dismantling the pots at the end of the experiment and analysing the ^{99}Tc content of the bulk soil, soil solution and remaining seaweed allowed the activity concentration of ^{99}Tc in each compartment to be quantified and the proportion of ^{99}Tc in the soil solution to be estimated.

To investigate whether the cutting up of the seaweed in the first pot experiment had speeded up microbial decomposition and release of ^{99}Tc into the soil, two sets of pots with seaweed fronds cut into large and small pieces were used in this experiment. Monitoring the CO_2 production and ^{99}Tc accumulation in these two sets of pots would highlight any effect of cutting the seaweed.

The plant species chosen to determine the bioavailability of released ^{99}Tc was spinach. This was chosen because it had been extensively used in previous plant uptake studies using soil artificially contaminated with ^{99}Tc, was known to have high activity concentration ratios for ^{99}Tc and was grown on the seaweed treated plots in Cumbria.

5.2 POT EXPERIMENT

5.2.1 Materials

In July 2001, *Fucus vesiculosus* was freshly cut from rocks and soil collected from the same locations as for the first experiment (See Section 3.3.1 and

3.3.2). Two varieties of spinach were grown, a summer cropping variety (Dominant, Johnston Seeds) and a winter cropping variety (Medania, Unwins Seeds Ltd). The fertiliser added to the pots was a general garden fertiliser (Growmore).

5.2.2 Procedure

A series of 25 litre plastic pots with removable air-tight lids were prepared by drilling drainage holes in the base and drilling a hole in the lid and fitting a rubber septum. A thin layer of gravel was placed in the bottom of 15 pots and this was covered with a permeable membrane to allow drainage of water. The pots were set up as shown in Figure 5:1. Ten pots were prepared with 7 kg of field moist soil mixed evenly with 1 kg (wet weight) *Fucus vesiculosus* (Figure 5:1a). The seaweed added to five of the pots had been chopped into small (4-6 cm) pieces, whilst that added to the remaining five pots was left in larger (20-25 cm) pieces. A further five pots contained only 7 kg of moist soil to act as controls.

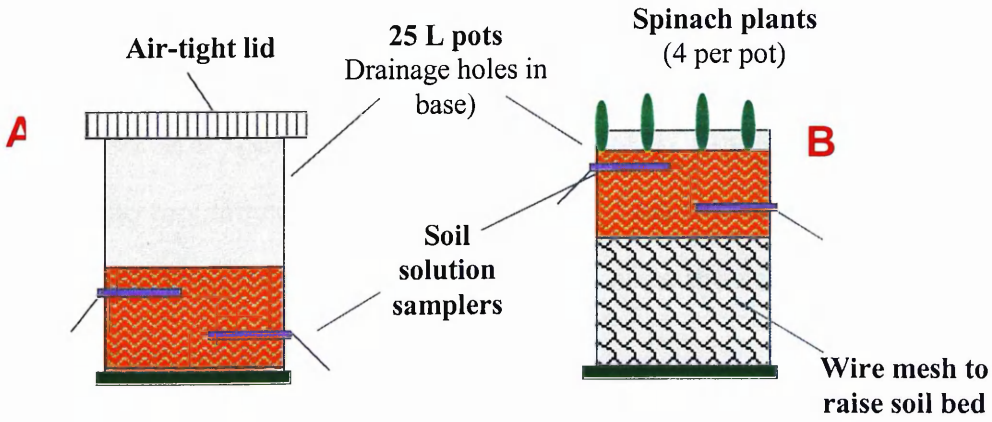


Figure 5:1: Experimental pots

The amount of material in each pot left around two-thirds of the pot empty, leaving a large air-space for the collection of CO_2 released by soil microbes. The final five pots were to be used for monitoring spinach uptake of ^{99}Tc from the soil solution, so the soil surface had to be raised to be close to the top of the pot. To minimise the weight of the pot, two-thirds of the pot was filled with chicken wire, covered with a permeable membrane. The pots were then filled with 7 kg of field moist soil mixed evenly with 1 kg (wet weight) *Fucus vesiculosus*, chopped into 4-6 cm pieces (Figure 5:1b). A sub-sample of seaweed was retained from each pot for ^{99}Tc analysis. Five grams of Growmore fertiliser was added to each pot which was equivalent to adding 0.35 g each of nitrogen, phosphorus and potassium. This represented the recommended application rate for the fertiliser on vegetable crops of 700 kg ha^{-1}. Holes were then drilled into each pot and the soil solution samplers inserted as described in Section 3.4.2. The pots were wrapped in black plastic to prevent algal growth on the inside of the pots.

The pots were set up at the beginning of July 2001 and kept at ambient temperature in an open-sided shelter at Stirling University (Grid reference NS817969), and maintained at 75% field capacity (measured gravimetrically) by watering regularly with collected rainwater. This increased moisture content, compared to Experiment 1, was necessary to allow the collection of a sufficient amount of soil solution for analysis.

At the end of July (3 weeks after seaweed addition to the pots), four spinach seedlings were planted into each of the five prepared pots in a square pattern, 8-9 cm apart and 8-9 cm from the edge of the pot. These plants were

harvested at the end of August 2001. A second batch of seedlings was planted out, in the same pattern, at the end of October 2001. This winter spinach crop was harvested in May 2002.

The pots were dismantled in March 2003.

5.2.3 Sampling regime and sample analyses

5.2.3.1 Basic soil parameters

Basic soil parameters (pH, organic matter content, moisture content and field capacity measurements) were analysed at the beginning of the experiment (See Section 3.4.1).

5.2.3.2 Carbon dioxide

At each of the sampling days, the pots were watered then left for 6-8 hours to equilibrate. CO_2 production by soil microbes over a 24-hr period was measured in both sets of treatment pots containing large and small seaweed pieces and the control pots, using the method described in Section 3.4.3. CO_2 production was monitored over 18 weeks from July to November 2001. Sampling was carried out at days 3, and 7 and from then weekly until 8 weeks, then at weeks 12 and 18. After the main period of monitoring, CO_2 production was measured periodically, with sampling carried out at 22, 28, 35, 42 and 48 weeks.

5.2.3.3 Soluble sugar and amino acid analysis

Soluble sugar and amino acid analyses were carried out on soil solution samples from the control and treatment pots (See Section 3.4.5 and 3.4.6 for methods). Analyses were carried out for samples collected on week 1,2,3,4,6,7,9, and 12.

5.2.3.4 Tc-99 analysis

Soil solution was collected over the following 24-48 hours from all pots after CO_2 measurements had been taken. The volume of soil solution acquired over that period varied greatly between pots and between dates, ranging from 0 – 10 ml. Soil solution was analysed for ^{99}Tc using the method described in Section 3.4.4. As all pots did not produce sufficient soil solution for analysis and because of time constraints, only three replicates from each treatment were analysed and samples were not analysed from every sampling date. Samples from the control and two types of treatment pots were analysed at 1, 2, 4, 6, 8, 12 and 18 weeks. Soil solution from the pots in which the spinach was grown was analysed prior to planting and after harvesting for each crop.

The spinach plants from both crops were weighed after harvesting then dried at 60°C overnight, ground to a fine powder and retained for ^{99}Tc analysis. The first crop (August) had bolted after only five weeks of growth, hence only the leaves from the four plants in each pot were pooled for analysis. The second crop (May) harvested after seven months growth showed better growth form, therefore individual plants (above ground biomass only) were analysed for ^{99}Tc.

In March 2003, a final set of soil solution samples was collected before the experimental pots were dismantled. The moist soil from each pot was passed through 8 mm and 4 mm sieves to remove the larger seaweed pieces, then dried overnight at 60°C. The oven dry soil was passed through 2 mm and 1 mm sieves to remove any remaining small fragments of seaweed. The soil was thoroughly mixed and three sub-samples from each pot were retained for ^{99}Tc analysis. The seaweed pieces removed from each pot were dried at 60°C overnight, weighed, then ground to a fine powder and kept for ^{99}Tc analysis.

5.2.3.5 Total carbon and nitrogen analysis

Two seaweed samples were retained, one when seaweed was added to the pots at the start of the experiment and another 21 months later, when the pots were dismantled. These samples were analysed for total carbon and nitrogen using a Carlo Erba 1500 C and N elemental analyser at the Agriculture and Agri-food Laboratories, Canada.

5.2.4 Statistical analysis

Statistical analysis was carried out using Minitab (Minitab, 2000). ANOVA was performed on square root transformed CO_2 production data multiplied by 10^6 and square root transformed ^{99}Tc in soil solution, using a General Linear Model (GLM), with 'sampling date', 'treatment' and 'treatment nested within pot number' as fixed factors and 'pot number' as a random factor. The data was further analysed using a Tukey's pairwise comparison (Family error rate 0.05) between sampling dates and treatments. ANOVA was performed on the soluble carbohydrate data, using a GLM with 'sampling date' and 'treatment' as factors. The assumptions for the GLMs were checked by drawing a normality plot of the residuals and plotting the residuals against the fitted values. The presence of any correlation between CO_2 production by soil microbes and soluble carbohydrate concentration in soil solution and the weight of the spinach plants and the uptake of ^{99}Tc per kg weight of the plants was tested using the Spearman's coefficient test.

5.3 RESULTS

5.3.1 Basic soil parameters

The < 2 mm fraction of the soil had a mean pH of 4.5 (SD ± 0.29) and an organic matter content of 2.96% (SD ± 0.47). The mean soil moisture content

(n=3) of the field moist soil on collection was 5.24% (SD ± 2.65). At field capacity the soil held an average (n=3) of 148.9 g (SD ± 48.5) of water per kilogram of soil (dry weight).

5.3.2 Carbon dioxide production

Carbon dioxide production was calculated as the number of moles of CO_2 per gram of soil (dry weight) produced in a 24-hour period (Table 5:1).

Table 5:1: CO_2 production in 24-hr period (moles CO_2 per gram dry weight soil) (n=5)

	Control pots		Treatment A (small)		Treatment B (Large)	
Week	**Mean**	**1 SD**	**Mean**	**1 SD**	**Mean**	**1 SD**
0.3	1.19×10^{-7}	3.20×10^{-8}	2.60×10^{-6}	3.86×10^{-7}	2.47×10^{-6}	2.85×10^{-7}
1	4.67×10^{-8}	0.00	1.40×10^{-6}	1.73×10^{-7}	1.28×10^{-6}	1.23×10^{-7}
2	4.09×10^{-7}	2.29×10^{-8}	1.97×10^{-6}	9.96×10^{-8}	2.02×10^{-6}	1.72×10^{-7}
3	1.02×10^{-7}	1.26×10^{-15}	1.73×10^{-6}	1.44×10^{-7}	1.57×10^{-6}	1.55×10^{-7}
4	1.69×10^{-8}	2.31×10^{-8}	1.21×10^{-6}	1.31×10^{-7}	1.19×10^{-6}	3.37×10^{-7}
5	3.80×10^{-8}	2.12×10^{-8}	1.15×10^{-6}	1.05×10^{-7}	1.11×10^{-6}	6.40×10^{-8}
6	1.56×10^{-7}	3.12×10^{-8}	1.79×10^{-6}	3.03×10^{-7}	1.44×10^{-6}	1.45×10^{-7}
7	6.36×10^{-8}	4.44×10^{-8}	9.52×10^{-7}	1.07×10^{-7}	9.14×10^{-7}	6.32×10^{-8}
8	5.73×10^{-8}	4.00×10^{-8}	8.33×10^{-7}	1.22×10^{-7}	7.90×10^{-7}	8.81×10^{-8}
12	3.84×10^{-8}	2.72×10^{-8}	4.24×10^{-7}	1.07×10^{-7}	4.17×10^{-7}	4.45×10^{-8}
18	1.82×10^{-8}	2.49×10^{-8}	1.53×10^{-7}	5.26×10^{-8}	1.21×10^{-7}	0.00
22	1.03×10^{-8}	2.30×10^{-8}	8.24×10^{-8}	2.05×10^{-8}	9.16×10^{-8}	3.24×10^{-8}
28	6.60×10^{-8}	0.00	1.64×10^{-7}	4.91×10^{-8}	9.39×10^{-8}	3.21×10^{-8}
35	5.27×10^{-8}	3.37×10^{-8}	1.20×10^{-7}	1.83×10^{-8}	1.00×10^{-7}	2.27×10^{-8}
42	5.38×10^{-8}	2.01×10^{-8}	2.39×10^{-7}	4.88×10^{-8}	2.47×10^{-7}	3.33×10^{-8}
48	5.93×10^{-8}	3.31×10^{-8}	2.24×10^{-7}	3.61×10^{-8}	2.24×10^{-7}	3.61×10^{-8}

The results from the main monitoring period (0-18 weeks) were plotted (Figure 5:2) to show the change in CO_2 production rate over time for the control and two types of treatment pots. The graph also shows the mean monthly ambient temperature over the period.

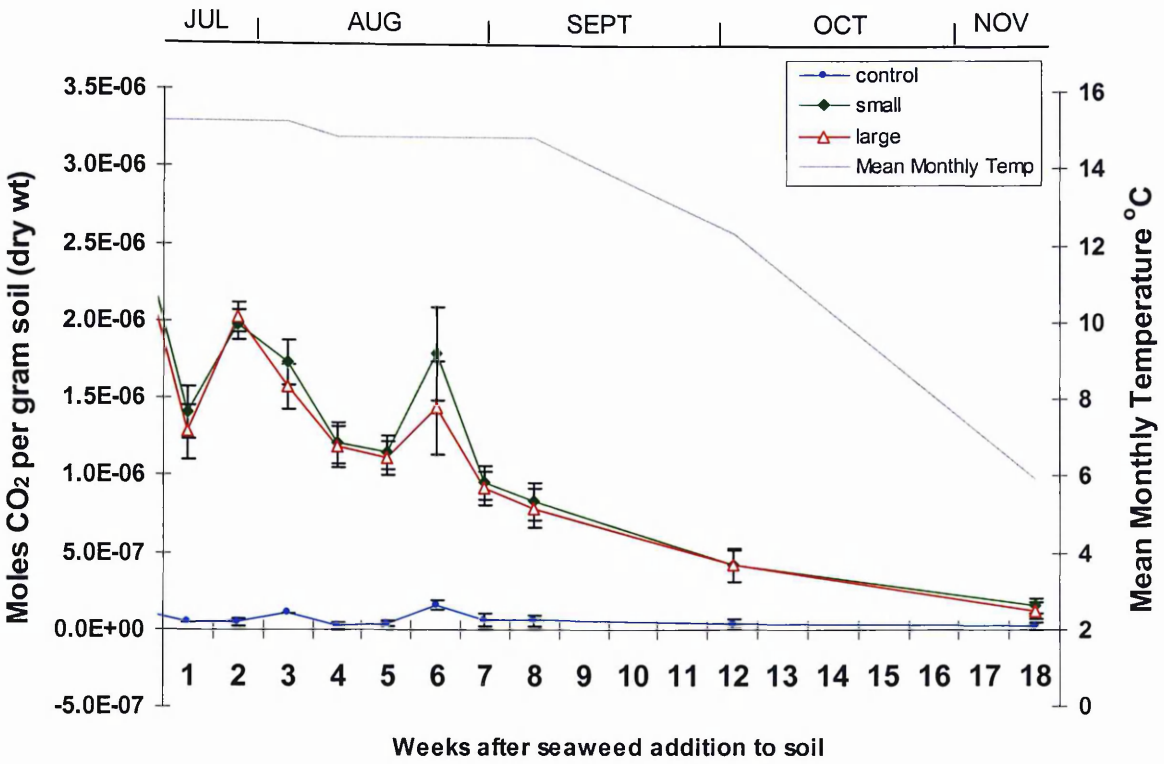


Figure 5:2: CO_2 production (moles per gram, dry weight soil, per 24-hrs) in treatment and control pots (n=5, Error bars = 1SD) and mean monthly temperature

The rate of CO_2 production in the control pots (without seaweed) remained low and fairly constant throughout the 18 weeks of the experiment. In both sets of treatment pots the CO_2 production was initially high, then appeared to undergo two cycles of falling and rising again over the first five weeks of sampling, before finally falling away steeply from the end of August (week 7) until the end of the experiment (week 18). Statistical analysis of square root transformed CO_2 data multiplied by 10^6 confirmed that there was no significant interaction between treatment and date for the two types of treatment pots ($\rho = 0.344$). Examination of the individual factors showed that there was a significant effect of time ($\rho < 0.001$). There was no significant effect of treatment ($\rho = 0.261$).

This suggests that the size of the seaweed pieces does not affect the rate of CO_2 production.

The results of the Tukey's pairwise comparison with a Family error rate of 0.05 between sampling dates for the two different treatment pot types are given in Table 5:2. The blue cells indicate that the value for the sampling date in the left hand column is significantly higher (difference of means has positive value) than that for the date in the top row whilst yellow cells indicate significantly lower values (difference of means has negative value).

Table 5:2: P values for Tukey's pairwise comparison between sampling dates for CO_2 production (Figures in bold = significant difference, For full statistical data see Appendix 2)

(a) Large seaweed pieces

Week	0.3	1	2	3	4	5	6	7	8	12
1	**0.0001**									
2	**0.0164**	**0.0001**								
3	**0.0001**	0.1400	**0.0032**							
4	**0.0001**	0.9991	**0.0001**	**0.0023**						
5	**0.0001**	0.9105	**0.0001**	**0.0002**	1.0000					
6	**0.0001**	0.9403	**0.0001**	0.9984	0.1609	**0.0254**				
7	**0.0001**	**0.0019**	**0.0001**	**0.0001**	0.1256	0.1034	**0.0001**			
8	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0003**	**0.0035**	**0.0001**	0.9599		
12	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	
18	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

(b) Small seaweed pieces

Week	0.3	1	2	3	4	5	6	7	8	12
1	0.0001									
2	0.0001	0.0001								
3	0.0001	0.0711	0.6679							
4	0.0001	0.7904	0.0001	0.0001						
5	0.0001	0.2588	0.0001	0.0001	1.0000					
6	0.0001	0.0160	0.9342	1.0000	0.0001	0.0001				
7	0.0001	0.0001	0.0001	0.0001	0.1215	0.5599	0.0001			
8	0.0001	0.0001	0.0001	0.0001	0.0005	0.0074	0.0001	0.9794		
12	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	
18	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

These results suggest that there are two distinct periods of significantly higher and lower CO_2 production over the first six weeks of sampling, whilst the latter stages show progressively significant falls in CO_2 production. The total amount of CO_2 released per pot over the 18-week period was 6.58×10^{-1} moles (small seaweed pieces) and 6.14×10^{-1} moles (large seaweed pieces).

The samples taken after the main monitoring period showed that the CO_2 production rate in both sets of treatment pots remained low, around the 18 week level, until week 35, after which it showed a slight increase (Figure 5:3).

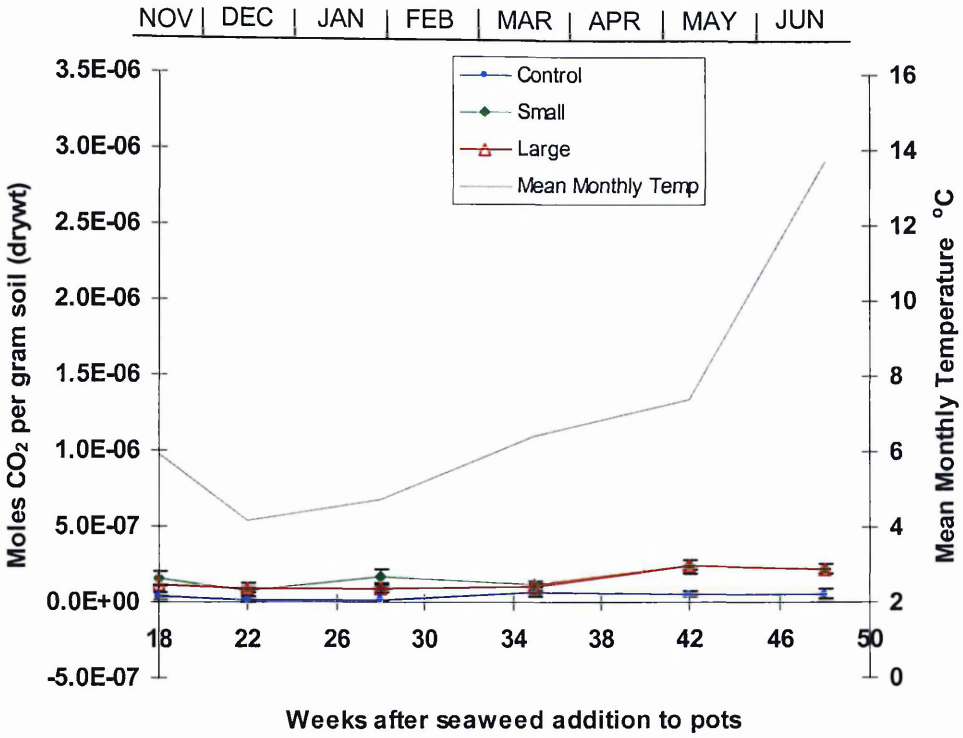


Figure 5:3: CO_2 production (moles per gram, dry weight soil, per 24-hrs) in treatment and control pots after main monitoring period (n=5, Error bars = 1SD) and mean monthly temperature

The total amount of CO_2 released per pot over this 48-week period was estimated to be 8.98 x 10^{-1} moles (small seaweed pieces) and 8.33 x 10^{-1} moles (large seaweed pieces).

5.3.3 Soluble sugar and amino acid analysis

The concentration of soluble sugars in the soil solution samples from the treatment pots over time is given in Table 5:3.

Table 5:3: Concentration of soluble carbohydrate in soil solution (mg l^{-1}) over time (n=3)

Week	Small pieces		Large pieces	
	Mean (mg l^{-1})	1 SD	Mean (mg l^{-1})	1 SD
1	118.75	54.39	245.97	NA
2	139.69	105.23	170.51	123.30
3	157.33	20.04	451.46	NA
4	716.78	76.99	246.48	92.39
6	486.26	118.48	248.12	72.68
7	433.16	18.29	234.90	92.79
9	335.28	113.61	193.80	103.92
12	325.73	NA	335.73	63.17

(NA = not available as only 1 sample analysed due to lack of soil solution collected)

Statistical analysis (ANOVA GLM) showed that there was an interaction of treatment and time (ρ = 0.002). The data was plotted along with the CO_2 production data over time to determine if there was any relationship between them.

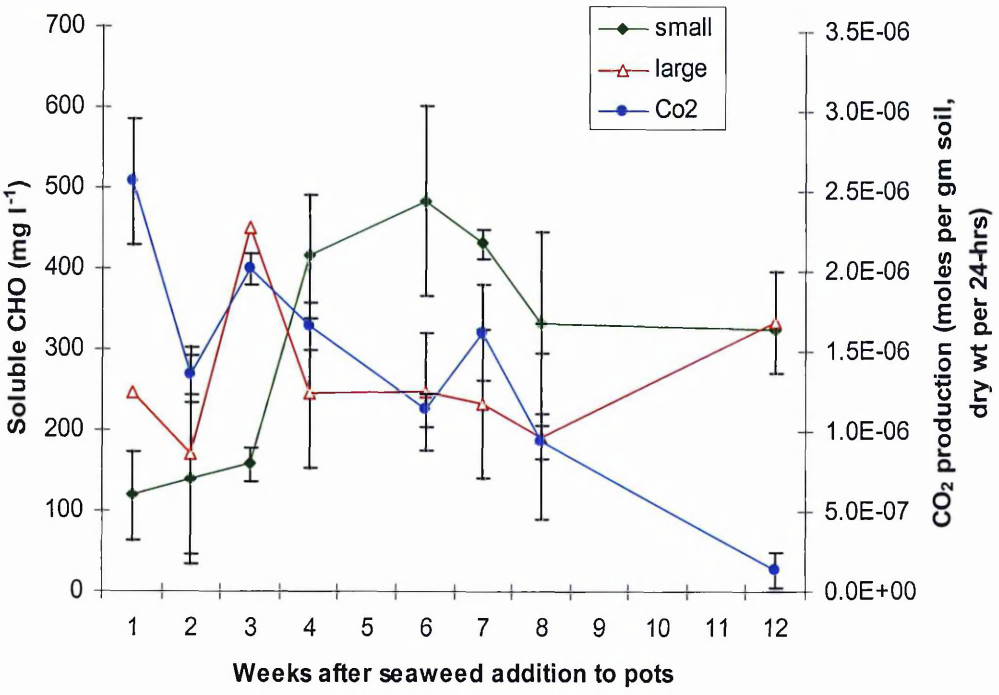


Figure 5:4: Relationship between CO_2 production by soil microbes and soluble carbohydrate concentration in soil solution in pots with large and small seaweed pieces over time

Statistical analysis shows that there was no correlation between the mean CO_2 production and the mean soluble carbohydrate concentration in the soil solution for both the pots with the large seaweed pieces and those with the small pieces) Spearman's rank coefficient 0.095 and -0.381 respectively.

The amino acid analysis of the soil solution samples found that the concentration was the same (greater than the highest standard) in all samples, including those from the control pots containing no seaweed. This means that the results from the treatment pots are meaningless and cannot be used as an indictor of microbial decomposition rate.

5.3.4 Tc-99 analysis

5.3.4.1 Initial seaweed samples

Fucus vesiculosus samples (retained when seaweed added to pots) had a mean activity concentration of 1.25 x 10^5 Bq kg^{-1} (dry weight) (2.5 x 10^4 Bq kg^{-1}, wet weight) (n=15, SD ± 0.4 x 10^4).

5.3.4.2 Soil solution samples

Measurements of ^{99}Tc activity concentration in the soil solution samples at each sampling date are given in Table 5:4. The activity concentrations are mean values from the soil solution extracted by two rhizon samplers for each of three pots. They assume that ^{99}Tc is evenly distributed within and between pots. The measurements show that on all sampling dates, between 0.3 and 18 weeks after the start of the experiment, ^{99}Tc was present in the soil solution.

Table 5:4 : Tc-99 activity concentration in soil solution (Bq l^{-1}) over time in pots with small and large seaweed pieces (n=3)

	Small Pieces		Large pieces	
Week	**Mean (Bq l^{-1})**	**1 SD**	**Mean (Bq l^{-1})**	**1 SD**
1	59.59	42.95	75.54	44.16
2	266.7	154.3	251.9	55.84
4	514.2	122.8	322.6	180.4
6	1076	463.3	354.0	207.6
8	598.8	297.5	387.7	163.1
12	1030	147.1	706.1	241.9
18	1153	153.7	1041	122.4

The ^{99}Tc present in the soil solution in each pot at each date was calculated as a percentage of that originally added with the seaweed and the results plotted to show the temporal change in the soil solution over time (Figure 5:5).

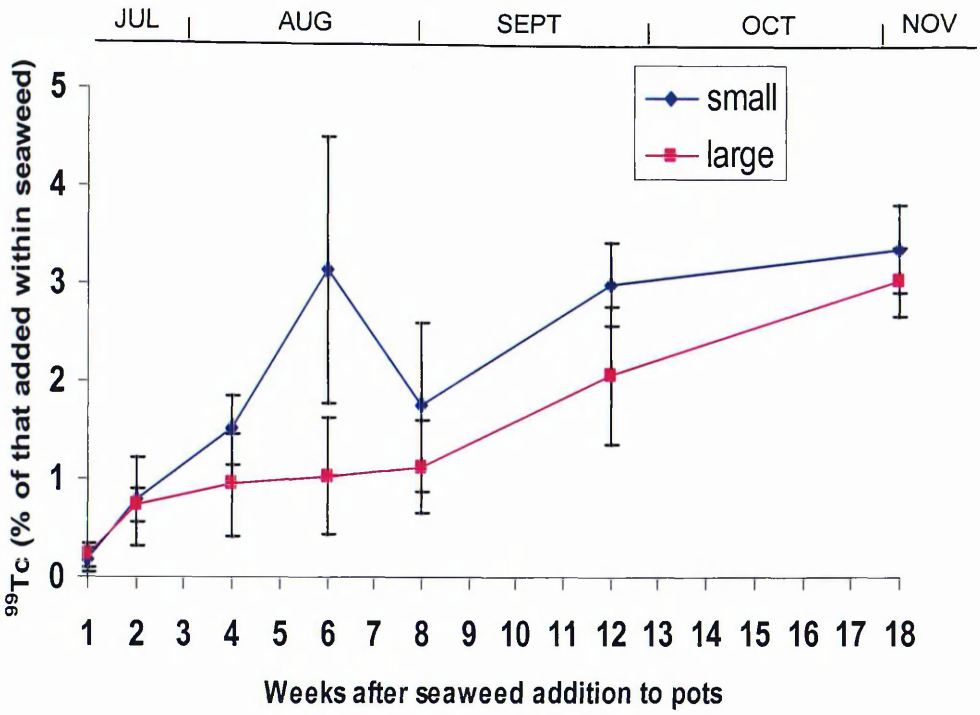


Figure 5:5: Tc-99 accumulation in soil solution over time (% of that added with seaweed) (n=3, Error bars = 1 SD)

For the pots containing small seaweed pieces, an average of 1.5% of the ^{99}Tc added to the pots with the seaweed was measurable in the soil solution over the first 4 weeks of the experiment. The mean value for the pots containing the larger seaweed pieces was 0.94%. By the 6-week sampling date the percentage present in the soil solution had risen to 3.15% (small pieces) and 1.03% (large pieces). Week 8 appeared to show a drop in ^{99}Tc activity concentration in the soil solution from pots with the small pieces of seaweed. At the 12-week sampling point the percentage of ^{99}Tc within the soil solution had risen again to 3.01 (small pieces) and 2.06 (large pieces), with a further increase seen at the 18-week sampling date. By the end point of the experiment 3.37% (small) and 3.04% (large) of the ^{99}Tc added within the seaweed was present in the soil solution.

Statistical analysis showed that there was a significant interaction between treatment and time (p=0.015). This suggests that the cutting up of the seaweed into small pieces has a time dependent effect on the accumulation of ^{99}Tc in the soil solution.

The results of the Tukey's pairwise comparison between sampling dates are given in Table 5:5. The blue shading indicates that the ^{99}Tc activity concentration on the sampling date in the left hand column is significantly higher than that taken on the sampling date in the top row.

Table 5:5: P values for Tukey's pairwise comparison between sampling dates for ^{99}Tc accumulation in soil solution (Figures in bold = significant difference, For confidence intervals see Appendix 2)

(a) Large seaweed pieces

Week	1	2	4	6	8	12
1						
2	0.1794					
4	0.0513	1.0000				
6	**0.0268**	0.9978	1.0000			
8	**0.0104**	0.9509	0.9998	1.0000		
12	**0.0004**	0.0783	0.2256	0.3505	0.5833	
18	**0.0000**	**0.0011**	**0.0035**	**0.0063**	**0.0144**	0.7802

(b) Small seaweed pieces

Week	1	2	4	6	8	12
1						
2	0.4978					
4	**0.0063**	0.3401				
6	**0.0000**	**0.0002**	**0.0305**			
8	**0.0090**	0.2510	1.0000	0.1961		
12	**0.0001**	**0.0009**	0.1093	1.0000	0.3276	
18	**0.0001**	**0.0003**	**0.0301**	1.0000	0.1160	1.0000

These results show that the values for the later sampling dates are significantly higher than those for the early dates, confirming that more ^{99}Tc is being released into the soil solution over time. The ^{99}Tc activity concentration in the soil solution in the pots with the small seaweed pieces shows a significant increase (from that in week 1) at an earlier date than that in the pots with the larger pieces (week 4 as compared to week 6). There is no significant difference between the later dates perhaps showing that equilibrium has been reached.

5.3.4.3 Spinach plants

The summer cropping spinach showed very poor growth, bolting to give leggy plants with small leaves and producing seed (Figure 5:6).

Figure 5:6: Summer cropping spinach showing poor growth form

The ^{99}Tc activity concentration for the spinach leaves spinach harvested at the end of August 2001 after 5 weeks growth is given in Table 5:6.

Table 5:6: Tc-99 activity concentration in summer cropping spinach plants (n=1, all 4 plants in each pot pooled to give 1 sample)

Pot	^{99}Tc kBq kg^{-1} (Dry weight)	Dry weight (leaves 4 plants) (g)	^{99}Tc Bq in 4 plants in pot
1	100.5	2.18	219.1
2	98.8	2.36	233.2
3	131.1	2.15	281.8
4	119.0	2.13	253.5
5	90.2	2.17	195.7

These results, despite the very poor growth of the spinach plants confirm that some of the ^{99}Tc released from *Fucus vesiculosus* is available for plant uptake.

The ^{99}Tc activity concentration in the above ground biomass of the winter cropping spinach harvested in May 2002 after 7 months growth (Figure 5:7) is given in Table 5:7. Whilst the growth of this variety was better than the summer cropping spinach, it was still low compared to what would be expected of plants growing in field conditions.

Figure 5:7: Winter cropping spinach

Table 5:7: Mean ^{99}Tc activity concentration (kBq kg^{-1}) winter cropping spinach (n=4)

Pot	^{99}Tc kBq kg^{-1} (Dry weight)	1 SD
1	146.2	38.6
2	107.9	56.6
3	96.7	23.0
4	63.3	52.0
5	52.2	34.3

The amount of ^{99}Tc present in the spinach was calculated as a percentage of that originally added within the seaweed and plotted in Figure 5:8.

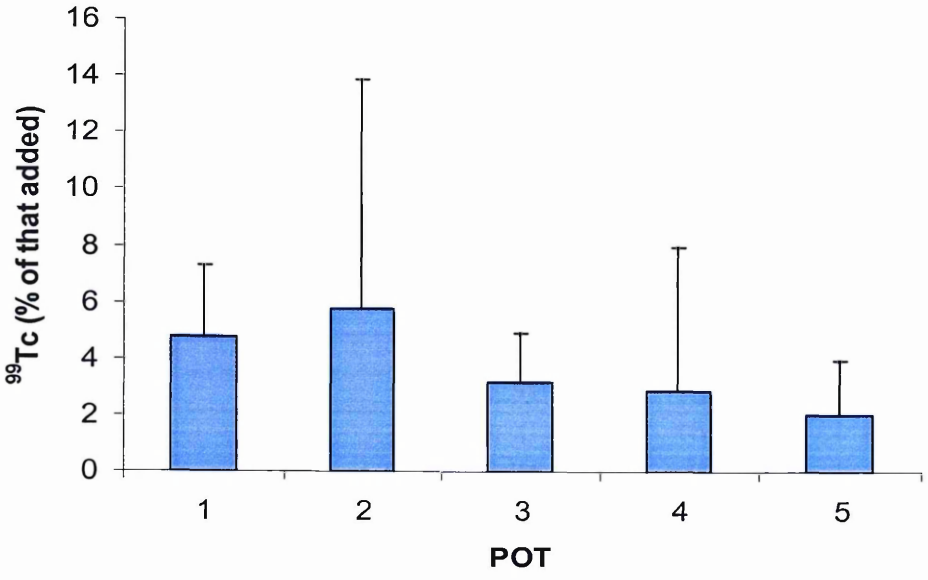


Figure 5:8: Tc-99 in winter cropping spinach plants (% of that added with seaweed) (n=4, Error bars = 1 SD)

After 7 months growth between 2 and 6% of the ^{99}Tc added was found within the spinach plants.

There was a large variation in the weight of the spinach plants and the ^{99}Tc activity concentration found within the plants (Table 5:8). The weight of the plants ranged from 0.9 g to 24.3 g (dry weight) but the mean value for each pot was similar, ranging from 6.65 g to 9.88 g. The total dry weight for each pot was also similar, ranging from 26.6 g to 39.5 g. Statistical analysis showed that there was a positive correlation between the ^{99}Tc activity concentration per kilogram of plant material and the weight of the plant (Spearman's rank coefficient = 0.620; p = 0.004).

Table 5:8 : Tc-99 activity concentration in individual plants, dry weight of plants and resulting concentration ratio

Pot	Plant	^{99}Tc kBq kg^{-1} (Dry weight)	Dry weight of plant (g)	^{99}Tc Bq per plant	CR
1	1	106.03	2.90	307.48	199
1	2	128.83	9.40	1211.05	242
1	3	196.22	9.20	1805.20	369
1	4	153.77	9.90	1522.28	289
2	1	70.62	3.70	261.29	156
2	2	187.05	24.30	4545.26	414
2	3	63.56	3.20	203.41	141
2	4	110.56	8.30	917.63	245
3	1	91.21	10.60	966.86	93
3	2	81.01	16.50	1336.64	82
3	3	130.62	2.20	287.37	132
3	4	83.85	7.70	645.62	85
4	1	27.23	3.30	89.85	28
4	2	35.57	3.10	110.28	37
4	3	139.87	19.30	2699.50	144
4	4	50.49	0.90	45.44	52
5	1	69.55	4.60	319.92	66
5	2	11.71	2.10	24.58	11
5	3	89.26	13.40	1196.05	84
5	4	76.06	7.20	547.64	72

The mean concentration ratio for ^{99}Tc uptake into the plants was calculated as approximately 120, with the range being 11 – 414, with the higher values generally associated with the plants with heavier weights.

5.3.4.4 Compartmentalisation of ^{99}Tc at end of experiment.

The ^{99}Tc activity present in each compartment (bulk soil, soil solution and remaining seaweed) when the experimental pots were dismantled is given in Table 5:9.

Table 5:9: Tc-99 in each compartment (Bq per pot) on dismantling experiment

	^{99}Tc per pot (Bq)		
Pot	**Bulk soil**	**Soil solution**	**Seaweed**
1	12396.7	1954.7	10509.8
2	12386.7	1238.4	14001.2
3	9123.3	724.2	11692.9
4	7019.2	1272.1	14257.7
5	11240.0	1024.8	14496.0
6	8523.4	752.0	11236.0
Mean	**10114.8**	**1161.0**	**12698.9**
1 SD	2224.1	452.8	1749.3

On average 11.6% of the ^{99}Tc released into the bulk soil is found within the soil solution. The distribution coefficient (K_d = ^{99}Tc on soil particles (Bq kg^{-1} dry wt) / ^{99}Tc in soil solution (Bq l^{-1}), which gives an indication of the proportion of ^{99}Tc absorbed to the soil particles in relation to that present in solution, at this time is 0.86. The ^{99}Tc activity in each compartment was calculated as a percentage of that added with the seaweed and is shown in Figure 5:9.

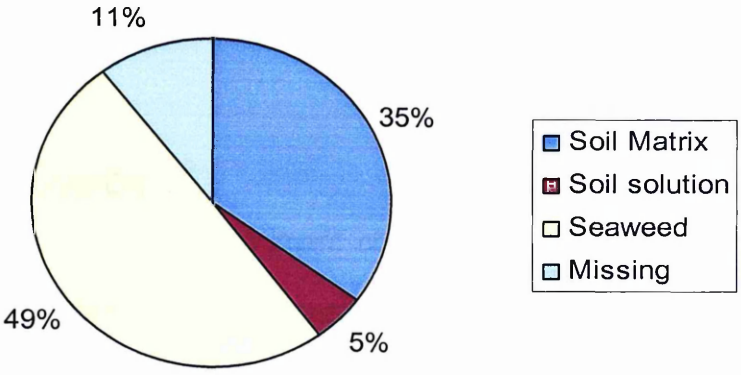


Figure 5:9: Tc-99 in each compartment on dismantling of pots (% of that added)

These results show that after 21 months, 49% of the ^{99}Tc still remains within the seaweed. Examination of the seaweed pieces remaining showed that on average 25% (SD ± 8.5%) of the seaweed (by weight) was recovered (n=6). Of that remaining, the majority (around 75%) was still in fairly large pieces (> 2 cm).

5.3.5 Total carbon and nitrogen analysis

The total carbon and nitrogen content, and C/N ratio of the fresh (Sample A) and post decomposition (Sample B) seaweed are given in Table 5:10.

Table 5:10: Total carbon and nitrogen content of seaweed

Sample	Total carbon (mg g^{-1})	Total nitrogen (mg g^{-1})	C/N ratio
A	345.4	17.03	20.3
B	168.2	11.52	14.6

The results show that just the total carbon content of the seaweed had fallen by over 50% and the nitrogen by around 33% over a period of 21 months in the soil.

5.4 DISCUSSION

The results from this experiment confirmed that ^{99}Tc was released into soil solution over time. Statistical analysis shows that a significant increase in ^{99}Tc activity concentration in the soil solution begins at four weeks after seaweed addition to the soil for the pots containing the small seaweed pieces, and at six weeks for the pots containing large pieces. This suggests that ^{99}Tc release from the smaller pieces is initially faster. However, analysis of the CO_2 production data showed that, whilst production was initially high, there was no significant difference between the two treatments. The faster rate of ^{99}Tc release in the pots with the small seaweed pieces compared to those with the large pieces, therefore, does not appear to be as a result of higher microbial decomposition. Whilst size of litter fragment is known to affect decomposition rate, with smaller size leading to increased surface area and more damaged cells which results in faster decomposition (Brady & Weil, 1999), in this case the difference may not be big enough to significantly influence the decomposition rate. In Cumbria, growers who put seaweed directly on to their plots do not cut the fronds at all, therefore the microbial decomposition rate may differ significantly from that in the experimental pots.

The high initial CO_2 production rate is due to the addition of fresh organic material to the soil (and the high ambient temperature) resulting in a rapid

increase in the activity of the opportunist (zymogeneous) microbes which may have been present in the soil in a dormant state (Brady & Weil, 1999). These microbes can rapidly multiply and deplete the pool of readily available decomposable material. Once their food supply is depleted many of the microbes die, with their cells providing a food source for other microbes to survive until a fresh release of material boost the population again (Brady & Weil, 1999). This would be reflected in periods of high and low CO_2 production as the number of microbes increase and decrease. The pattern of CO_2 production can be seen over the first six weeks after the seaweed is added to the soil. It was hoped that the analysis of the soluble sugar content of the soil solution would help to confirm increases and decreases in microbial activity, with a reduction in concentration associated with an increase in microbial activity, as the microbes utilise the carbohydrate as a food source. However no association was found. The soil solution is a very dynamic medium and perhaps more frequent sampling over a shorter time period would produce clearer results.

Over the first six weeks, after introduction of the ^{99}Tc within the seaweed to the pots, just over 3% was present in the soil solution of the pots with the small seaweed pieces and over 1% in the pots with the large pieces. Leaching is likely to be the predominant mechanism for ^{99}Tc release over this period and the rate of leaching is likely to be higher in the smaller pieces of seaweed as cutting would produce more damaged cells from which soluble compounds can escape. Microbial decomposition may play a subsidiary role, with the high initial rates leading to more damaged cells from which soluble material can leach.

After week six, the CO_2 production rate fell, possibly due to the depletion of readily decomposable material and/ or (especially after September (week 12)) in response to the falling ambient temperature. By week 18, around 11% of the carbon in the seaweed had been metabolised as CO_2. In the pots with the large seaweed pieces the ^{99}Tc activity concentration in the soil solution continued to rise slowly between week eight and 18. By this point in the experiment, just over 3% of that added with the seaweed was present in the soil solution. In the pots with the small seaweed pieces, the ^{99}Tc activity concentration in the soil solution appeared to be lower at week eight compared to week six. This may be due to experimental error or unequal distribution of ^{99}Tc throughout the total soil solution volume giving unusually high values for the previous date or low values for this date. Alternatively it may be due to adsorption of ^{99}Tc to organic material in the soil, thus removing it from the soil solution. At the next sampling date (week 12) the activity concentration had risen again to a level similar to that found at week six and it rose again slightly at week 18. However, there was no significant difference between the activity concentrations, from either sets of treatment pots, between the samples taken at week 8, 12 and 18 suggesting that either ^{99}Tc had stopped being released from the seaweed in significant amounts or equilibrium between ^{99}Tc in solution and that bound to soil particles or organic matter had been reached. As there seems to be little microbial decomposition after week six, leaching once again is likely to be the predominant mechanism for ^{99}Tc release from the seaweed. At the end of the monitoring period (week 18), there is no significant difference between the activity concentration of ^{99}Tc in the soil solution of the two types of treatment pot. This suggests that although the initial rate of ^{99}Tc release

appears to be faster from the small seaweed pieces, over the long term there may be little, if any difference in the rate of release. If this assumption is applied to field conditions it means that from whole seaweed frond, the initial rate of ^{99}Tc release may be slower but in the long term any differences in overall release rates may be minimal.

Monitoring of the CO_2 production between 22 and 48 weeks showed that the production rate remained low, only showing a slight increase in the later dates, most likely as a response to increasing ambient temperature. The production rate however did not rise to anywhere near the levels produced during the earlier phase of the monitoring period. By week 48, the percentage of carbon metabolised as CO_2 had increased to around 15% (only an increase of 4% since week 18). This sustained low level of CO_2 production suggests that most of the readily decomposable material has been utilised and that any microbial activity is due to the specialised autochthonous microbes which increase in the latter stages of decomposition as available resources decrease (Kilham, 1994).

Analysis of the percentage of ^{99}Tc in each compartment (soil matrix, soil solution, seaweed) when the pots were dismantled, 21 months after the seaweed was added to the soil, showed that around 46% of the ^{99}Tc added within the seaweed had accumulated in the soil, just over 11% of which was present in the soil solution. This was lower than the 50% predicted from the results of ^{99}Tc lost from artificially contaminated plant material in previous studies. This may be due to ^{99}Tc being bound differently in the plant cells of environmentally and artificially contaminated seaweed so that more of the ^{99}Tc is released in an insoluble form. The lower percentage in the soil solution may

also be due to the removal of soluble forms from the soil solution due to the reduction of pertechnetate in anaerobic microsites and its subsequent binding to soil particles and organic matter. A small amount of ^{99}Tc may also have been adsorbed or taken up by soil microbes. It has to be taken into consideration that the values obtained were from samples representing less than one percent of the total soil solution volume, therefore there is a large error associated with the calculation of the ^{99}Tc activity concentration in the total soil solution.

Although only 25% by weight of the seaweed remained in the pot, 49% of the initial ^{99}Tc was present within this. The total carbon content (dry weight) of this remaining seaweed was about 50% of that of the seaweed (dry weight) added to the pots The carbohydrates remaining are likely to be structural carbohydrates which are more resistant to decomposition. This could mean that around half the ^{99}Tc in the seaweed is associated with structural components of the seaweed, such as cell walls or chloroplasts, but this seems to contradict previous research which shows that 60-70% of ^{99}Tc is present in a soluble form (most likely in the cytosol) of brown seaweed and unicellular algae (Vandecasteele *et al.,* 1986; Bonotto *et al.*, 1986). It is possible that some of the ^{99}Tc associated with the seaweed is ^{99}Tc that has been released but has subsequently been adsorbed to the exterior of the seaweed after it was converted to its reduced form (Tc (IV)) in anaerobic microsites. In this form ^{99}Tc can form ligands with organic matter (Van Loon *et al.*, 1986a; Stalmans *et al.*, 1986; Bodietti & Garten 1986). As the seaweed is the major component of organic matter in this sandy coastal form, it is most likely the material to which any reduced ^{99}Tc would bind. In the field, this ^{99}Tc could become re-oxidized

under favourable conditions (Stalmans *et al.*, 1986; Nicholson *et al.*, 1990), e.g. if the soil is dug or hoed over, introducing oxygen, making it available once again in a form suitable for plant uptake. As such a large percentage of ^{99}Tc is still associated with the seaweed, and if the seaweed does not fully decompose, before the addition of the next batch the following year, the amount of ^{99}Tc within the system could continue to increase, year after year, despite possible losses through plant uptake or leaching down through the soil profile.

The ^{99}Tc labelled as 'missing' on dismantling the pots could be due to one or more of the following reasons:

(a) Experimental error in the measurement of ^{99}Tc in each compartment

(b) The variability in the distribution of ^{99}Tc in the soil solution or initial seaweed samples

(c) Loss of ^{99}Tc from the pot through the extraction of soil solution samples over the monitoring period.

(d) Loss of ^{99}Tc from the pot into the layer of gravel placed in the base to aid drainage (No water drained through into the containers on which pots were sitting)

The presence of ^{99}Tc in the spinach plants confirms that at least a proportion of ^{99}Tc released from the seaweed into the soil solution, is in a form that is suitable for plant uptake, i.e. pertechnetate (Van Loon, 1986). The summer

cropping spinach showed poor growth, bolting soon after their planting out in the pots. This was due to the high ambient temperature and the poor quality of the soil, as unlike the soils on the garden plots, it was unimproved and contained little organic matter. The winter cropping spinach, because of its slower growth pattern over the colder winter months showed a compact, much improved growth form. After seven months growth between 2 and 6% of the ^{99}Tc added to the pots within the seaweed was found in the spinach plants with the mean activity concentration of the plants being 9.3×10^5 Bq kg^{-1} (dry wt) which equates to 1.86×10^5 Bq kg^{-1} (wet wt), using a dry to wet conversion factor of 0.2 (calculated by weighing plants fresh and after oven drying, this factor also quoted for leafy vegetables in Staven *et al.*, (2003). This is about double the ^{99}Tc activity concentration found in spinach plants on garden plots in Cumbria by Camplin *et al.,* (1999), but allowing for variations between growing seasons this is a fairly close match.

The calculated concentration ratio for spinach uptake of ^{99}Tc is only an approximation, calculated by estimating the total soil (dry weight) activity concentration of ^{99}Tc in the pots prior to planting the winter spinach crop by assuming that the partitioning of the ^{99}Tc between soil matrix and soil solution was identical to that found when the pots were dismantled (i.e. 11% of total soil ^{99}Tc was present in soil solution). This estimation was necessary to compare the calculated value with the standard set by the International Atomic Energy Agency's Handbook of Parameter Values for the Prediction of Radionuclide Transfer in Temperate Environments which is calculated as Bq kg^{-1} dry crop edible product / Bq kg^{-1} dry soil in the upper 20cm (IAEA, 1994). The mean concentration ratios calculated in this experiment, 120 is lower than that found

in the IAEA handbook (95% confidence range 260 – 7800, mean 2600) but the highest value in the range calculated for this experiment (414) is within the IAEA 95% confidence range. The IAEA figures are based on the measurement of uptake from artificially contaminated soil, so the ^{99}Tc may be present in different chemical forms in the soil in this study. The lower values calculated for this experiment may be due to errors in the assumptions used to estimate the total soil concentration as the system is a dynamic one, the activity concentration in the soil solution will vary with time, as it is released from seaweed and taken up by plants or adsorbed onto organic matter, so it is difficult to put a single figure on it. The distribution of ^{99}Tc throughout the soil can affect its uptake into plants. The root system of these plants was poorly established and remained close to the surface of the pots. If the majority of ^{99}Tc has been washed down the soil profile below the rooting zone, it would have been unavailable to the plants. The poor yield from each plant in this study may also be a reason for the low concentration ratios. This is supported by the higher concentration ratios associated with the heavier plants. If the plants were to grow to a harvestable size the concentration ratios might have been closer to those found by the IAEA.

The positive correlation between ^{99}Tc activity concentration per kilogram of plant material and the weight of the plant may be linked to the higher active growth increasing the concentration ratio for the faster growing plants. The relative growth rate of a plant is one of the factors that influence the concentration ratio in the model for soil-to-plant transfer of ^{99}Tc developed by Van Loon (1986). Whilst in the model, relative growth rate is considered to be a parameter characteristic for the plant species, it could also follow that

variations in uptake within a species could also be affected by the relative growth rates of individual plants.

5.5 CONCLUSION

This experiment has confirmed that ^{99}Tc is released from *Fucus vesiculosus* into soil solution over time and that at least some of this ^{99}Tc is in a form that is available for uptake into spinach plants. Whilst the decomposition rate was high at the beginning of the monitoring period due to the warm summer conditions it does not seem to be the major factor in the release of ^{99}Tc from the seaweed. There was no statistical difference between CO_2 production in the pots with the large and those with the small seaweed pieces (therefore no difference in microbial decomposition rate), but the rate of ^{99}Tc release into the soil solution was faster in the pots with the small pieces. This suggests that a factor other than decomposition was influencing ^{99}Tc release from seaweed. The predominant mechanism appears to be leaching with microbial decomposition playing a minor role. Around 30% of the ^{99}Tc contained within the seaweed was released into the soil between July and November and this increased to around 46%, 17 months later. Roughly 11% of the released ^{99}Tc was found in the soil solution. The presence of un-decomposed seaweed, still containing around 50% of the initial ^{99}Tc after 21 months in the soil may be an important issue in the field situation. If new batches of contaminated seaweed are added year after year, the activity concentration of ^{99}Tc in this compartment of the system may continually increase. This could increase the amount of ^{99}Tc available for plant uptake each year.

The ^{99}Tc in the soil solution was readily available for uptake by spinach plants, with an average concentration ratio calculated as 120. This suggests that

some ^{99}Tc is present in the soil solution is in the pertechnetate form. Further investigations are required to determine the percentage of ^{99}Tc in the soil solution that is pertechnetate. These will be detailed in Chapter 7.

As release of ^{99}Tc from *Fucus vesiculosus* into soil seems to occur predominantly by leaching, it is important to investigate this mechanism further. Chapter 6 details an experiment designed to investigate the rate of loss of ^{99}Tc from *Fucus vesiculosus* through leaching.

CHAPTER 6 - EXPERIMENT 3: ESTIMATION OF PERCENTAGE OF ^{99}Tc IN *FUCUS VESICULOSUS* READILY EXTRACTABLE WITH WATER

6.1 INTRODUCTION

6.1.1 Rationale

The results of the first two experiments suggest that the major mechanism responsible for the release of ^{99}Tc from *Fucus vesiculosus* into soil is the abiotic process of leaching. To further investigate the capacity of water to remove ^{99}Tc from seaweed, an experiment was designed to measure the amount of ^{99}Tc that could be extracted from fresh seaweed samples by shaking in water over a period of time. The rate of release of ^{99}Tc could then be compared with the release of other ions, such as potassium, calcium and magnesium.

Two-compartment kinetics has been observed for the loss of ^{99}Tc from artificially contaminated seaweed when soaked in water. The biological half lives for the turnover of technetium from the fast and slow compartments for four species of brown seaweed (not *Fucus vesiculosus*) were around 11-19 hours and 27-75 days respectively (Benco *et al.*, 1986). A further study also reported this pattern with biological half-lives of 1-3 days and 16-196 days for the fast and slow compartments (Beasley & Lorz, 1986). The ^{99}Tc lost through soaking the seaweed in water is likely to be present within the seaweed in a soluble form. Using an extraction method developed by (Bowen *et al.*, 1962), it has been shown that 54% of ^{99}Tc in *Fucus vesiculosus* was in a readily extractable form whilst the remainder was either poorly extractable or remained in the residue (Vandecasteele *et al.*, 1986). They also showed that 60% of the

6. ^{99}Tc could be removed by three successive overnight soakings of chopped seaweed in water.

The mechanisms influencing the loss of chemical elements from plant litter through leaching are poorly understood (Laskowski *et al.*, 1995) but it has been shown that different compounds leach out of the litter at different rates. The rate at which a given compound or ion will leach is dependent on the type of litter, how they are bound within the plant cells and environmental conditions, but in most cases a general order can be observed. The first molecules to be lost include soluble sugars, amino acids and sodium and potassium ions whilst calcium and magnesium tend to lost at a slower rate. The slowest leaching rates have been found with iron, zinc and lead (Mason, 1977).

6.1.2 Aim

The aim of this experiment was to determine the rate of loss of ^{99}Tc through leaching and compare that rate with the loss of other ions and compounds. This position of ^{99}Tc in the general order then may indicate how important the process of leaching is in the release of ^{99}Tc from seaweed.

6.1.3 Hypotheses

The hypothesis that this experiment was designed to test was:

Around 50% of ^{99}Tc present in *Fucus vesiculosus* will be readily extractable with water

6.1.4 Design choice

A shaking experiment was chosen to estimate the readily leachable fraction of ^{99}Tc within the seaweed. As the pots from the first two experiments had been

watered with collected rainwater, it was preferential to use the same water in this experiment. The experiment was carried out in a cold room at 4°C to minimise any microbial metabolism which could have had an effect on the amount of ^{99}Tc transferred to the rainwater through leaching.

6.2 EXPERIMENT

6.2.1 Materials

Fucus vesiculosus was freshly cut in July 2003 from the same site, close to Sellafield, as for the previous experiments (Grid reference NY 018034). Rain water was collected in the same butt in the grounds of Stirling University (Grid reference NS817969), used to water the pots in the first two experiments.

6.2.2 Method

Fifty gram (wet weight) samples of freshly collected seaweed, chopped into 4-6 cm pieces, were placed in three loosely capped plastic bottles, each containing 500 ml of rainwater that had been kept overnight in the refrigerator. The bottles were placed upright on a shaker in a cold room kept at 4°C and gently agitated (50 times per minute).

6.2.3 Sampling regime and sample analyses

At intervals (0.5, 1, 2, 4 and 6 days), all the water was removed from the bottles and replaced with the same volume of fresh rainwater stored at 4°C in a refrigerator. The water samples were stored in the freezer for future analysis. Sample of fresh seaweed and initial rainwater were also retained for analysis.

Seaweed and water sub-samples were analysed for ^{99}Tc using the same method as in previous experiments (See Section 3.4.4). The samples were also analysed for soluble sugars and the ions, calcium (Ca), magnesium (Mg),

potassium (K) and sodium (Na) using the methods detailed in Sections 3.4.5and 3.4.7. The analyses were carried out in triplicate for each date. In addition, seaweed samples retained when the experimental pots from Experiment 1 were dismantled at each sampling date were analysed for the same ions. These samples had been collected at 5, 12 days, 3, 8 12 and 15 weeks.

6.2.4 Statistical analysis

Statistical analysis was carried out in Minitab (Minitab 2000). The cumulative ^{99}Tc and soluble sugar data was analysed using a one-way ANOVA with 'sample date' as the factor. The data was further analysed using a Tukey's pairwise comparison (Family error rate 0.05) between sample dates. ANOVA was carried out on percentage of initial ion concentration, using a General Linear Model (GLM), with 'sampling date' and 'ion type' as fixed factors and 'sample' as a random factor. The data was further analysed using a Tukey's pairwise comparison (Family error rate 0.05) between sampling dates and the different ions. The assumptions for the ANOVA and GLM were checked by drawing a normality plot of the residuals and plotting the residuals against the fitted values.

6.3 RESULTS

6.3.1 ^{99}Tc analysis

The mean activity concentration of ^{99}Tc in the fresh seaweed samples was 9.9 x 10^4 Bq l^{-1} (SD = 2.95 x 10^4). Analysis of the initial rainwater samples confirmed that they did not contain ^{99}Tc. The mean ^{99}Tc activity concentrations in the rainwater samples retained after agitation with seaweed are shown in Figure 6:1.

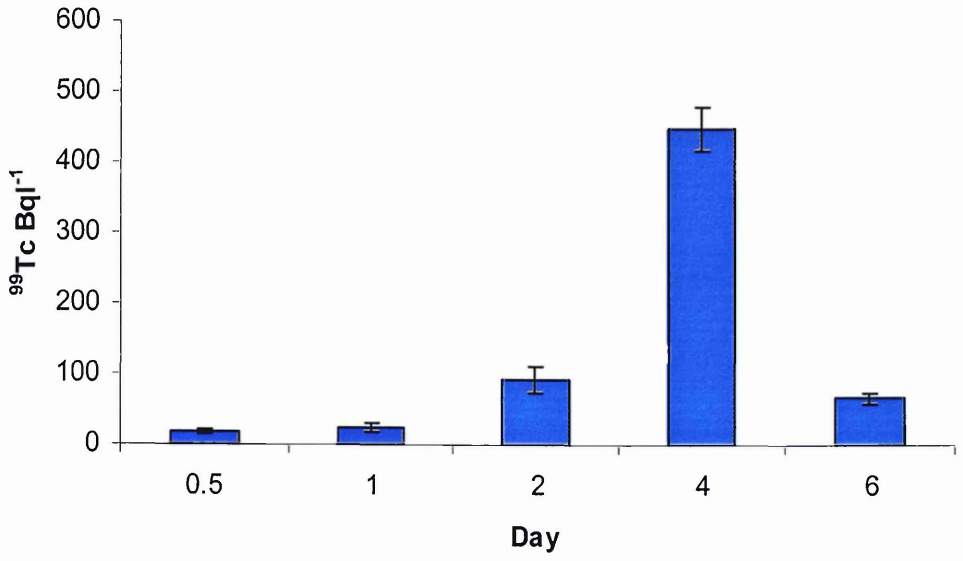


Figure 6:1: Tc-99 activity concentration (Bq l^{-1}) in rainwater samples agitated with seaweed over time (n=3, Error bars = 1SD).

These results show that ^{99}Tc can be rapidly leached from the seaweed by shaking it in water and shows that the majority (69% of that removed) of the ^{99}Tc was leached from the seaweed between day two and four. The ^{99}Tc lost from the seaweed through leaching was calculated as a percentage of that contained within the fresh seaweed samples and plotted as the cumulative loss over time (See Figure 6:2).

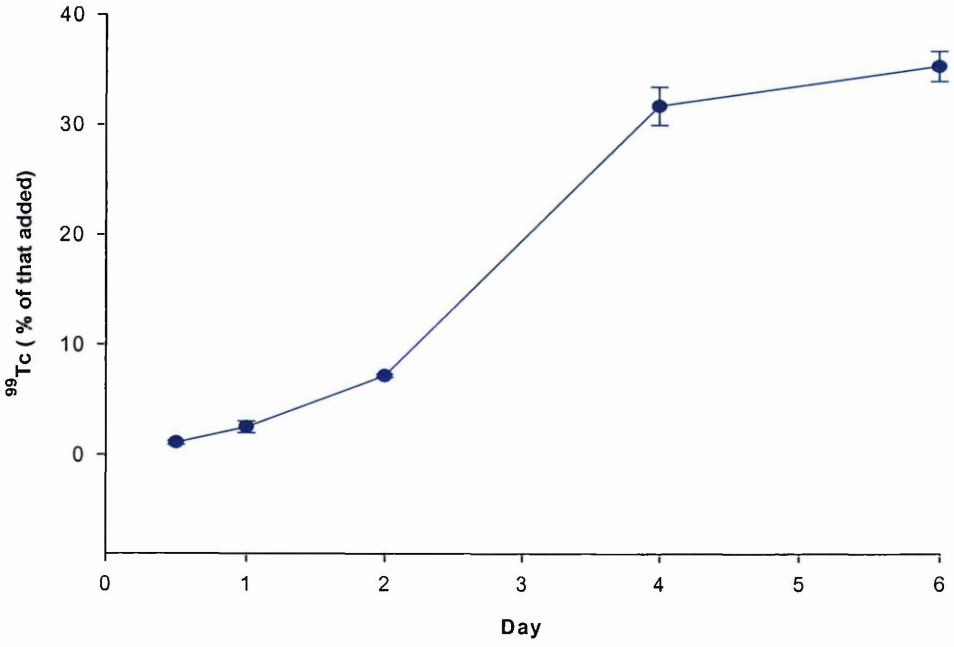


Figure 6:2: Tc-99 lost from seaweed through leaching (cumulative percentage of that present in seaweed (n=3, Error bars = 1SD)

The graph shows that the initial loss of ^{99}Tc is slow over the first two days (around 7%). This period is followed by a large increase in ^{99}Tc lost through leaching by day four. By this point around 32% of the ^{99}Tc had leached from the seaweed into the rainwater. The rate of leaching then slowed again so that at day six, the end point of the experiment, the amount of ^{99}Tc leached had risen another 4% to just under 36%. Statistical analysis confirmed that there was a significant effect of time ($\rho < 0.005$). The results from the Tukey's pairwise comparison is shown in Table 6:1.

Table 6:1: P-values for Tukey's pairwise comparison between sampling days for cumulative ^{99}Tc activity concentration (figures in bold = significant difference, For confidence intervals see Appendix 2)

Day	0.5	1	2	4
1	0.473			
2	**0.000**	**0.002**		
4	**0.000**	**0.000**	**0.000**	
6	**0.000**	**0.000**	**0.000**	**0.009**

The results show that after the first 24 hours the ^{99}Tc activity concentrations were significantly higher on each subsequent sampling date.

6.3.2 Soluble sugar analyses

The rainwater samples contained minimal amounts of soluble carbohydrate. The concentration of soluble sugars in the water samples from the shaking experiment are given in Figure 6:3 (Measured values minus rainwater values).

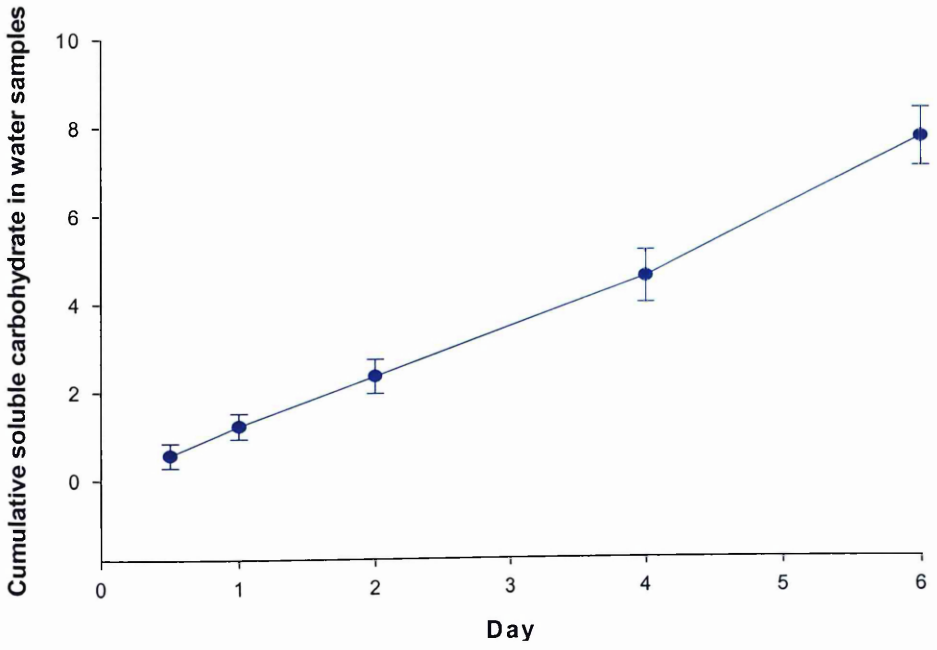


Figure 6:3: Cumulative concentration of soluble sugars in water samples (mg l^{-1}) over time (n=3)

The rate of release remains steady over the first two days then increases over the following four days. This suggests that the main release of soluble sugars begins between days two and four and they continue to be released up to day six. Statistical analysis confirmed that there was a significant effect of time ($\rho < 0.001$). The results from the Tukey's pairwise analysis (Table 6:2) show that the soluble sugar concentrations in the earlier dates were not significantly different from each other but the later dates were significantly higher.

Table 6:2: P-values for Tukey's pairwise comaprison between sampling days for cumulative soluble sugar concentration (Figures in bold = significant difference, For confidence intervals see Appendix 2)

Day	0.5	1	2	4
0.5				
1	0.4312			
2	**0.0051**	0.0771		
4	**0.0000**	**0.0000**	**0.0009**	
6	**0.0000**	**0.0000**	**0.0000**	**0.0001**

6.3.3 Metal ion analyses

6.3.3.1 Reference material

The concentration of Ca, Mg, K and Na present in the reference material (SRM 1515) are given in Table 6:3.

Table 6:3: Concentration of metal ions in reference material samples (SRM 1515) and certified concentrations (mg g^{-1})

Metal ion	Concentration mg g^{-1}	Certified concentration mg g^{-1}
Calcium	8.47	15.26 ± 0.15
Magnesium	1.85	2.71 ± 0.08
Potassium	16.07	16.1 ± 0.11
Sodium	0.02	0.024 ± 0.001

6.3.3.2 *Initial seaweed samples*

The concentration of Ca, Mg, K and Na present in the seaweed samples at the beginning of the experiment are given in Table 6:4.

Table 6:4: Metal ion concentration in seaweed samples at beginning of shaking experiment (mg g^{-1}) n=3

Metal ion	Concentration mg g^{-1}	1 SD
Calcium	8.17	0.44
Magnesium	5.83	0.48
Potassium	33.36	7.64
Sodium	38.11	2.77

6.3.3.3 *Water samples*

The concentration of the ions present in the water samples collected on each of the sampling dates was calculated as a percentage of that present in the seaweed added to each bottle. The results were plotted, along with the ^{99}Tc data, to show the percentage remaining within the seaweed over the time period (Figure 6:4).

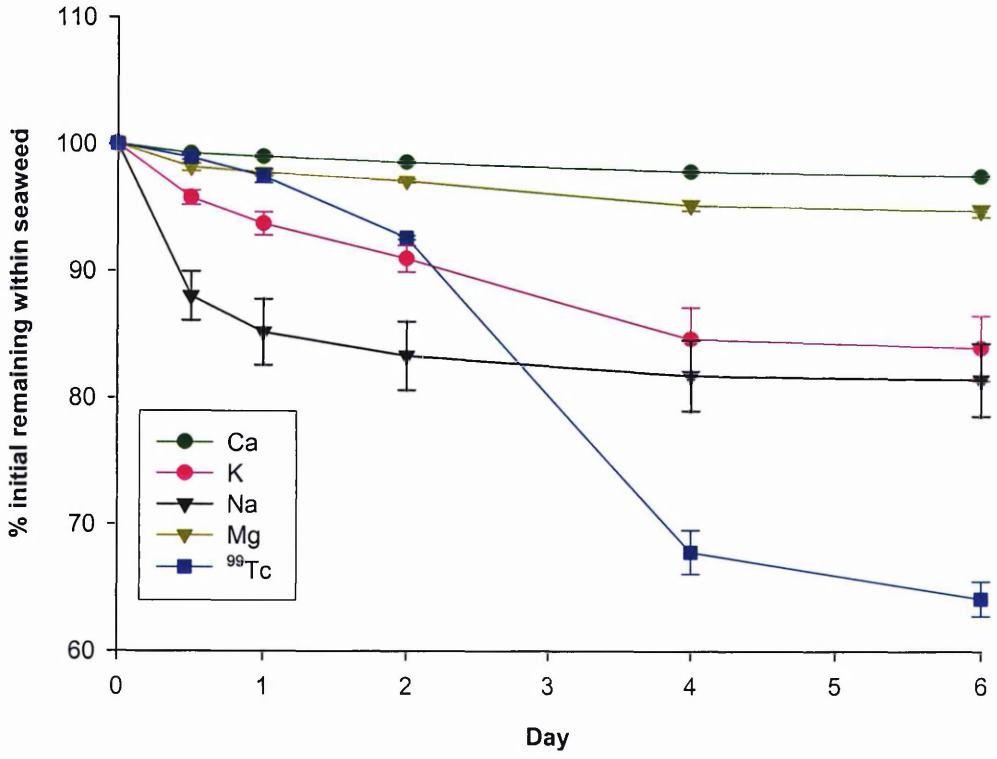


Figure 6:4: Temporal change in metal ion and ^{99}Tc concentration in seaweed after shaking in water (% initial) (n=3, error bars = 1SD)

Over the first 24-hrs sodium appears to be lost at the most rapid rate, followed by potassium. There is little or no loss of calcium, magnesium or ^{99}Tc over this period. Between days two and four there is rapid leaching of ^{99}Tc from the seaweed, but little further loss of the other ions. By the end point of the experiment (day six), the percentage losses from the seaweed of calcium, magnesium, potassium, sodium and ^{99}Tc were 2.1%, 4.9% 15.8%, 18.3% and 36% respectively. Statistical analysis showed that there was a significant interaction of ion type and sampling date (ρ <0.01) and also ion type and sample (ρ <0.01). Comparisons showed no significant effect of sampling date in the calcium and magnesium values (all ρ>0.05), suggesting very slow release of these ions from the seaweed. The results for sodium and

potassium are shown in Table 6:5. The yellow cells indicate that the value for the sampling date in the left hand column is significantly lower than that for the date in the top row.

Table 6:5: P values for Tukey's Pairwise Comparison between sampling dates for potassium (a) and sodium (b) (Figures in bold = significant difference, For full statistical data see Appendix 2)

Day	0.5	1	2	4
1	0.5830			
2	**0.0002**	0.1515		
4	**0.0001**	**0.0001**	**0.0001**	
6	**0.0001**	**0.0001**	**0.0001**	1.0000

(a) Potassium

Day	0.5	1	2	4
1	0.0938			
2	**0.0003**	0.7750		
4	**0.0001**	**0.0269**	0.9578	
6	**0.0001**	**0.0105**	0.8228	1.0000

(b) Sodium

The results show that there was no significant difference between the first two sampling dates but the values from the later dates were significantly lower than the earlier dates. There is no significant difference between the later dates.

Comparisons between the values for each ion on each sampling date showed that sodium values were statistically lower (thus the rate of release is faster) than calcium and magnesium on all sampling dates, whilst those for potassium were lower than calcium on all dates and lower than magnesium on all dates except for day 0.5. Sodium values were lower than potassium levels over the first three sampling dates but there was no difference over the

later dates. This suggests that the rate of release for sodium is initially faster but at the end of the period the rates become similar (For statistical tables – See appendix 2).

The results from the Tukey's Pairwise Comparisons between ^{99}Tc and the other ions on each sampling date are given in Table 6:6. The blue cells indicate that the values for ^{99}Tc are significantly higher than those for the ion in the top row, whilst yellow cells indicate that the value for ^{99}Tc is lower.

Table 6:6: P- values for Tukey's pairwise comparison between ^{99}Tc and other ions on all sampling dates. (Figures in bold = significant difference, For full statistical data see Appendix 2)

Day	K	Na	Mg	Ca
0.5	**0.0151**	**0.0001**	1.0000	1.0000
1	**0.0015**	**0.0001**	1.0000	0.8876
2	0.8019	**0.0001**	**0.0001**	**0.0001**
4	**0.0001**	**0.0001**	**0.0001**	**0.0001**
6	**0.0001**	**0.0001**	**0.0001**	**0.0001**

These results suggest that over the first 24 to 48-hrs the rate of ^{99}Tc release is slower than that of sodium or potassium but after that period the rate of ^{99}Tc release increases and becomes faster than that for all other ions including potassium and sodium.

6.3.3.4 Seaweed samples from Experiment 1

The concentration of Ca, Mg, K and Na in the seaweed added to the pots at the beginning of Experiment 1 are given in Table 6:7.

Table 6:7: Metal ion concentration in seaweed added to pots at beginning of Experiment 1 (mg g^{-1}) n=3

Metal ion	Concentration mg g^{-1}	1 SD
Calcium	8.22	0.47
Magnesium	5.22	0.07
Potassium	35.44	6.65
Sodium	30.41	1.70

The metal ion concentration of the seaweed at each sampling date was calculated as a percentage of the seaweed added to the pots at the beginning of the experiment. The results were plotted to show the temporal change in concentration (Figure 6:5).

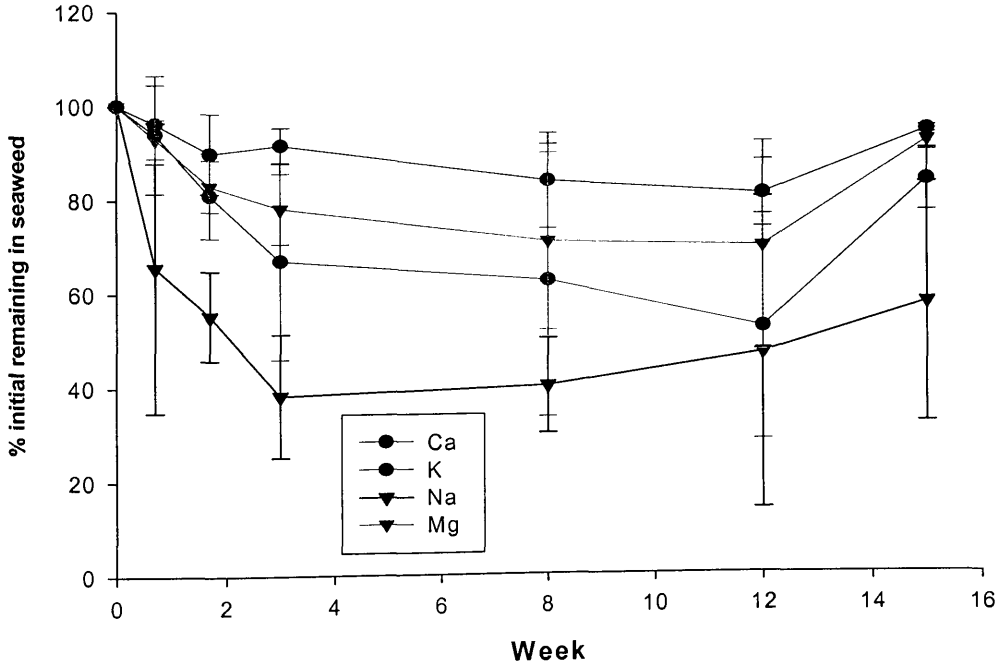


Figure 6:5 : Temporal change in metal ion concentration in seaweed in Experiment 1(% original) (n=3, error bars =1 SD)

The graph shows a similar pattern of ion loss as Figure 6:4, with sodium appearing to leach most rapidly, followed by potassium, magnesium then calcium. The size of the error bars shows that at each sampling date there was a wide variation in the values obtained. Statistical analysis showed that there was no significant effect of the interaction of sampling date and ion type ($\rho = 0.848$) or of sample and ion type ($\rho = 0.239$). Examination of the individual factors showed that there was no significant effect of sampling date ($\rho = 0.168$) but there was a significant effect of ion type ($\rho = 0.001$). The significant effect of ion type arises because, globally there is the overall loss pattern of Na > K > Mg > Ca (confirmed by the main effects plot). Tukeys pairwise comparisons showed that the values for sodium at the week three and four sampling dates were significantly lower than those for calcium and that at week three was also significantly lower than for magnesium. There is no significant difference between all other ions at all dates (For full results of statistical analysis, see appendix 2). A clear pattern for the loss of each ion through leaching over this time period from the seaweed cannot be identified.

6.4 DISCUSSION

Around 36% of the ^{99}Tc within *Fucus vesiculosus* was extracted with water over a six-day period. This is similar to a previous study of the loss from other brown seaweed species (*Sargassum vulgare*, *Cystoseira complexa*, *Dictyota dichotoma implexa* & *Dictopteris membranacea*) which found that around 30% of ^{99}Tc was lost over a seven day period (Benco *et al.*, 1986). It is, however, less than that observed in a study of *Fucus vesiculosus*, which found that 54% was in a readily extractable form and that three overnight soakings of chopped seaweed with water removed around 60% of the ^{99}Tc (Vandecasteele *et al.*,

1986). This may be due to the seaweed in that experiment being artificially contaminated, thus the ^{99}Tc may not be bound within the cells of the seaweed in the same manner as in the environmentally contaminated plants and/ or the seaweed being chopped into smaller pieces thus aiding the leaching process.

The majority of the ^{99}Tc was removed from the seaweed between days two and four. This is a similar time scale to the 'fast compartment' fraction of ^{99}Tc, which was reported as having a biological half-life of one to three days (Beasley & Lorz, 1986). This fraction of the ^{99}Tc within the seaweed is likely to be present within the plant in a soluble form, most likely within the cell cytosol or within the sieve tubes, which are the specialised cells which transport compounds throughout the seaweed fronds (Lobban & Harrison, 1994). Although there has been no study which confirms that ^{99}Tc is transported throughout the seaweed frond in this manner, it has been shown that it is concentrated in the young rapidly growing parts of the plants (Benco *et al.*, 1986; Beasley & Lorz, 1986), indicating that there must be some movement of ^{99}Tc within the frond. In addition it is thought that ^{99}Tc is transported in vascular plants as soluble pertechnetate in the xylem (specialised transport vessels) (Cataldo *et al.*, 1986) and it is reasonable to assume that a similar method of transport exists in seaweed.

In the shaking experiment, the rate at which ^{99}Tc is lost from the seaweed through leaching, whilst slower than sodium and potassium over the initial 24-hrs, was the fastest over the six day period. The initial loss of sodium and potassium may be due to the washing off of salt from the exterior of the fresh seaweed plants as the plants were not rinsed prior to the addition to the bottles.

The total loss of ^{99}Tc over the six-day period was around twice that of sodium and potassium, which are known to be among the first elements lost through leaching from plant material (Mason, 1977; Wagner & Wolfe, 1998). The timing of the majority of the ^{99}Tc loss is similar to that of the soluble sugars (between 2 and 6 days), most of which are present in the cell cytosol of the plants. This suggests that a considerable proportion of the ^{99}Tc is present in the seaweed in a soluble form and that it can be easily removed from the plant by leaching. The patterns of ion loss in the samples retained from Experiment 1, whilst similar to those seen in the shaking experiment, show too large a variation between the replicates on each date to provide a conclusive result. The large variation is due to the 18 samples from Experiment 1 coming from individual pots, whilst the 15 samples from the shaking experiment came from repeated measurements from three bottles. The results show that the shaking experiment gives a clearer measurement of the leaching rate.

6.5 CONCLUSION

Around 36% of the ^{99}Tc in Fucus vesiculosus can be leached out when the seaweed is shaken in water for six days. The majority of this was lost from the seaweed between days two and four, which is similar to the time period when soluble sugars are lost. Whilst the rate of ^{99}Tc lost is slower than sodium and potassium over the initial 24-hours, the total loss of ^{99}Tc was around twice that of these ions. These results suggest that a considerable proportion of ^{99}Tc is present in the seaweed in a soluble form, possibly in the cell cystol that can be easily removed by water.

CHAPTER 7 - INVESTIGATION OF THE SPECIATION OF ^{99}Tc IN SOIL SOLUTION BY GEL CHROMATOGRAPHY

7.1 INTRODUCTION

7.1.1 Rationale

The research programme so far supports the conclusion that leaching is a major mechanism for the release of ^{99}Tc from *Fucus vesiculosus* into soil. As leaching is the removal of soluble compounds (Mason, 1977), the leached fraction of ^{99}Tc is likely to be pertechnetate ions or complexes with small molecular weight ligands (Wilgung *et al.*, 1977; Bondietti & Garten, 1986). Tc-99 incorporated into wheat leaves is readily extractable, mobile and as bioavailable to plants as pertechnetate added in solution (Echevarria *et al.*, 1997). Experiment 2 in this study has confirmed that at least some of the ^{99}Tc released from seaweed is present in the soil solution in a form that is available for plant uptake. As plants take up ^{99}Tc as pertechnetate (Wildung et al., 1977; Routsen & Cataldo, 1978; Cataldo *et al.*, 1986; Garten *et al.*, 1986; Lembrechts, 1986; Van Loon, 1986; Van Loon *et al.*, 1986b; Vandecasteele *et al.*, 1986; Cataldo *et al.*, 1987; Echevarria *et al.*, 1997), and to a much lesser extent, as small Tc-X complexes (Van Loon *et al.*, 1989), some of the ^{99}Tc released from the seaweed is likely to be present within the soil solution as pertechnetate.

7.1.2 Aim

This investigation was carried out to try to identify the species of ^{99}Tc present within the soil solution to confirm the presence of pertechnetate.

7.1.3 Hypothesis

The hypothesis this experiment was designed to test was:

7. Tc-99 released into soil solution will be in the pertechnetate form

7.1.4 Design choice

The method chosen to identify the species of ^{99}Tc within the soil solution in this investigation was gel chromatography. This method had been successfully used in previous studies (Lembrechts, 1986; Van Loon 1986) to separate and identify species of ^{99}Tc in soil, soil solution and plant material. However, these studies were carried out on artificially contaminated soil or plant material and the level of contamination was on average 20-fold higher than that obtained in this experiment using environmental samples. The aim was to try to identify the species of ^{99}Tc present in soil solution samples collected during Experiment 2.

7.2 PRINCIPLES OF GEL CHROMATOGRAPHY

Gel chromatography is a process which involves separating molecules based upon their size. The method involves passing the solution containing the molecules to be separated through a column packed with a gel. The gel comprises a three-dimensional network of gel grains (often cross-linked polymers) suspended in a solute that is compatible with the solution to be separated. The principle of the separation of the different sized molecules is shown in Figure 7:1.

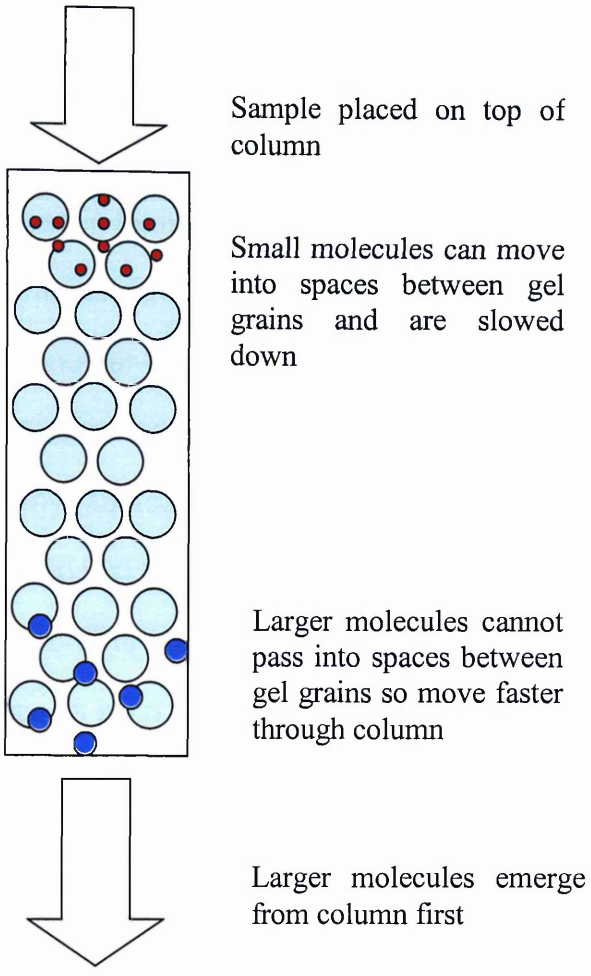


Figure 7:1: Principles of gel chromatography (adapted from Fischer, 1980)

The speed at which the molecules pass through the column is proportional to the amount of time spent in the spaces between the gel grains, thus small molecules which can penetrate the spaces travel at a slower speed than larger ones which can not. There are two main types of separation possible using gel chromatography. Fractionation is the separation of molecules into a range of sizes whilst group separation is a more crude separation of the molecules into two groups, high and low molecular weight molecules (Fischer, 1980).

There are numerous types of gel used in this type of chromatography. These gels are inert, chemically stable and have no electrical charge (Fischer, 1980). The gel column can be described in terms of a number of properties including bed height, i.e the length of the column of gel within the tube, and total bed volume (V_t) (Fischer, 1980). Another important variable is the separation or fractionation range which determines what size of molecules can be successfully separated by any particular gel e.g. Superdex 200 has a separation range of 10,000 – 600,000 M whilst Superdex peptide 10/30 has a separation range of 100 – 7000. The former is useful for clear separation of larger molecular weight molecules such as proteins, whilst the latter is better for smaller molecular weight molecules such as peptides. The solution is washed through the column using an eluant which is a solution that is similar to the liquid between the gel grains. This flow is maintained using a mechanical pump which provides a steady flow rate through the column. The solution passing through the column is collected in fractions of a predetermined size. The fractions can be analysed for conductivity, absorbance at various wavelengths and for the concentration of various substances e.g. proteins, ^{99}Tc. Thus, data is used to plot the concentration of the substance against the volume of eluant that has passed through the column, creating an elution curve for the particular substance. From this curve the elution volume, i.e. the average volume of eluant required to carry the molecules through the column, can be calculated. This volume can be used to characterize a substance (Fischer, 1980). Often instead of using the elution volume (V_e) directly, the variable V_e / V_t is calculated as it decreases

the influence that the geometry of the bed and the way the column is packed have on the results.

7.3 METHOD

Various types of gel columns were used to try to separate and identify the species of ^{99}Tc present in soil solution samples collected during Experiment 2. Section 7.3.1 gives the details of the columns used in the trials and 7.3.2 outlines the basic procedures used.

7.3.1 Columns

Pre-packed columns, supplied by Amersham were used. In addition one column was packed with gel in the laboratory. The properties of the columns are shown in Table 7:1.

Table 7:1: Properties of columns used in trials

	Superdex 200 HR 10/30	**Superose 6 HR 10/30**	**Superdex peptide HR 10/30**	**Sephadex G-25M**	**Sephadex G-25M**
Prepacked	Yes	Yes	Yes	No	Yes
Bed Height	300-310mm	300 -310 mm	300–310 mm	370 mm	50 mm
Bed Volume	24 ml	24 ml	24 ml	29 ml	8.8 ml
Separation range	10,000 – 600,000 M	5000 – 5 x 10^6 M	100 – 7000 M	1000 – 5000 M	1000 – 5000 M

7.3.2 Procedure

The first three columns were run using an ACTA FPLC (Amersham) operated Unicorn v 4.0 computer package (Amersham). This system collected one-ml fractions and measured the conductivity in each one to detect the presence of ions and the absorbance at 275 nm to detect proteins and/or 214 nm to detect

peptides. These measurements were recorded and a chromatogram of the conductivity and absorbance against fraction number was drawn. The flow rate was set at 0.5 ml min^{-1} for the Superdex 200 and Superose 6 columns and 0.25 ml min^{-1} for the Superdex peptide column. The eluent was 10mM sodium phosphate (NaPi) or 10mM NaPi + 150 mM NaCl, both pH 7.5. One ml samples were added to the top surface of the gel and a total of around 35, one ml fractions were collected.

The sephadex G-25 columns were run on a system comprising a Gilson miniplus 3 pump and a Pharmacia LKB Redifrac fraction collector as shown in Figure 7:2.

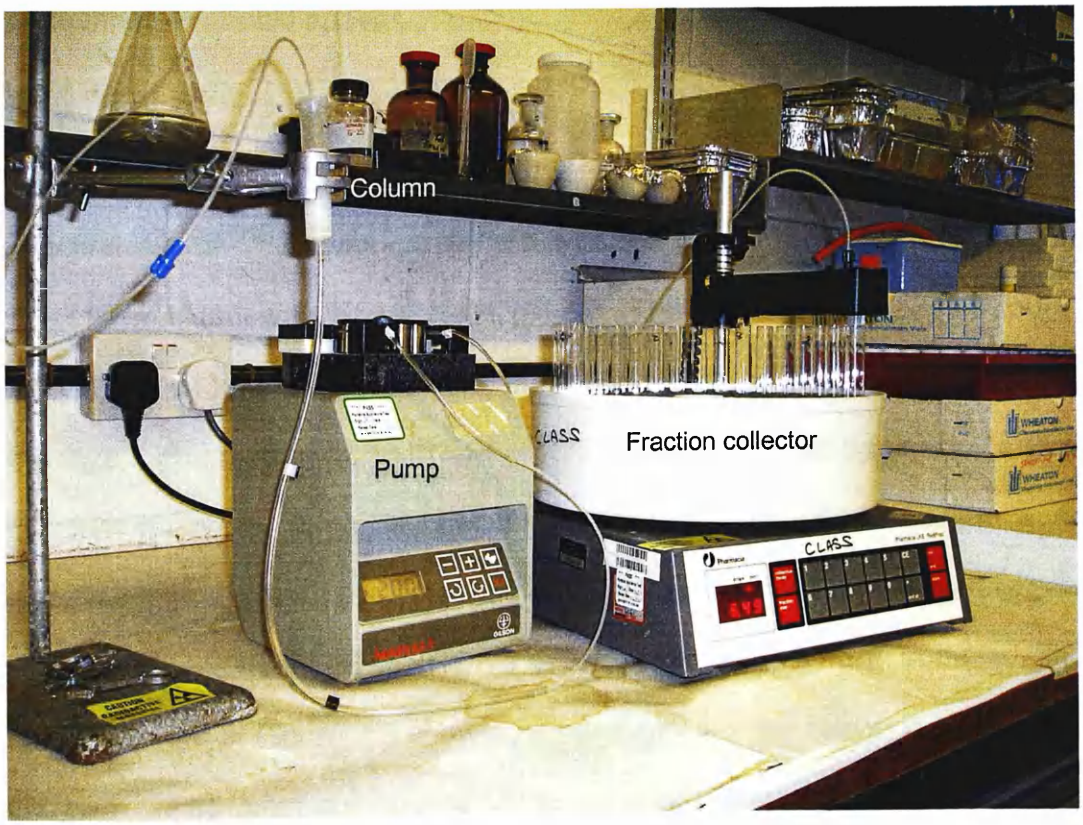


Figure 7:2: Equipment for gel chromatography

The flow rate was set at 12 ml per hour and a one-ml sample was added to the surface of the gel. The eluent was 10 mM NaPi + 150mM NaCl, pH 7.5. A total of around 15, two-ml fractions were collected. The conductivity of the fractions was measured using a Model P335 conductivity meter (Portland Electronics LTD) and the absorbance at 275 and 214 measured using an Ultraspec 2100 *pro* spectrophotometer.

For each column, to determine which fraction(s) pertechnetate would appear in, the ^{99m}Tc tracer used for ^{99}Tc analysis was passed through the column. The tracer solution consisted of ^{99m}Tc as sodium pertechnetate, so its position in the sequence of fractions could be used to predict where ^{99} Tc as pertechnetate should appear.

7.4 TRIALS

7.4.1 Superdex 200

The first run of ^{99m}Tc was carried out with the conditions set as detailed in 7.3.2. The eluant was 10 mM NaPi, pH 7.5. The ^{99m}Tc failed to pass through the column so after washing the column out a further sample was applied and the eluant changed to 10 nM NaPi + 150 nM NaCl. It has been shown that the movement of some technetium compounds through gel columns is dependent on the NaCl concentration of the eluant, with higher concentrations producing better separation (Harms *et al.*, 1996). This run produced the chromatogram in Figure 7:3 showing a conductivity peak followed by a small UV peak. The main component of the conductivity peak is likely to be the NaCl added to prevent the ^{99m}Tc interacting with the gel.

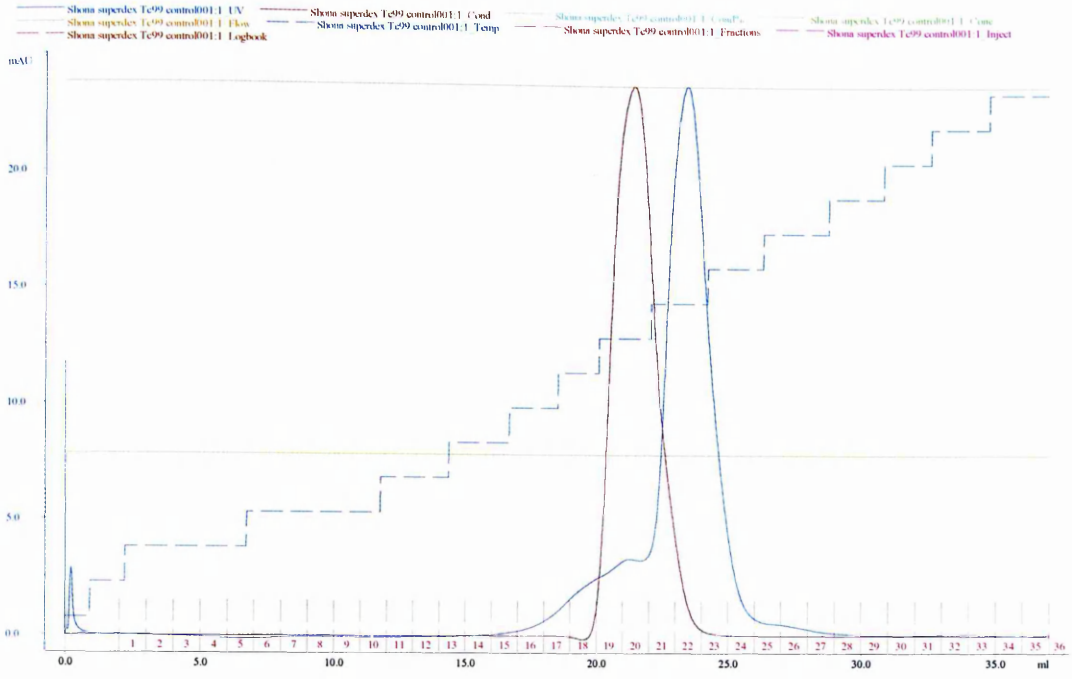


Figure 7:3: Chromatogram of conductivity and absorbance at 275 nm for ^{99m}Tc on Superdex 200 (Blue peak = UV 275 nm; brown peak = conductivity)

The ^{99m}Tc activity concentration in the fractions was measured and calculated as a percentage of the total (Figure 7:4).

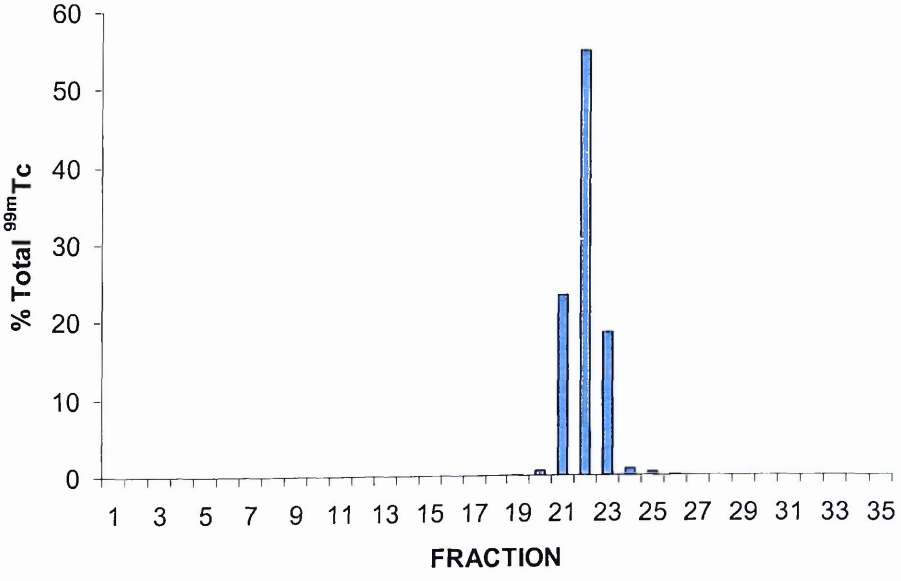


Figure 7:4: Percentage of total ^{99m}Tc in fractions from Superdex 200 column

Fifty eight percent of the ^{99}Tc appeared in fraction 22, with 23% and 18% appearing in fractions 21 and 23 respectively. This was associated with the UV peak in Figure 7:3. However, the size of the 'peak' is actually very small (25 mAU) so the ^{99m}Tc is unlikely to be associated with protein. This may be a contaminant in the column, as the column was also being used in the department for the separation of other materials. The V_e/V_t for pertechnetate can be calculated as 0.92.

A soil solution sample taken one month after seaweed was added to the soil was applied to the column and the sample run under the same conditions as for the second run of ^{99m}Tc. This provided the chromatogram shown in Figure 7:5. The chromatogram shows a small protein peak followed by a conductivity peak. Due to the long procedure for ^{99}Tc analysis it was impossible to analyse every individual fraction so they were bulked together to form five samples for ^{99}Tc analyses as detailed in Table 7:2.

Table 7:2: Soil solution sample fractions analysed for ^{99}Tc

Sample	Fractions	Associated features on trace
1	1-7	Before UV or conductivity peaks
2	8-13	Before UV or conductivity peaks
3	14-18	UV peak
4	19-24	Conductivity peak
5	25-35	Post UV and conductivity peaks

The results from the ^{99}Tc analysis are shown in Figure 7:6.

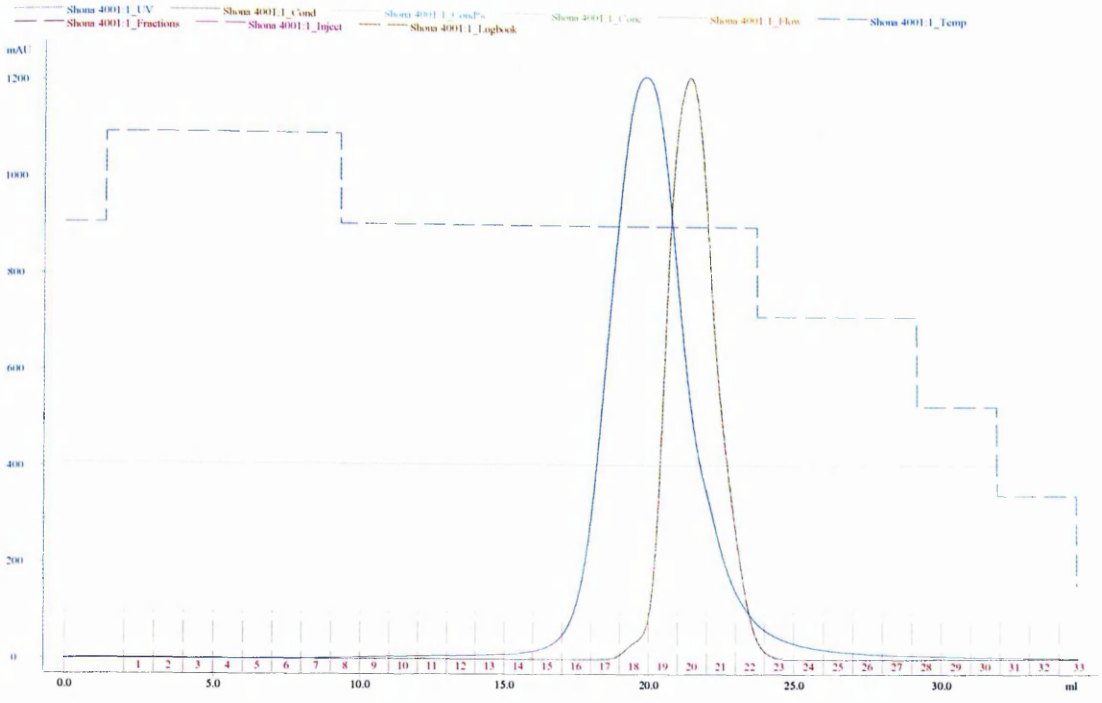


Figure 7:5: Chromatogram for soil solution sample on Superdex 200 (Blue peak = UV 275 nm; brown peak = conductivity)

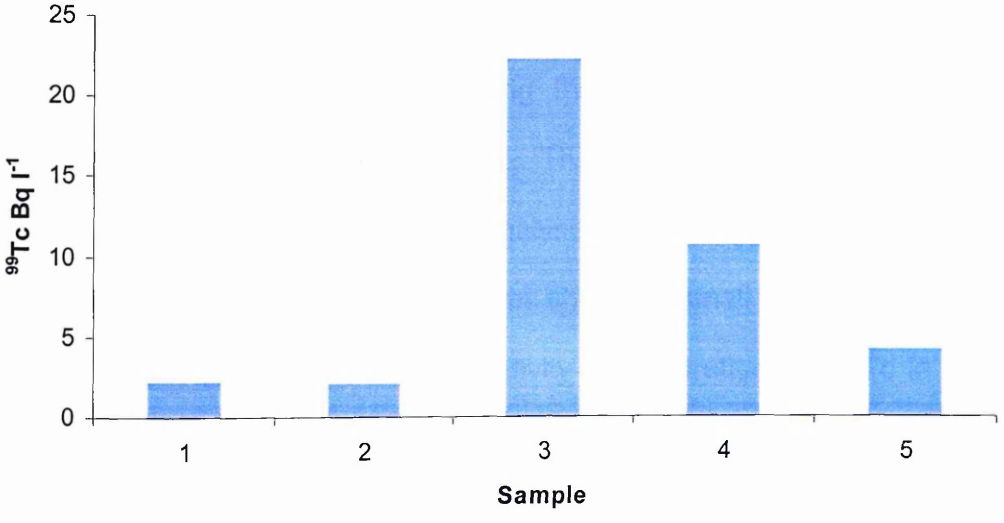


Figure 7:6: Tc-99 activity concentration in collective samples from fractions from soil solution using Superdex 200.

The largest activity concentration (about 54% of total) was found in sample 3 which was made up of fractions 14 -18 and is associated with the UV peak.

Around 26% of the total was found in sample 4 which is associated with the conductivity peak. The V_e/V_t variables for each sample are 0.66 (Sample 3) and 0.90 (Sample 4). The value for sample 4 is comparable with that calculated for pertechnetate. The presence of the UV peak in relation to sample 3 suggests that some proteins may be present but the peak is very low (1200 mAU). The absorbance at 214 nm was measured for the fractions associated with the UV 275 peak and that suggested that the solution was rich in peptides.

A sub-sample of sample 3 was sent for mass spectroscopy to determine the nature of the molecules it contained. However the analysis was unsuccessful as the concentration of the molecules in the sample was too low.

7.4.2 Superose 6

The second trial was carried out using the Superose 6 column. The conditions were set as described in 7.3.2. The eluant was 10 nm NaPi. The ^{99m}Tc tracer was the first sample to be analysed producing the chromatogram shown in Figure 7:7. The chromatogram shows a small UV peak (quickly followed by a conductivity peak. The activity concentration of ^{99m}Tc in each fraction was measured and plotted as a percentage of the total (Figure 7:8). This shows that the largest percentage (51%) was found in fraction 20, with 22% and 17% in fractions 19 and 21 respectively. These fractions are associated with the conductivity peak. The V_e/V_t for pertechnetate was calculated to be 0.83.

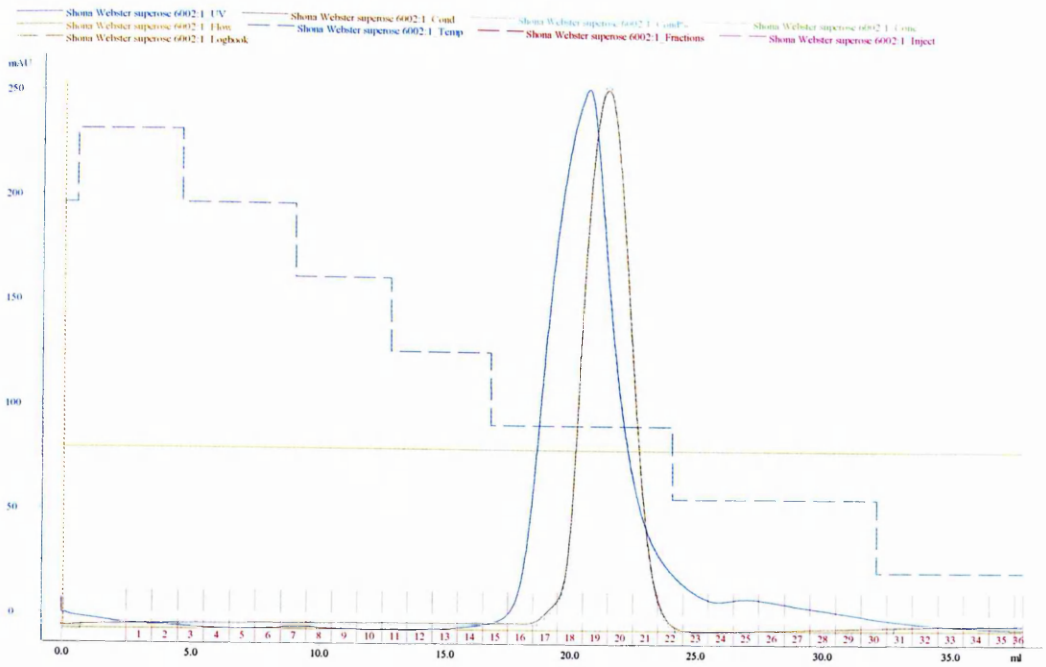


Figure 7:7: Chromatogram for ^{99m}Tc on Superose 6 (Blue peak = UV 275 nm; brown peak = conductivity)

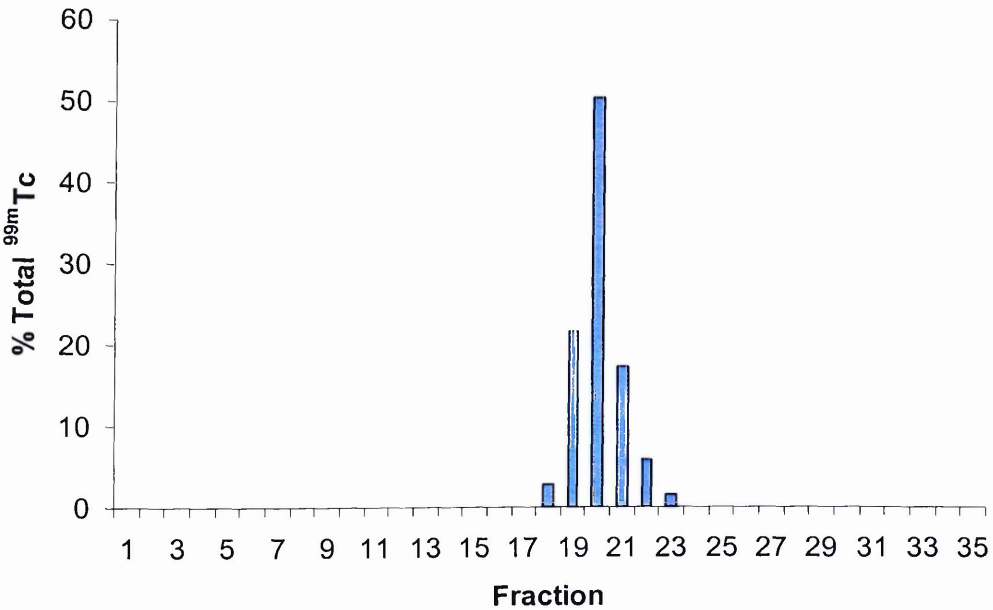


Figure 7:8: Percentage of ^{99m}Tc in individual fractions from Superose 6 column

A soil solution sample collected one month after seaweed addition to the soil was passed through the column. This produced the chromatogram shown in

Figure 7:9. As it was not possible to analyse fractions individually for ^{99}Tc they were bulked into five samples for analysis (Table 7:3).

Table 7:3: Soil solution fractions analysed for ^{99}Tc from Superose 6 column

Sample	Fractions	Associated features on trace
1	1-7	Before UV or conductivity peaks
2	8-16	Before UV or conductivity peaks
3	17-19	UV peak
4	20-23	Conductivity peak
5	24-30	Post UV and conductivity peaks
6	31-35	Post UV and conductivity peaks

The results from the ^{99} Tc analyses are shown in Figure 7:10. The largest activity concentration (about 40% of total) was found in Sample 4 (Fractions 20-23) which is associated with the conductivity peak. Around 38% was found in Sample 3 (Fractions 17-19) which is associated with the UV 275 nm peak. These two samples also cover the fractions in which pertechnetate ions in the ^{99m}Tc tracer were found. Once again the height of the protein peak is actually very small (1400 mAu) so there are unlikely to be noteworthy amounts of protein present. The approximate V_e/V_t variables for each of the samples were calculated to be 0.75 (Sample 3) and 0.90 (Sample 4).

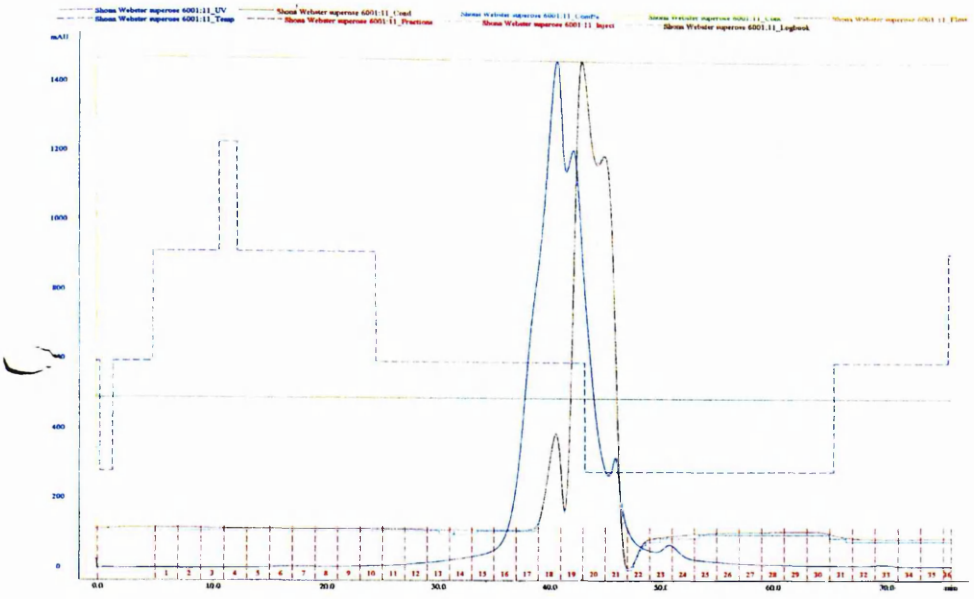


Figure 7:9: Chromatogram for soil solution sample on Superose 6 (Blue peak = UV 275; brown peak = conductivity)

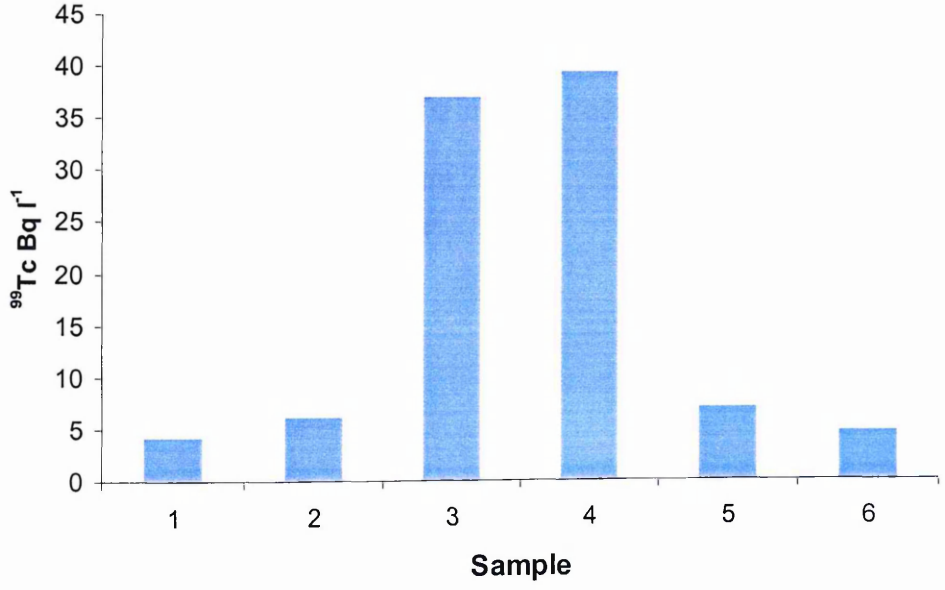


Figure 7:10: Tc-99 activity concentration in collective samples from fractions of soil solution using Superose 6

7.4.3 Superdex peptide

The Superdex 200 and Superose 6 columns did not provide clear definition between the UV and conductivity peaks on the chromatograms. This was due to the separation range for the gel in these columns (usually used for separating proteins) being two wide for the size of the compounds present in the soil solution. To try to separate the compounds further a gel was used with a lower and narrower separation range (used to separate peptides).

First the ^{99m}Tc tracer was run through the column with the conditions set as detailed in 7.3.2. The eluent was 10 nM NaPi + 150 nM NaCl. The chromatogram is shown in Figure 7:11. The protein peak (UV 275) is again very low (around 15 mAU) with the peptide peak slightly higher 20 mAU). The conductivity peak is again likely to be mostly the NaCl added to the eluant. The activity concentration of ^{99m}Tc in each fraction was measured and plotted as a percentage of the total (Figure 7:12).

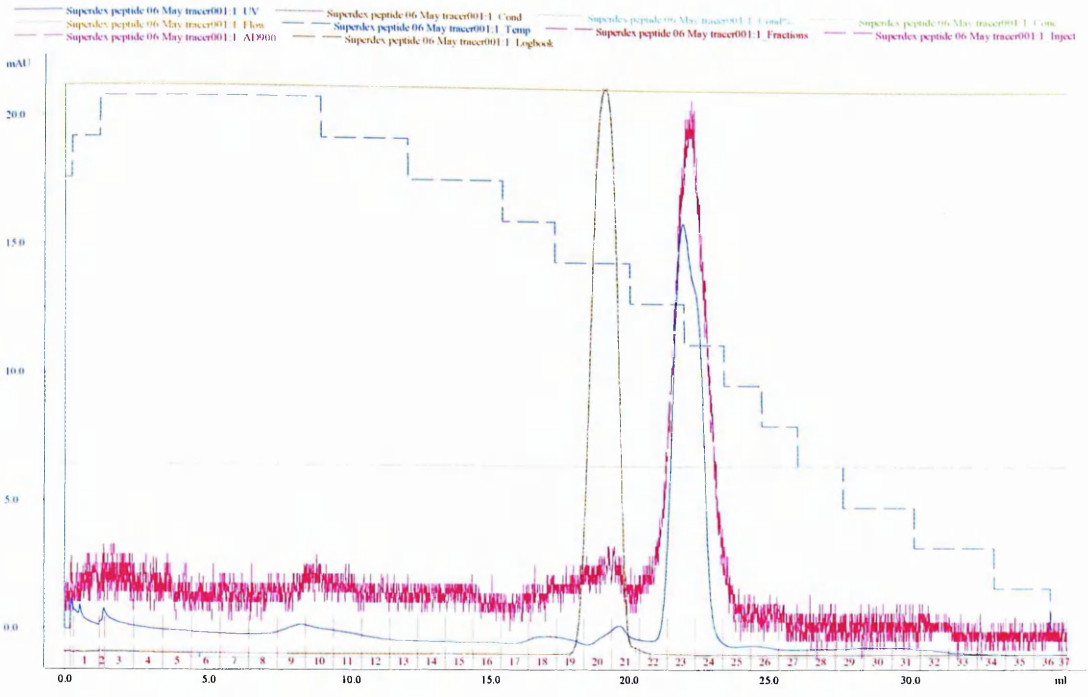


Figure 7:11: Chromatogram for ^{99m}Tc for Superdex peptide column (Blue = UV 275: Pink = UV 214 and Brown = conductivity)

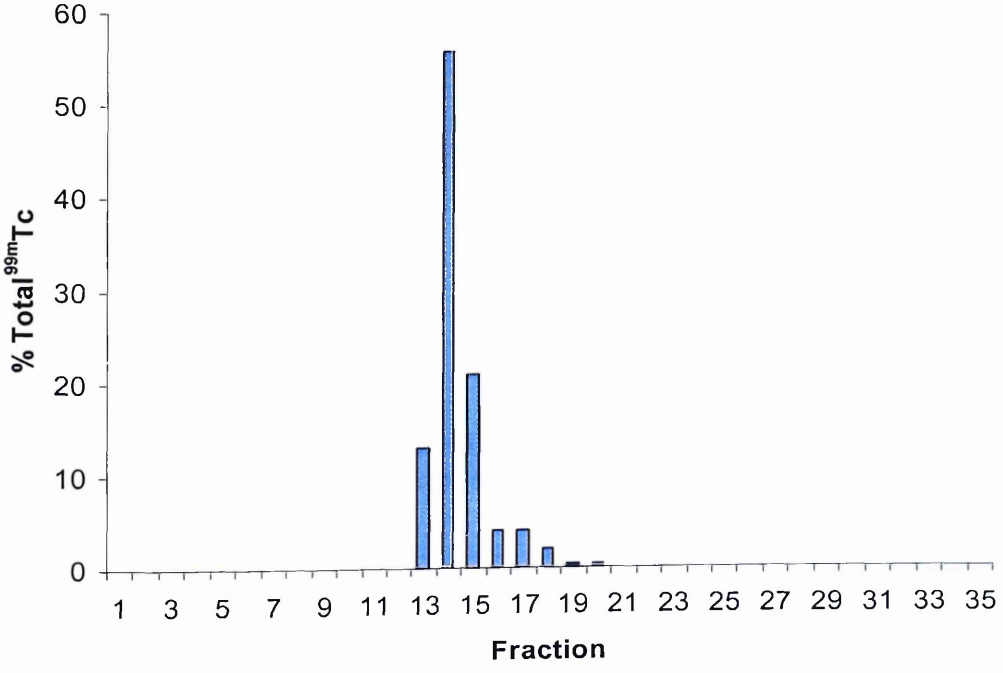


Figure 7:12: Percentage of ^{99m}Tc in individual fractions from Superdex peptide column

The highest activity concentration of ^{99m}Tc was found in fraction 14 which is earlier in the sequence than either of the UV peaks or the conductivity peak. Further inquiries discovered that the tracer supplied was not pertechnetate but ^{99m}Tc attached to gamma globulin (a protein found in blood plasma).

Soil solution collected two months after seaweed addition to the soil was added to the column and the sample run under the same conditions as for the tracer. The resulting chromatogram is shown in Figure 7:13.

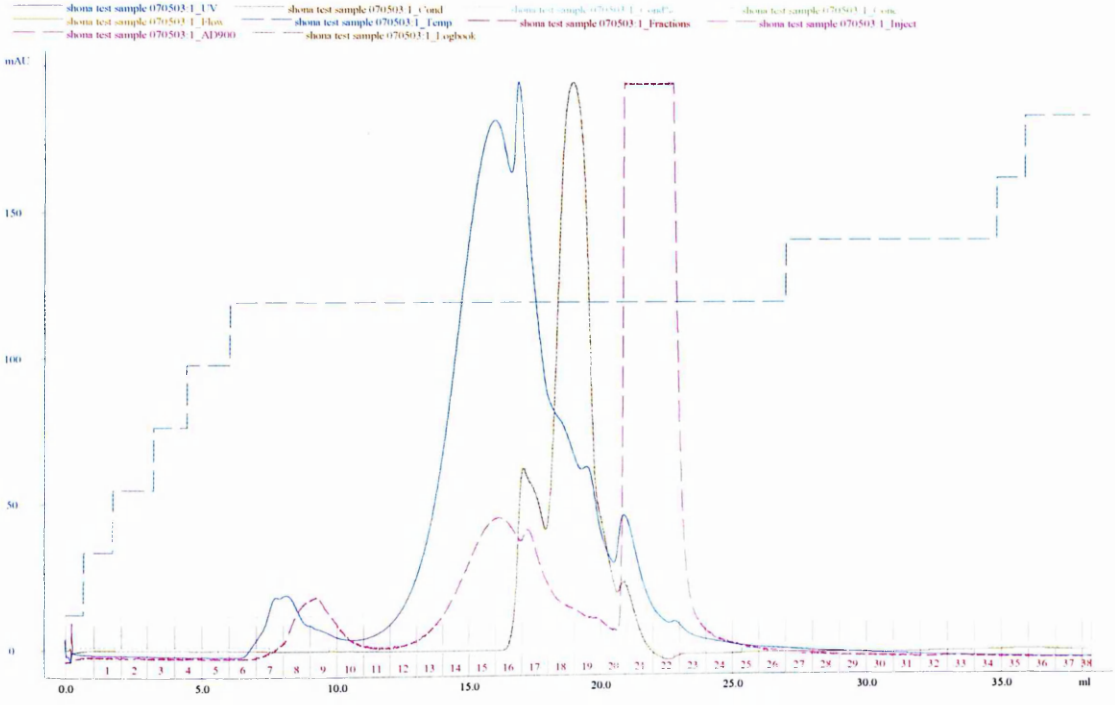


Figure 7:13: Chromatogram for soil solution sample for Superdex peptide column (Blue = UV 275: Pink = UV 214 and Brown = conductivity)

This shows a small protein peak followed by a conductivity peak and finally a higher peptide peak. In an attempt to identify the molecules present in fractions that indicated the presence of peptides, (9, 10, 16, 17, 18, 19, 20, 22 and 23) the fractions were run on a SDS-PAGE (Sodium Dodecyl Sulphate – PolyAcrylamide Gel Electrophoresis) gel. This is a method that separates

proteins and peptides according to size. A solution containing molecular weight markers was also run. On staining the gel with coomassie blue to highlight the bands of peptides, only those from the marker molecules were visable. This could be due either to the concentration of peptides in the samples being too low or they contained peptides that were too small to be observed on the gel. The fractions were bulked for ^{99}Tc analysis as shown in Table 7:4.

Table 7:4: Fractions analysed for ^{99}Tc from Superdex peptide column

Sample	Fractions	Associated features on trace
1	1-6	Pre UV and conductivity peak
2	7-9	Small protein and peptide peaks
3	10-12	Between peaks
4	13-15	Protein peak
5	16-19	Conductivity peak + protein peak
6	20-23	Peptide peak
7	24-35	Post conductivity and UV peak

Tc-99 analyses failed to detect any ^{99}Tc present in any of the samples. Further trials using the ^{99m}Tc tracer showed that it also failed to move through the column.

7.4.4 Sephadex G-25M

This gel had been used previously to separate pertechnetate from ^{99}Tc bound to organic matter in soil (Van Loon, 1986).

7.4.4.1 Long column (37 cm)

The ^{99m}Tc tracer was added to the column with the conditions set as described in Section 7.3.2. The eluant was 10 nM NaPi + 150 nM NaCl. The ^{99m}Tc failed to emerge from the column. Tracing the activity with a hand held counter

indicated that the ^{99m}Tc 'stuck' about half-way down the column. This suggests that ^{99}Tc was interacting with the gel. This can be an advantage as the system can be miniaturised, i.e. the column can be shortened (Van Loon, 1986).

7.4.4.2 Short columns (5 cm)

As the ^{99}Tc was interacting with the gel, a shorter column was used as described in Table 7:1. Short columns can be used for group separation. This should allow the separation of pertechnetate from any ^{99}Tc bound to ligands. The conditions and eluant were set as before and a 1 ml sample of the tracer solution was applied to the column. The procedure was repeated. The ^{99m}Tc activity concentration within each fraction was measured and the results are shown in Figure 7:14.

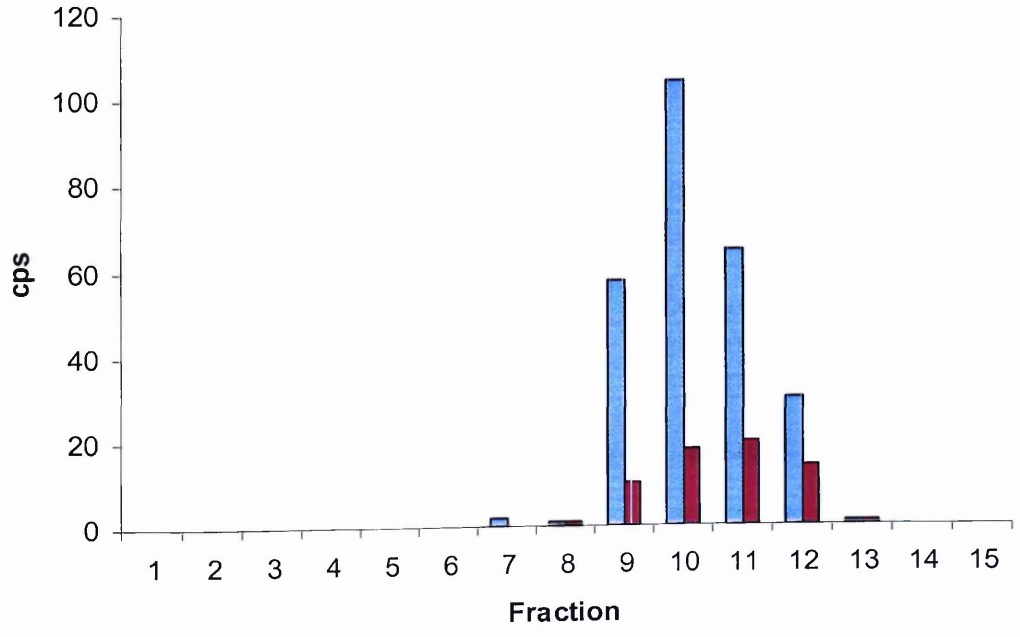


Figure 7:14: Tc-99m activity concentration in fractions from Sephadex G-25M columns (Blue colour = first run, brown colour = second run)

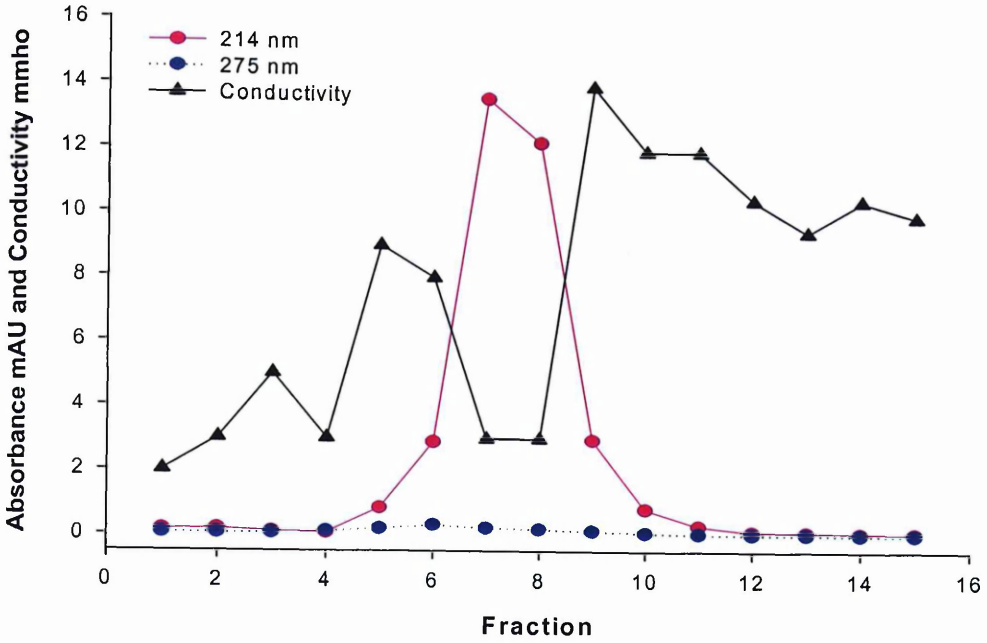
The lower activity concentrations in the second run are due to the short half-life of the isotope resulting in a reduction in the activity added to the column. The V_e/V_t variable for pertechnetate was calculated as around 2.3 to 2.5.

Three soil solution samples collected 5-6 months after seaweed added to the soil were passed through the column. The conductivity and UV absorbance at 275 nm and 214 nm were measured (Figures 7:15a, 7:16a and 7:17a). The UV 214 nm peaks suggest that there are peptides present in Fractions 6-9 in all samples. There was no noteworthy peak for absorbance at 275 nm suggesting there were no large protein molecules present. The rise in conductivity around fractions 10-12 suggests the presence of ions. Once again it was impossible to analyse each fraction individually for ^{99}Tc so the fractions were again bulked together as shown in Table 7:5.

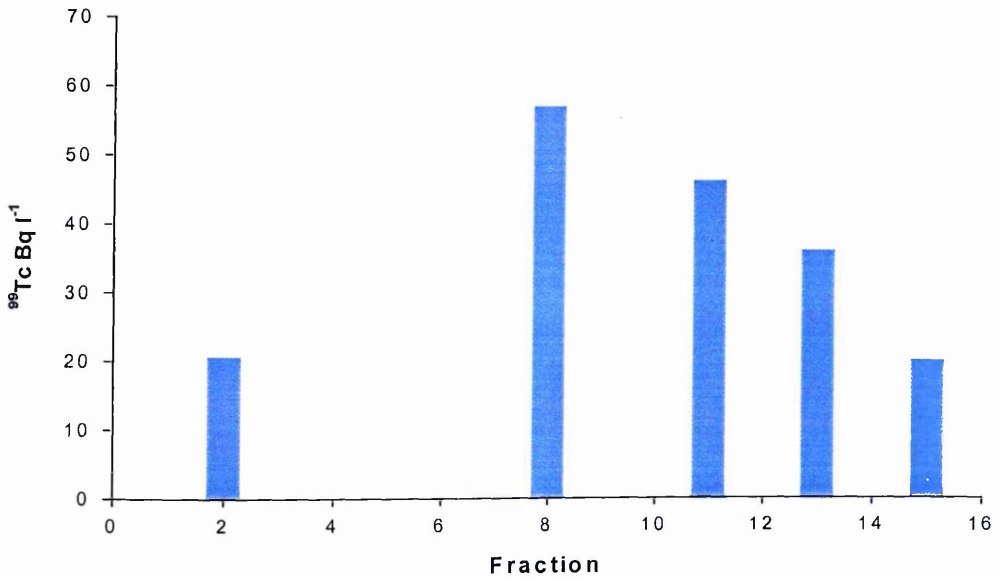
Table 7:5: Samples from Sephadex G-25 column analysed for ^{99}Tc

Sample	Fractions	Associated features on trace
1	1-5	Prior to conductivity and UV peaks
2	6-9	UV 214 nm Peak
3	10-11	Conductivity peak
4	12-13	Post conductivity peak
5	14-15	Post conductivity peak

The activity concentration within each sample was measured and the results are shown in Figures 7:15b, 7:16b and 7:17b.

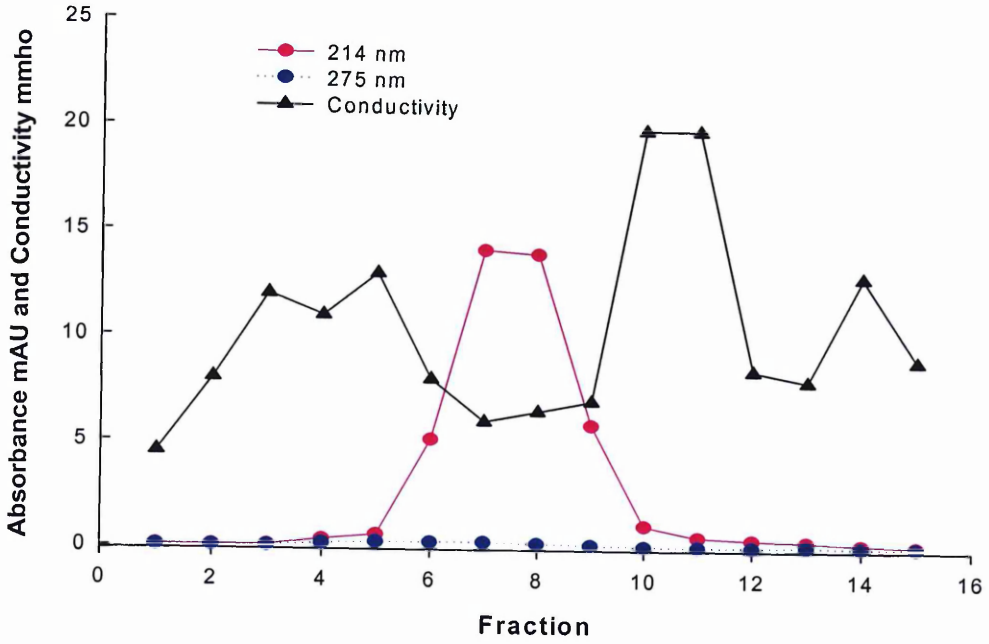


(a)

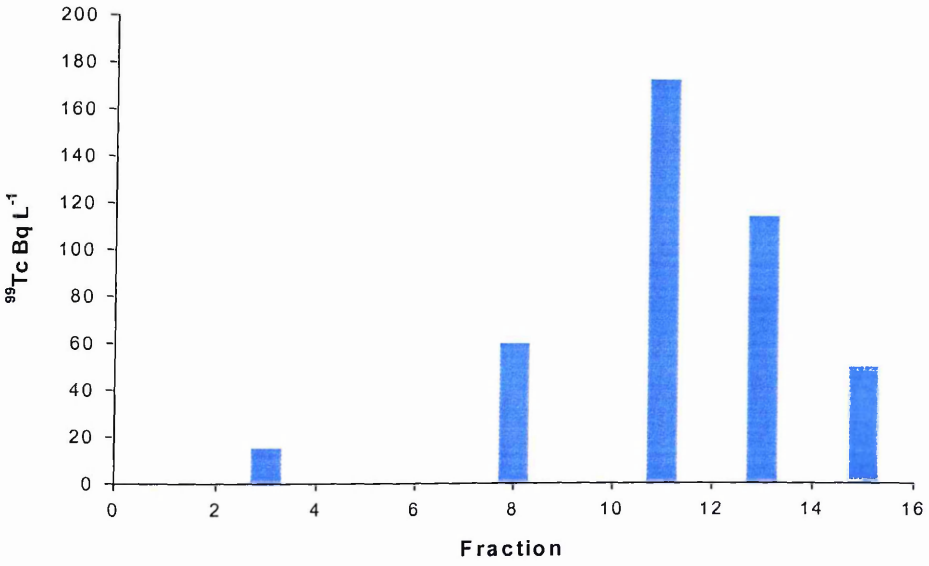


(b)

Figure 7:15: Conductivity, UV and ^{99}Tc activity concentration measurements of soil solution A (collected 5 months after seaweed addition to soil) from Sephadex G-25 column (^{99}Tc activity concentration of collective fractions shown as single measurement for fraction in centre of bulked sample range)

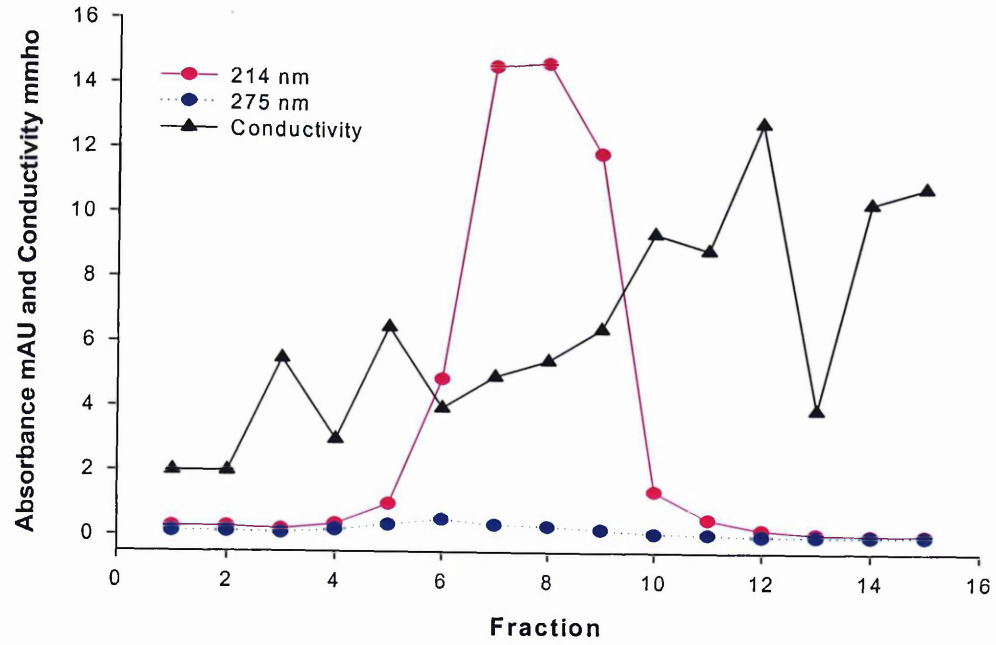


(a)

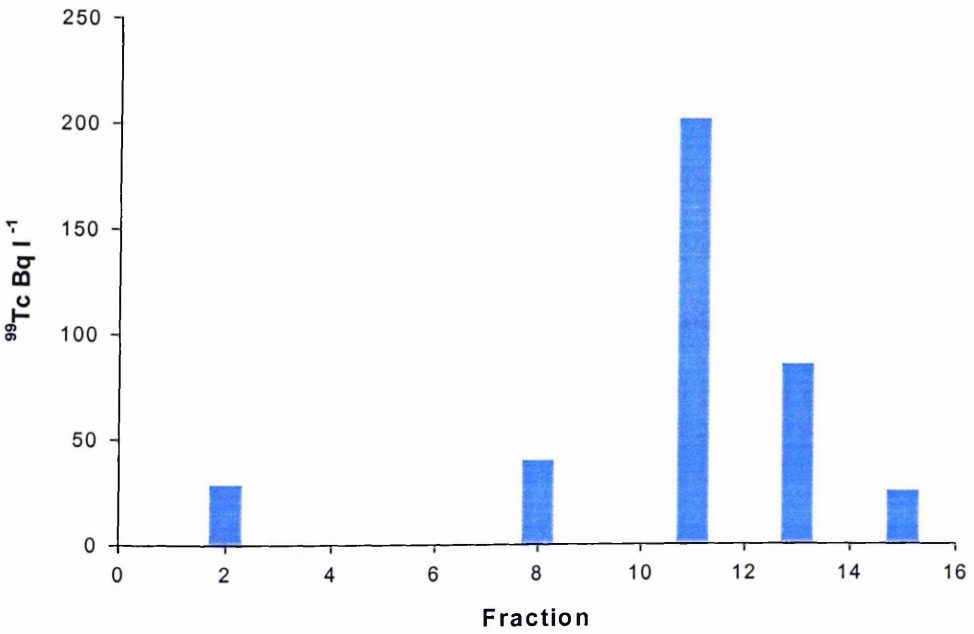


(b)

Figure 7:16 Conductivity, UV and ^{99}Tc activity concentration measurements of soil solution B (collected 6 months after seaweed addition to soil) from Sephadex G-25 column (^{99}Tc activity concentration of collective fractions shown as single measurement for fraction in centre of bulked sample range)



(a)



(b)

Figure 7:17: Conductivity, UV and ^{99}Tc activity concentration measurements of soil solution C (collected 6 months after seaweed addition to soil) from Sephadex G-25 column (^{99}Tc activity concentration of collective fractions shown as single measurement for fraction in centre of bulked sample range)

The results show that for soil solution sample A the highest activity concentrations (32% of total) were found in sample 2 (Fractions 6-9) followed by 26% in sample 3 (Fractions 10-12) and 20% in Sample 4. For soil solution samples B and C the highest activity concentrations were found in sample 3 (42% and 53% respectively), followed by sample 4 (28% and 22%). The V_e/V_t for each samples 2, 3 and 4 were calculated to be approximately 1.7, 2.4 and 2.8 respectively. As the V_e/V_t variable for Samples 3 and 4 are similar to those obtained for the pertechnetate tracer, it is assumed that these samples (plus Sample 5) contain the pertechnetate ion form of ^{99}Tc.

7.5 DISCUSSION

The attempts to separate the various species of ^{99}Tc present in the soil solution had variable success depending on the method used. The Superdex 200 and Superose 6 columns did not produce adequate definition between the UV and conductivity peaks, but did allow ^{99}Tc to pass through. However the results from these columns did suggest that only small molecules were present in the soil solution. This would seem likely as only soluble molecules would be present in solution and they are likely to be small in size e.g. sugars, amino acids, peptides.

The Superdex 200 column appears to show two species of ^{99}Tc in the soil solution sample. One (sample 4) has a V_e/V_t variable similar to that of the pertechnetate in the tracer (0.90 compared to 0.92) suggesting that that this and most likely the following sample (5) contains pertechnetate. This means that around 36% of the total ^{99}Tc in the soil solution sample, one month after seaweed addition to the soil, is in the pertechnetate form. The other species

(found in sample 3) was eluted before pertechnetate (Ve/Vt = 0.66). As this sample was found to be rich in peptides, it can be reasonably assumed that the ^{99}Tc is attached to the peptide molecules. This species comprised 54% of the total ^{99}Tc in the soil solution sample. In plant material, it has been found that 50-70% of ^{99}Tc was associated with soluble plant macromolecules (Cataldo *et al.*, 1986).

A similar pattern was seen with the Superose 6 column, where again two groups of molecules seemed to be present. Comparison between the V_e/V_t variables for the pertechnetate tracer and those for the samples in the soil solution are not quite as clear as those found for the Superdex 200 column. This may be due to the way the fractions were bulked together for ^{99}Tc analysis. The V_e/V_t for Sample 4 was approximately 0.90 (V_e was calculated as the 21.5 mls, i.e. the middle of the sample made up of fractions 20-23) whilst that for the pertechnetate tracer was 0.83 (V_e was taken at the highest point of the peak, i.e. fraction 20). If most of the ^{99}Tc in Sample 4 was present in fractions 20 and 21, the variable would be closer to that of the pertechnetate in the tracer. If Sample 4 and the two following samples contain pertechnetate, around 50% of the total ^{99}Tc in this soil solution, collected one month after seaweed addition to the soil, is present as pertechnetate.

As the UV and conductivity peaks on both the Superdex 200 and the Superose 6 gel columns overlapped, it was difficult to determine whether the ^{99}Tc was associated with both of these features or only one of them. In an attempt to separate the species of ^{99}Tc further and because UV absorption at 214 nm had suggested the presence of peptides in the soil solution, a gel which was

designed to separate peptides was used. Whilst the chromatogram seemed to show the presence of several peaks at 214 nm, analysis failed to show the presence of ^{99}Tc in any of the samples. This may be because:

1. The activity concentration of ^{99}Tc in the fractions was too low to be detected by the analysis method.
2. The ^{99}Tc was interacting with the gel
3. The ^{99}Tc was interacting with another compound which had interacted with the gel (The column was also being used for the separation of peptides of collagen)

The results from the SDS-PAGE did indicate that the concentration of peptides in the individual fractions was very low but as fractions were bulked to make five samples, explanation 1 is unlikely. Explanation 2 is also unlikely, as the first tracer sample did emerge from the gel. The most likely explanation is therefore that the ^{99}Tc is interacting in some manner with some collagen fractions that have interacted with the gel and are trapped in the column. Trials with this column therefore had to be abandoned.

The final trials were carried out using Sephadex G-25, which had been successfully used to separate pertechnetate from ^{99}Tc bound to organic matter. The trial with the longer column was unsuccessful, because the ^{99m}Tc tracer failed to move through the column at a sufficient rate. After eight hours the tracer had only moved half way down the 37 cm column. As the tracer only has a half life of just over 6 hours, the activity would be immeasurable by the time it emerged from the column. The interaction of ^{99}Tc with the gel allows miniaturisation of the system with the pertechnetate form of ^{99}Tc eluting at a

volume that is greater than the total volume of the gel column (Van Loon, 1986). Trials with the short columns (5 cm long) showed that the pertechnetate tracer did elute behind the total volume of the column (V_e/V_t = 2.3 to 2.5), in a time that was fast enough to allow measurement of the activity concentration in the fractions.

The short columns allowed the separation of pertechnetate from bound ^{99}Tc (Desmet *et al.*, 1991). The chromatograms appear to show a slightly better distinction between the UV and conductivity peaks. The results from the ^{99}Tc analysis of soil solution samples showed that for sample A aken five months after seaweed addition to the soil, around 57% (Samples 4, 5 and 6) of the ^{99}Tc present in the soil solution is in the pertechnetate form, with the remainder being complexed to small molecules possibly peptides. For the samples B and C taken one month later, 82 -89% of the ^{99}Tc present was in the pertechnetate form, with the remainder complexed with small molecules possibly peptides. The small number of replicates makes it difficult to determine if the difference between the samples taken on different dates are true differences or are the result of experimental variation. It is possible that ^{99}Tc released complexed to small molecules is converted to pertechnetate in the soil over time through the action of soil microbes breaking down the organic molecules. It has to be remembered that the system under investigation is a dynamic one, with ^{99}Tc likely to be added to the soil solution through its release from the seaweed and possibly by the breakdown of insoluble Tc-organic matter complexes that have been released into the bulk soil and removed from solution either by soil microbes or by forming insoluble complexes with organic matter. Each sample taken, therefore, is only a snapshot of the system at any particular point in time.

7.6 CONCLUSION

The investigation of the chemical forms of ^{99}Tc present in the soil solution showed that a substantial proportion of ^{99}Tc present in soil solution has a V_e/V_t similar to that of the pertechnetate in the tracer and so is likely to be in the pertechnetate ion form and available for plant uptake. The proportion varied with sampling date from 36-50% (one month after seaweed addition to soil) to over 80% (five months after addition). More replicates, using the same column type for different dates are needed to determine if these differences show a true pattern or if they are due to experimental variation. Analysis of individual fractions would have perhaps shown a clearer distinction between pertechnetate and bound forms of ^{99}Tc but the time required for the analysis prevented this. As the short columns (5 cm, sephadex G-25) only allows group separation (pertechnetate from Tc-organic matter complexes), trials with other gel columns could be carried out to try to achieve fractionation of the species present.

CHAPTER 8 - SUMMARY DISCUSSION

8.1 INTRODUCTION

The existence of a potential pathway for the transfer of ^{99}Tc from marine to terrestrial ecosystems and onto man through the use of contaminated seaweed as a soil conditioner was identified in the late 1990s. The pathway itself has been poorly researched, but some components can be verified through studies of uptake of ^{99}Tc by brown seaweed and uptake of ^{99}Tc by plants. The literature review at the beginning of this research programme identified major gaps in knowledge due to a lack of research into the rate and mechanics of the release of ^{99}Tc from seaweed into soil. Hence the aim of this research programme was to quantify the amount of ^{99}Tc released from the brown seaweed species, *Fucus vesiculosus* into soil over time, investigate the mechanisms involved and determine the bioavailability of the released ^{99}Tc.

As the investigation of ^{99}Tc release from seaweed involves studying a complex, dynamic natural system it was necessary to focus the investigation on a key aspect: the mechanisms and dynamics of ^{99}Tc release from seaweed into soil. The investigation comprised a series of pot and laboratory experiments designed to provide controlled conditions to fully test the hypotheses formed.

This chapter provides an overview of the results from all experiments in relation to the hypotheses formed and assess the implications of these results for practices in the field. A critical appraisal of the methods used in this investigation, a discussion of problems encountered and suggestions for further work are also included.

8.2 EXPERIMENTAL APPROACH

Pot experiments were designed to mimic field conditions as closely as practically possible. This involved using contaminated seaweed collected from the shore close to BNFL, Sellafield, a soil representative of those to which seaweed is added and similar application rates and techniques to those used on the garden plots in Cumbria. The ^{99}Tc activity concentration in the environmentally contaminated seaweed varied in response to the levels of ^{99}Tc discharged from BNFL, Sellafield. The seaweed collected in July 2001 for experiment 2 had a higher ^{99}Tc activity concentration than that collected in January 2001 for the first experiment (25,000 Bq kg^{-1} compared to 11,800 Bq kg^{-1}, wet wt). This could reflect the increase in ^{99}Tc discharge into the Irish Sea during 2001 compared to 2000 (79 TBq versus 44.4 TBq) (FSA & SEPA, 2002). Whilst the use of environmentally rather than artificially contaminated seaweed posed some difficulties, as discussed in Section 8.5, it was deemed very important to use seaweed that was naturally contaminated because artificial contamination might affect the release of ^{99}Tc from seaweed.

Pot and laboratory based experiments were chosen rather than field experiments as they provided controlled conditions to fully test the hypotheses formed.

8.3 HYPOTHESES

The aims of this research programme were to quantify the amount of ^{99}Tc released from environmentally contaminated *Fucus vesiculosus* into soil, investigate the mechanisms involved and determine the bioavailability of the released ^{99}Tc.

Four initial hypotheses were formed on the basis of information gathered in the literature review and a further three were formed as the investigation progressed.

8.3.1 *Hypothesis 1: Tc-99 within Fucus vesiculosus will be released into the soil over time*

For the sea-to-land pathway to exist, the ^{99}Tc bioaccumulated by the seaweed and put onto the land must be released into soil over time. This study is the first to investigate the release of ^{99}Tc from environmentally contaminated seaweed into soil. The first pot experiment confirmed that ^{99}Tc was released into the soil over time with between 54 and 71% of the ^{99}Tc contained within the seaweed found in the bulk soil, 15 weeks after seaweed addition to the soil. There was a significant overall effect of time on the ^{99}Tc activity concentration in the soil, with the concentration on later dates significantly higher than the earlier dates. The release of ^{99}Tc into soil over time was also confirmed in experiment 2, but this showed that 21 months after seaweed addition to the soil, 46% of the ^{99}Tc added with the seaweed was present in the soil. Whilst this value is lower than the values obtained in the first experiment, the differences in the experimental conditions (time, temperature etc.) make the two sets of values difficult to compare. The retention of around 50% of the initial ^{99}Tc in the seaweed 21 months after its addition to the soil suggests that a substantial proportion may be associated with material that is resistant to decomposition.

Confirmation that ^{99}Tc is released from seaweed into soil over time means that Hypothesis 1 can be accepted.

8.3.2 *Hypothesis 2: The temporal release pattern will be dependent on decomposition processes, leaching and microbial decomposition*

In experiment 1, over 20% of the ^{99}Tc added within the seaweed was present in the soil three weeks after the addition of seaweed to the soil. Over this period there was no sign that microbial decomposition was taking place (no increase in CO_2 production), suggesting that the abiotic process of leaching was the predominant mechanism. Leaching appeared to be the dominant mechanism throughout most of the monitoring period as the rate of ^{99}Tc release slowed down and appeared to cease. However in the later stages, a rise in CO_2 production was associated with a further increase in the release of ^{99}Tc into the bulk soil. The increase in CO_2 production may also have been a response to an increase in ambient temperature but may have been due to the start of the decomposition of more resistant material within the seaweed, e.g. cellulose. The amount of carbon released over the 15-week period equalled around 8% of the total carbon content of the seaweed, suggesting that large-scale decomposition of the structural and storage carbohydrates had not yet taken place.

Further investigation of the mechanisms responsible for the release of ^{99}Tc from seaweed was carried out in experiment 2. Despite higher rates of CO_2 production at the start of the monitoring period (this experiment was started in July as opposed to January for experiment 1 so ambient temperatures were higher) the rate of ^{99}Tc release did not increase. At 18 weeks after seaweed addition to the soil, around 11% of the carbon in the seaweed had been metabolised as CO_2 but only 3% of the ^{99}Tc added with the seaweed was present in the soil solution. If the percentage of the total soil activity

concentration present in the soil solution is similar at 18 weeks to that found at 21 months (11%) then less than 30% of the ^{99}Tc added within the seaweed is present in the soil at 18 weeks. This compares to 54 – 71% released over 15 weeks in experiment 1 when 8% of the carbon in the seaweed had been released as CO_2. An increase in microbial activity in the soil therefore appears to decrease the amount of ^{99}Tc released from the seaweed into the soil. Whilst this may be due to fluctuations in the natural system or experimental varaition, it may indicate the adsorption of released ^{99}Tc back onto the exterior of the seaweed pieces. The increased microbial activity can create anaerobic microsites (Sparkes & Long, 1986). promoting the reduction of pertechnetate (Tc(VII) to Tc(IV)), which can then adsorb on to soil mineral or more likely organic matter fragments (Wildung *et al.*, 1986; Stalmans *et al.*, 1986; Nicholson *et al.*, 1990). In this system, as the coastal sandy soil has very little organic matter (< 3%), the main organic component is the seaweed. It is reasonable to assume that any reduced ^{99}Tc could be bound to the exterior of the seaweed pieces. This could help to explain why such a large proportion of the initial ^{99}Tc was still associated with the seaweed pieces 21 months after their addition to the soil.

Leaching appears to be the predominant mechanism for the release of ^{99}Tc from seaweed into soil, with microbial decomposition perhaps playing a subsidiary role in the later stages. Hypothesis 2 can therefore be accepted in part. More work is required to investigate further the role of microbial decomposition.

8.3.3 *Hypothesis 3: In aerobic, sandy soils around half of the ^{99}Tc within Fucus vesiculosus will be released in a soluble form and remain within the soil solution*

Hypothesis 1 confirmed that ^{99}Tc was released from environmentally contaminated seaweed into soil, but for it to be available for plant uptake, it was necessary for at least some of the released ^{99}Tc to be present in the soil solution. This is because its bioavailability is dependent on the concentration of pertechnetate in soil solution rather than total soil concentration of ^{99}Tc (Van Loon, 1986). Previous research on artificially contaminated seaweed had indicated that around 60% was present in the plant in a soluble form and could be removed by shaking the seaweed in water. However, there have been no studies, until now, that have investigated the release of ^{99}Tc from environmentally contaminated seaweed into soil and soil solution.

Tc-99 analysis of soil solution samples collected during experiment 2 confirmed that ^{99}Tc was released into soil solution over time. Eighteen weeks after seaweed addition to the soil, just over 3% of the ^{99}Tc added within the seaweed was present in the soil solution. Tc-99 activity concentration of the samples on the later dates were significantly higher than those on the earlier dates.

Analysis of the percentage of ^{99}Tc within the bulk soil and soil solution, 21 months after seaweed addition to the soil, showed that around 11% of the ^{99}Tc in the soil was present within the soil solution. This was lower than the 50% predicted. It is possible that some of the soluble ^{99}Tc was adsorbed or taken up by soil microbes. Anaerobic and sulphate reducing bacteria, which could be present in reduced microsites within the soil, can bioaccumulate ^{99}Tc to a high degree (C_r 50-600 and 6974-9311 respectively (Henrot, 1999)). However, the

low mass of bacteria compared to the mass of the soil suggests that the bacteria play only a small role in removing ^{99}Tc from solution. Removal of ^{99}Tc from the soil solution in this system would involve its adsorption to soil particles or organic matter. As the sandy soil has little organic matter (disregarding the added seaweed) adsorption to the soil particles is required to account for the increased ^{99}Tc activity concentration found in the bulk soil compared to that in the soil solution. Pertechnetate is poorly adsorbed to mineral soil particles under aerobic conditions (k_d values close to zero for sandy soils) (Routsen *et al.*, 1977; Sheppard *et al.*, 1983) but under anaerobic conditions, pertechnetate is reduced and its ability to adsorb to the soil matrix increases (Leiser, 1993). This process can take place in anaerobic microsites created by high levels of soil microbial metabolism (Henrot, 1989). Tc-99 released into the soil as pertechnetate thus converts to insoluble Tc(IV) over time and is adsorbed to soil particles or organic matter. As soil solution is a dynamic medium, it is likely that the concentration of soluble ^{99}Tc will fluctuate over time as conditions e.g. extent of anaerobic microsites, alter the proportion of insoluble and soluble ^{99}Tc present within the system. Continual altering of redox conditions can lead to a re-oxidation of readily oxidisable forms of ^{99}Tc (Stalmans *et al.*, 1986).

It is also possible that a lower percentage of ^{99}Tc released from environmentally contaminated seaweed is in a soluble form. This was further investigated via Hypothesis 6.

The presence of ^{99}Tc in the soil solution means that Hypothesis 3 can be partially accepted but the percentage is considerably lower than predicted.

8.3.4 *Hypothesis 4: The majority of ^{99}Tc released into the soil solution will be available for plant uptake.*

The presence of ^{99}Tc in the soil solution creates the potential for plant uptake. In experiment 2, spinach plants grown in pots containing contaminated *Fucus vesiculosus* mixed with the sandy coastal soil took up ^{99}Tc, with the concentration ratio calculated as approximately 120. This confirms that at least some of the soluble ^{99}Tc present within the soil solution is in a bioavailable form, namely pertechnetate. The winter spinach plants contained between 2 and 6% of the ^{99}Tc added to the pots within the seaweed. There was a positive correlation between ^{99}Tc activity concentration per kilogram of plant material and the weight of the plant suggesting that a higher growth rate resulted in an increase in ^{99}Tc uptake.

The uptake of ^{99}Tc that has been released by seaweed into the soil solution by the spinach plants means that hypothesis 4 can be accepted. This also provides circumstantial evidence that a substantial proportion of the ^{99}Tc present in the soil solution is in the pertechnetate form.

8.3.5 *Hypothesis 5: The rate of ^{99}Tc release from Fucus vesiculosus will be faster from small seaweed pieces than large ones due to increased leaching and microbial decomposition*

The size of plant litter particles generally has an effect on decomposition rate with the rate increasing as particle size decreases (Brady & Weil, 1999). The cutting up of the seaweed fronds in experiment 1 may have speeded up the release of ^{99}Tc from the seaweed due to increased rates of leaching and microbial decomposition. Cutting would create a large number of damaged cells from which soluble ^{99}Tc could leach more rapidly and also create a larger surface area for microbes to attack.

However in experiment 2 there was no difference in the rate of CO_2 production between pots with small seaweed pieces and those with larger pieces. Cutting up of the seaweed, therefore, does not seem to affect microbial decomposition rates. The increase in surface area may not have been great enough to cause a significant increase in microbial activity.

Measurements of ^{99}Tc in the soil solution over time showed no overall significant difference between the amounts of ^{99}Tc released in the pots with the small and the large pieces, despite the rate of release being initially faster in the pots with the small pieces of seaweed. This initial faster ^{99}Tc release is most likely due to increased leaching and not increased microbial decomposition. Hypothesis 6 therefore can only be accepted in part.

8.3.6 Hypothesis 6: Fifty percent of the ^{99}Tc present in Fucus vesiculosus will be readily extractable with water

Leaching appears to be the predominant mechanism by which ^{99}Tc is released from *Fucus vesiculosus* into soil. Experiment 3 was carried out to support this assumption and investigate the percentage of ^{99}Tc that could readily be extracted by shaking the seaweed in water. After six days around 36% of the ^{99}Tc in the seaweed had leached from the seaweed into the water, 69% of which was released between days two and four. This was lower than the 50% predicted from the results of previous studies using artificially contaminated seaweed. This suggests that a larger percentage of ^{99}Tc was present in the plant cells in an insoluble form. Tc-99 will have been present in the plant cells of environmentally contaminated seaweed for a much longer time than in artificially contaminated seaweed (years rather than days or weeks). The opportunity arises, therefore for more of the ^{99}Tc to become bound within the

cells, for example in the chloroplasts. Tc-99 is thought to compete with the electron acceptor molecule $NADP^+$ in the electron transport chain of photosynthesis which takes place in the chloroplasts (Lembrechts, 1986). As time progresses, it is possible that an increasing amount of ^{99}Tc will become bound within the chloroplasts. This would leave less soluble ^{99}Tc in the cell cytosol which could be removed through shaking in water. The timing of the loss of ^{99}Tc was similar to that of soluble sugars, most of which are present within the cell cytosol. The total percentage loss of ^{99}Tc was greater than that of sodium and potassium ions, which are amongst the most easily leached molecules from plant material (Mason, 1977).

The percentage of ^{99}Tc lost through leaching in this shaking experiment was far greater than the percentage of ^{99}Tc found in the soil solution in the second pot experiment. This may be due to agitation of the samples through shaking accelerating the leaching process. Alternatively pertechnetate may have been removed from the soil solution due to the formation of insoluble Tc(IV) complexes with e.g. ligands on the exterior surface of the seaweed or onto the soil particles. The percentage of ^{99}Tc leached during the shaking experiment is comparable to the total ^{99}Tc released into the bulk soil in experiments 1 and 2. This suggests that ^{99}Tc released in a soluble form may be converted at times to insoluble Tc(IV), if the conditions within the soil are suitable, e.g. presence of anaerobic microsites (Henrot, 1989).

While the results from experiment 3 show that a substantial proportion of ^{99}Tc in environmentally contaminated *Fucus vesiculosus* is readily extractable with

water, the percentage was considerably lower than predicted. Hypothesis 6 therefore cannot be accepted.

8.3.7 *Hypothesis 7: Tc-99 present in the soil solution will be in the pertechnetate form*

The plant availability of ^{99}Tc in the soil solution was backed up by the speciation trials which suggested that a substantial proportion of the ^{99}Tc in the soil solution was likely to be in the pertechnetate form. The percentage of ^{99}Tc likely to be in the pertechnetate form varied from 36-50%, one month after seaweed addition to the soil, to over 80% (five months after addition). There were not enough replicates analysed to determine if the increase over time was a trend or experimental variation. It is important to emphasise that the samples only represent a snapshot of a dynamic system at the point of sampling. Tc-99 as pertechnetate is readily taken up by plants (Wildung *et al.*, 1977; Routsen & Cataldo, 1978; Garten *et al.,* 1984; Cataldo *et a*l., 1986; Lembrechts, 1986; Van Loon 1986; Van Loon *et al.,* 1986b; Echevarria *et al.*, 1997) , thus the presence of pertechnetate in the soil solution confirms that some of the ^{99}Tc released into the soil solution will be available for plant uptake.

The ^{99}Tc not identified as pertechnetate was associated with small soluble molecules, possibly peptides and may therefore also be available for plant uptake, perhaps at a slower rate than pertechnetate.

Hypothesis 7 can be accepted since a considerable proportion of ^{99}Tc is present in the soil solution as pertechnetate, the remainder being associated with small soluble molecules possibly peptides.

This research programme has fulfilled the aims set out at the beginning of the investigation. The findings can now be related to the situation in the field.

8.4 THE FIELD SITUATION

8.4.1 Confirmation of the sea-to-land-to plant pathway

This study has provided evidence of the sea-to-land-to-plant pathway for ^{99}Tc through the use of contaminated seaweed as a soil conditioner. Whilst vegetables grown on garden plots, to which contaminated seaweed had been added, were known to contain ^{99}Tc (Camplin *et al.*, 1999) there had been no study, until now, of the mechanisms and dynamics of the release of ^{99}Tc from artificially contaminated seaweed into soil. The high release rate of ^{99}Tc from the brown seaweed species, *Fucus vesiculosus,* into soil and the subsequent presence of bioavailable pertechnetate in the soil solution confirm that the practice of using contaminated seaweed as a soil conditioner is a potentially important pathway for the sea-to-land-to-plant transfer for ^{99}Tc. There is the potential, therefore, for human exposure to ^{99}Tc on consumption of vegetables grown on plots conditioned with contaminated seaweed. The calculated dose that individuals using seaweed in this way received due to consumption of vegetables varied from 20-90 microsieverts (10^{-6} sieverts) (Camplin *et al.*, 1989). Whilst this included all radionuclides present in the vegetables, over 60% of the dose in each case was due to ^{99}Tc. Considering that the total average dose of radiation for the general public in the UK is around 2.6 millisieverts (10^{-3} sieverts), 85% of which comes from natural sources e.g. radon gas, air travel (DEFRA, 2003), the dose from the consumption of vegetables grown on these plots is very small. Tc-99 has been shown to accumulate in the thyroids of adult rats and their foetuses with possible

carcinogenic effects (Gerber *et al.*, 1989) and transferred to breast milk and sperm in humans (Day *et al.*, 2003). So, whilst the risk appears to be very small, continued long term monitoring of critical groups is required.

8.4.2 Implication of results on practices in the field

Even under winter conditions in the first experiment, a considerable percentage of the ^{99}Tc was released from seaweed into soil, predominantly through leaching.

The ease with which ^{99}Tc can leach from seaweed has important implications for the eventual transfer of ^{99}Tc to crop plants on plots conditioned with contaminated seaweed. As much of the seaweed used is drift weed collected from the shoreline after storms have cast it up on to the beach (Anonymous, 2001), it may have been lying on the shore exposed to rainfall. As the results of experiment 3 have shown, around one-third of the ^{99}Tc can be leached out of the seaweed in about two to four days. The ^{99}Tc activity concentration in the collected seaweed could therefore be considerably lower than that of fresh weed and if the most readily leached fraction had already been lost, the remaining ^{99}Tc may not be released so readily into the soil. Collecting drift seaweed after several days of heavy rain could become a recommendation for people who wish to use seaweed on their garden plots but minimise its ^{99}Tc content and bioavailability, although this may mean that the soluble sugar and mobile ion (such as potassium) content of the seaweed is reduced. This may also be a recommendation for individuals who compost the seaweed before applying it to the soil as it will minimise the activity concentration of ^{99}Tc in the finished compost. Keeping the compost aerated and allowing any fluid to drain

away may also reduce the ^{99}Tc content of the seaweed, as any ^{99}Tc released in a soluble form could drain out of the compost.

The weather conditions on application of seaweed to the soil could also affect the amount of ^{99}Tc available to crops planted on the plots. If the seaweed still contains substantial amounts of readily leachable ^{99}Tc, periods of heavy rainfall could leach this out and wash it down the soil profile so that it is no longer within the rooting zone of the plants. This could have implications for the contamination of groundwater in areas where seaweed is used to condition soil.

In experiment 2, around 50% of the initial ^{99}Tc remained associated with the seaweed 21 months after its addition to the soil (25% of the seaweed, by weight, was recovered from the soil at this time). This may have repercussions in the field situation. If new batches of contaminated seaweed are added, year after year before the existing seaweed decomposes completely, the activity concentration of ^{99}Tc within the seaweed compartment in the soil may continually increase. When the older, more resistant, material eventually begins to decay, releasing its ^{99}Tc, this along with the readily released fraction from the freshly applied seaweed will lead to an increase in the amount of ^{99}Tc entering the soil at that time. This could increase the amount of ^{99}Tc available for plant uptake over that growing season.

The results of this research programme have confirmed the existence of the sea-to-land-to-plant pathway of ^{99}Tc through the use of seaweed as a soil conditioner. If BNFL is successful with the new treatment of liquid waste and reduces the amount of ^{99}Tc discharged to the Irish Sea by 90% (BNFL, 2004) this would result in lower activity concentrations of ^{99}Tc in seaweed applied to

the soil. The radiation dose received by people eating produce grown on soil conditioned with contaminated seaweed should also fall. Monitoring of the individuals who use seaweed on their plots should be continued, as some adverse health effects may take a long time to appear.

8.5 CRITICAL APPRAISAL OF METHODS USED

The pot experiments were designed so that the results could be related to the field situation. Carrying out the investigation in field plots was an alternative approach but the use of pot experiments allowed better replication and control over environmental conditions such as water content of the soil which can influence the release of ^{99}Tc from the seaweed and the species of ^{99}Tc present. Mimicking field conditions as closely as possible involved using seaweed collected from the shore close to BNFL, Sellafield, replicating seaweed application rates and techniques, using a soil representative of those to which seaweed is added and growing a crop that has been grown on some of the plots in Cumbria.

Using environmentally contaminated seaweed meant that before the activity concentration of ^{99}Tc could be measured, contaminants which would interfere with the counting had to be removed and the ^{99}Tc concentrated to allow its detection. This involved a lengthy procedure which allowed a maximum of eight samples per week to be analysed (including a blank and a standard). This reduced the number of dates and replicates that could be analysed from the second experiment and led to the bulking of samples from the fractions collected from the columns in the speciation experiment. The use of artificially contaminated seaweed would have allowed quicker analysis and therefore more data to be collected. However, the results may not relate to the field

situation so clearly. For instance, ^{99}Tc was not as readily extractable from environmentally contaminated seaweed with water over a six day period in experiment 3 as from artificially contaminated seaweed in a previous investigation. This suggests that the forms of ^{99}Tc present in the seaweed may differ according to how it was contaminated and the length of time the seaweed has been in contact with the ^{99}Tc. This in turn could change the rate at which ^{99}Tc is released and the chemical form that is released, altering the results of all the experiments carried out in this investigation. To relate the results to the field situation, the use of environmentally contaminated seaweed was essential.

The seaweed application method and rates were replicated as closely as possible. The application rate had to be halved from that used in some field situations to allow the incorporation of seaweed in the small amounts of soil in the pots in experiment 1 and they were kept at that level for experiment 2 so that the results could be compared. However, as the amount of seaweed applied to the plots will vary over the area and from year to year, the difference in the amounts added to the pots may be of little consequence. The timing of the application was more realistic in the first experiment and it may have been better to wait and set up the second experiment at the same time of year in 2002. However, the long time required for ^{99}Tc analysis meant that the experiment had to be set up as quickly as possible after the first experiment to allow maximum time for analysis. Setting up the experiment in the summer rather than winter months, however, did show that increasing microbial decomposition did not speed up ^{99}Tc release, thus highlighting the role that leaching plays in ^{99}Tc release from the seaweed.

The main problem encountered during this investigation was the failure of ^{99}Tc to move through the initial columns used to try to separate the different chemical forms present in the soil solution. The failure to get any results from the attempts at SDS-PAGE and mass spectroscopy suggests that part of the problem is the low activity concentration of ^{99}Tc present in the soil solution. The fractioning of this small amount of ^{99}Tc into several samples made the detection of ^{99}Tc impossible. The amount of seaweed added to the soil in the pots may need to be increased to try to elevate the ^{99}Tc activity concentration in the soil solution if further speciation investigations are to be carried out.

Overall, the approach used in this research programme has successfully produced results which can be related to the situation in the field.

8.6 FURTHER WORK

Further experiments could be carried out to investigate the mechanisms and dynamics of ^{99}Tc release from seaweed into soil. The ^{99}Tc release from seaweed into soil under sterile conditions could be investigated e.g. by using high activity ultra violet light to sterilize the soil and seaweed samples and placing them in a microcosm bathed in UV light to prevent microbial growth. This could investigate whether microbial decomposition does play a subsidiary role in the later stages of ^{99}Tc release into soil or if its release is purely due to leaching.

The role which the incorporated seaweed plays in binding released ^{99}Tc under anaerobic conditions could be explored. This could be done by setting up pots where the seaweed instead of being incorporated into the soil, was layered on the surface and separated from the soil by a permeable membrane. The pots

could be regularly watered and the ^{99}Tc activity concentration in the soil solution monitored over time. The results could be compared to pots in which the seaweed was mixed evenly into the soil to observe if the ^{99}Tc activity concentration differs between the two sets of pots. If the ^{99}Tc is binding to the exterior of the seaweed, the activity concentration would be expected to be lower in the soil solution of the pots where the seaweed is incorporated into the soil.

The loss of ^{99}Tc through leaching from drift seaweed before application to the soil could be further investigated. The relative importance of loss through the action of rain and washing by wave or tidal action could be studied by determining the relative rates of leaching of ^{99}Tc from seaweed into rain and sea water. The optimum time that the seaweed should be left lying on the shore or in heaps in the garden before collection for addition to the soil to minimise the ^{99}Tc content and bioavailability could be recommended. The effect of composting the seaweed on the release and subsequent bioavailability of ^{99}Tc also needs to be explored.

The release dynamics of the fraction of ^{99}Tc that is not so readily leached could be investigated by first removing the readily leached fraction by shaking the seaweed in water for six days then adding the seaweed to soil and monitoring the release of the remaining fraction. This could also help to determine whether microbial decomposition plays an important role in the later stages of release.

Further work is also required to identify the chemical species of ^{99}Tc present in the soil solution over time. Using the small Sephadex G-25M columns, a series

of samples, e.g. at weekly intervals, need to be examined to determine if the percentage of ^{99}Tc present as pertechnetate alters with time, e.g. is ^{99}Tc released as pertechnetate and is converted to insoluble forms over time or vice versa. A clearer definition between bound ^{99}Tc and pertechnetate may be seen if individual fractions are analysed for ^{99}Tc rather than bulking samples. The amount of seaweed added to the pots may need to be increased so that the ^{99}Tc activity concentration is at a measurable level.

Finally the location of ^{99}Tc in environmentally contaminated seaweed could be compared with artificially contaminated plants to determine if the different lengths of exposure affects where ^{99}Tc is located in the plant cells. This could be carried out using autoradiography to locate the ^{99}Tc throughout the plant. The location of ^{99}Tc within the seaweed could have important implications on how it is bound within the plant cells. The chemical species present in environmentally and artificially contaminated plants could then be investigated using gel chromatography. The location of ^{99}Tc and the chemical species present in the plant cells of environmentally and artificially contaminated seaweed could have important implications for its subsequent bioavailability on addition to soil. This could be investigated by setting up pot experiments and growing plants in soil contaminated with seaweed contaminated by both methods.

8.7 CONCLUSION

The potential for the existence of a sea-to-land-to-plant pathway for ^{99}Tc through the use of contaminated seaweed as a soil conditioner was recognised over a decade ago. The existence of the pathway has been inferred from research of individual processes that, for instance, show that ^{99}Tc is taken up

by brown seaweed from seawater or that ^{99}Tc when added to soil as pertechnetate solution is available for plant uptake. However, until now, there has been no research into the mechanisms and dynamics of the processes involved, in the context of the pathway itself. In particular, there has been no research into the release of ^{99}Tc from contaminated seaweed into soil and its subsequent bioavailability to plants. This information is crucial to confirm the existence of this pathway and to predict any risk to humans and the environment.

The research detailed in this thesis is unique as it is the first to investigate the release of ^{99}Tc from environmentally, rather than artificially contaminated seaweed into soil, which allowed the results to be related more closely to the field situation. The high rate of ^{99}Tc release from *Fucus vesiculosus* into the soil, the subsequent presence of bioavailable pertechnetate in the soil solution and its uptake into spinach plants shown by the series of experiments in this research programme confirm that the practice of using contaminated seaweed as a soil conditioner creates a pathway for the sea-to-land-to-plant transfer of ^{99}Tc. There is, therefore, the potential for human exposure to ^{99}Tc on consumption of vegetables grown on plots conditioned with contaminated seaweed. While the dose that these individuals receive from consumption of these ^{99}Tc contaminated vegetables is very small compared to that from natural resources such as x-rays or radon, the long term monitoring of these individuals is necessary because very little is known about the long term effects of low levels of radiation on human health.

Comparison of the rate of release of soluble ^{99}Tc from environmentally contaminated seaweed in this investigation with those from published data which used artificially contaminated plant material and seaweed suggests that there is a considerable difference in how ^{99}Tc is bound in the two groups, with ^{99}Tc more readily released from artificially contaminated material. This could have implications for the output of models which are predicting, for instance, soil activity concentration or plant uptake in systems where plant material containing ^{99}Tc is added to soils. If the parameter values are based on data from experiments using artificially contaminated material, the models could over-estimate the release of ^{99}Tc into soil and therefore uptake into plants.

Whilst the use of contaminated seaweed as a soil conditioner does expose humans to a small dose of ^{99}Tc, the risk can be reduced if the seaweed is left exposed to heavy rain for several days before its application to the soil. This will allow the readily leachable fraction of ^{99}Tc to be lost prior to application. There seems no reason at this time to stop the practice of using seaweed to condition garden vegetable plots.

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QUESTIONNAIRE FOR SEAWEED USERS

1. What type of seaweed is applied and where do you collect it?
2. Is the seaweed collected fresh or partially decomposed?
3. At what time of year is it collected?
4. Is it applied to the land immediately or stored before use?
5. Is it composted with any other material?
6. Is it applied as whole fronds or chopped up?
7. Is it surface applied or dug / ploughed in?
8. How often is the seaweed applied?
9. What is the soil type in the area?
10. What kinds of crops are grown?
11. What benefits are gained from adding seaweed to the soil?
12. How long has this practice been taking place on this piece of land?

Name:

Address:

APPENDIX 2: STATISTICS

A EXPERIMENT 1

A.1 CO_2 production data

A two way ANOVA was performed on square root (SQRT) transformed CO_2 production data, using a GLM with 'sampling date' (i.e. time) and 'treatment' (i.e. with or without seaweed) as factors. The assumptions for the GLM were checked by drawing a normal probability plot of the residuals and plotting the residuals against the fitted values

Table A1: ANOVA table for SQRT transformed CO_2 production data

Source	df	SS	MS	*F*	*P*
Sample date	5	0.0000016	0.0000003	71.87	0.000
Treatment	1	0.0000035	0.0000035	804.92	0.000
Sample date*treatment	5	0.0000010	0.0000002	44.79	0.000
Error	24	0.0000001	0.0000000		
Total	35	0.0000062			

d.f = degrees of freedom

SS = Sum of squares

MS = Mean square

F = Ratio of within group variation

P = Probability of hypothesis being true

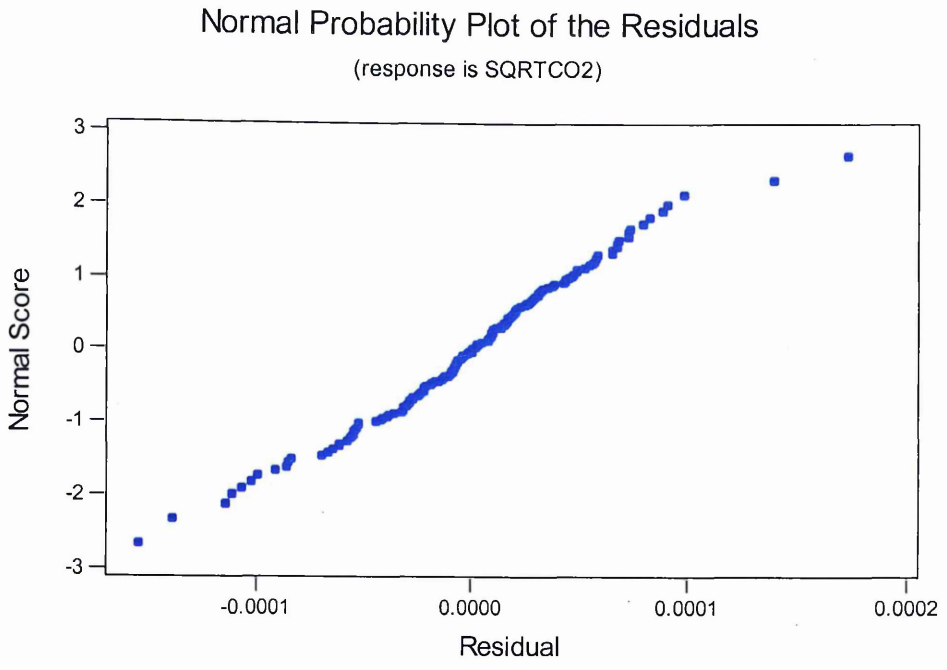

Figure A1: Normal probability plot for residuals for SQRT transformed CO_2 production data

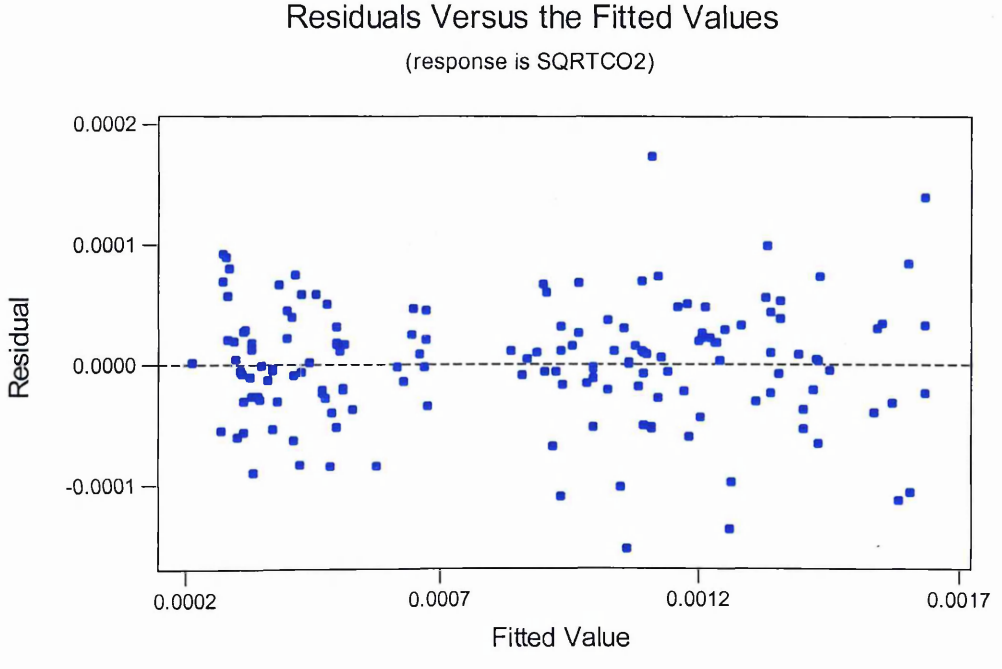

Figure A2: Plot of residuals v fitted values for SQRT transformed CO_2 production data

The data was further analysed using a Tukey's pairwise comparison (Family error rate 0.05) between sampling dates.

Table A2: 95% lower and upper confidence intervals for Tukey's pairwise comparison between sampling dates for SQRT transformed CO_2 production data

Week	0.7	1.7	3	8	12
1.7	- 0.000124 0.000113				
3	- 0.000155 0.000082	- 0.000149 0.000087			
8	0.000301 0.000538	0.000307 0.000543	0.000338 0.000574		
12	0.000053 0.000289	0.000059 0.000295	0.000090 0.000326	- 0.000366 - 0.000130	
15	0.000365 0.000601	0.000370 0.000607	0.000401 0.000638	- 0.000055 0.000182	0.000193 0.000430

A.2 ^{99}Tc accumulation data

A one way ANOVA was performed on SQRT transformed ^{99}Tc in soil (% of that added with seaweed) using 'sampling date' as the factor. The assumptions for the ANOVA were carried out by drawing a normal probability plot of the residuals and plotting the residuals against the fitted values.

Table A3: ANOVA table for SQRT transformed ^{99}Tc accumulation data

Source	d.f.	SS	MS	*F*	*P*
Sample date	5	362.663	72.533	14.19	0.000
Error	11	56.236	5.112		
Total	16	418.899			

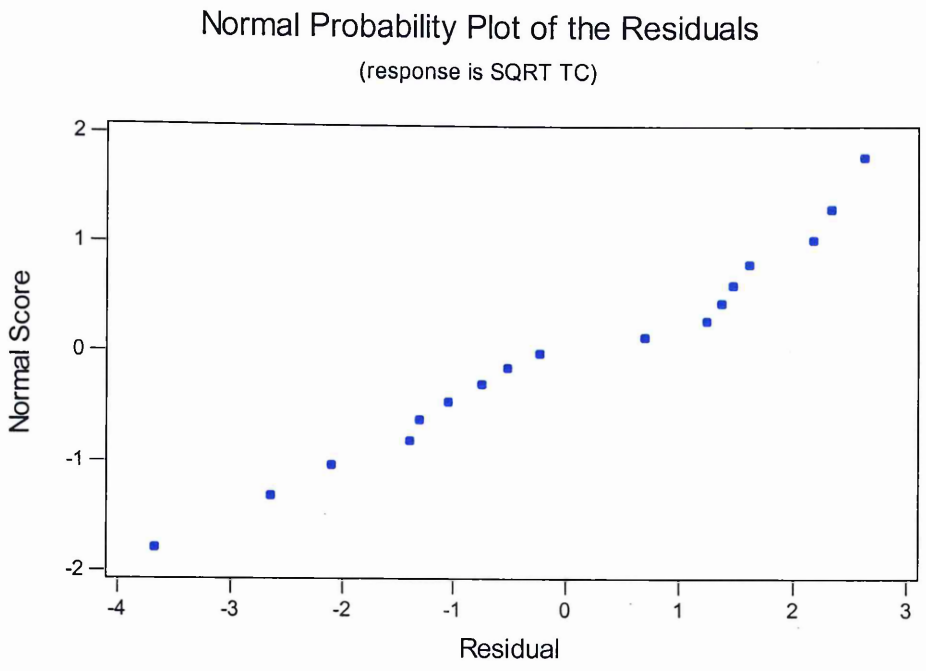

Figure A3: Normal probability plot of residuals for SQRT transformed ^{99}Tc accumulation data

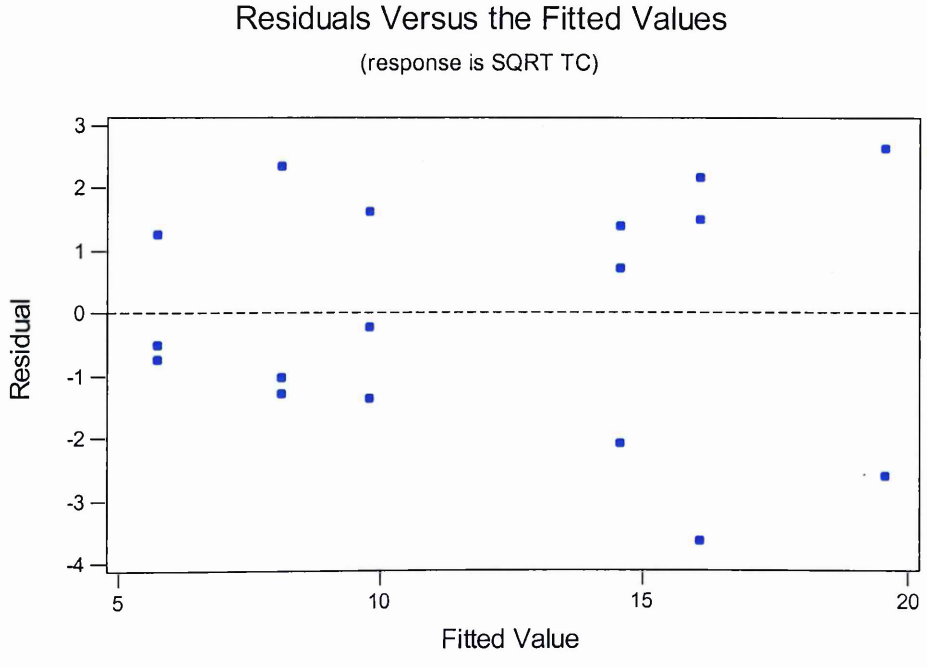

Figure A4: Plot of residuals v fitted values for SQRT transformed Tc accumulation data

The data was further analysed using a Tukey's pairwise comparison (Family error rate 0.05) between sampling dates.

Table A4: 95% lower and upper confidence intervals for for Tukey's pairwise comparison between sampling dates for SQRT transformed ^{99}Tc accumulation data

Week	0.7	1.7	3	8	12
1.7	- 3.923 8.662				
3	- 2.238 10.347	- 4.607 7.977			
8	4.067 16.651	1.697 14.282	0.102 12.60		
12	2.551 15.136	0.182 12.766	- 1.503 11.08	- 7.808 4.777	
15	6.804 20.874	4.434 18.504	2.749 16.82	- 3.555 10.515	- 2.039 12.03

B. EXPERIMENT 2

B.1 CO_2 production data

ANOVA was carried out on SQRT transformed CO_2 production data multiplied by 10^6, using a GLM with 'sampling date', 'treatment', and 'treatment nested within pot number' as fixed factors and 'pot number' as a random factor. The assumptions of the ANOVA were checked by drawing a normality plot of the residuals and plotting the residuals against the fitted values.

Table A5: ANOVA table for SQRT transformed CO_2 production data multiplied by 10^6

Source	d.f.	SS	MS	*F*	*P*
Treatment	1	0.03358	0.03358	1.46	0.261
Date	10	11.94324	1.19432	352.67	0.000
Treatment*Date	10	0.03860	0.00386	1.14	0.344
Pot(treatment)	8	0.18376	0.02297	6.78	
Error	80	0.27092	0.00339		
Total	109	12.4011			

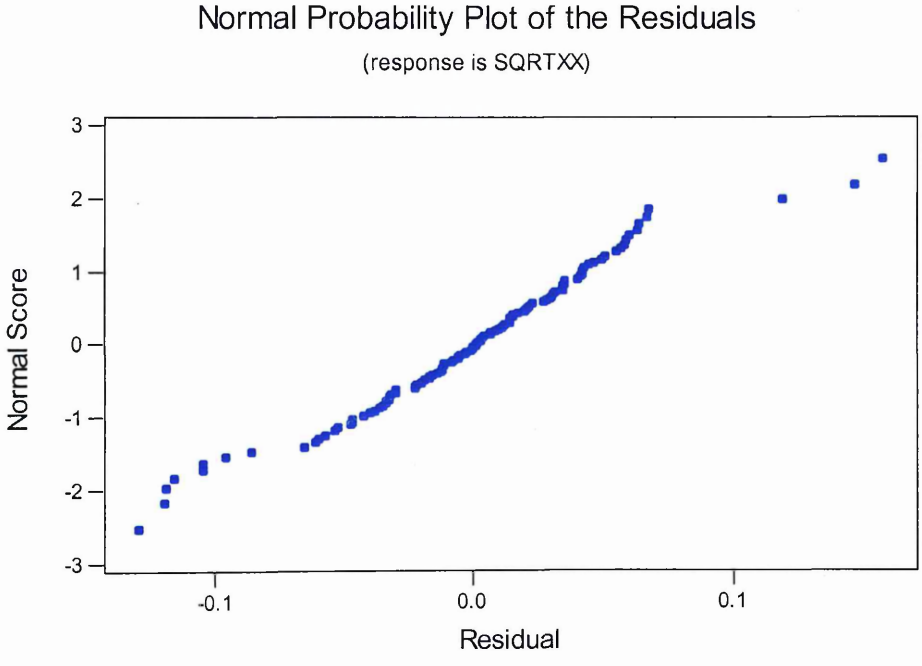

Figure A5: Normal probability plot of residuals for SQRT transformed CO_2 data multiplied by 10^6

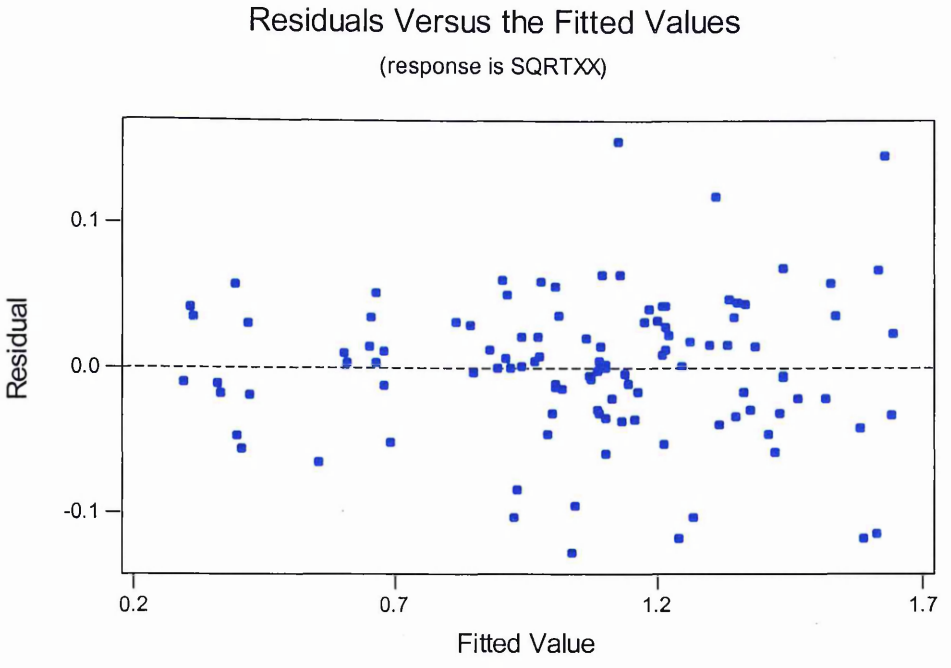

Figure A6: Plot of residuals v fitted values for SQRT transformed CO_2 data multiplied by 10^6

The data was further analysed using a Tukey's pairwise comparison (Family error rate 0.05) between sampling dates.

Table A6: 95% lower and upper confidence intervals for Tukey's pairwise comparison between sampling dates for SQRT transformed CO_2 production data multiplied by 10^6 for pots with large seaweed pieces

Week	0.3	1	2	3	4	5	6	7	8	12
1	- 0.578 - 0.374									
2	- 0.287 -0.014	0.154 0.428								
3	- 0.456 - 0.182	- 0.014 0.260	- 0.305 - 0.031							
4	- 0.628 -0.354	- 0.186 0.087	-0.477 - 0.203	- 0.309 - 0.035						
5	- 0.652 - 0.379	- 0.211 0.063	- 0.502 - 0.228	- 0.334 - 0.060	- 0.162 0.112					
6	- 0.507 - 0.233	- 0.066 0.208	- 0.357 - 0.083	- 0.189 0.085	- 0.017 0.257	0.008 0.282				
7	- 0.752 - 0.478	-0.311 -0.037	- 0.601 - 0.328	- 0.433 - 0.159	- 0.261 0.013	- 0.236 0.038	- 0.382 - 0.1.8			
8	- 0.820 - 0.546	- 0.379 - 0.105	- 0.669 - 0.396	- 0.501 -0.227	- 0.329 - 0.055	- 0.304 - 0.030	- 0.450 - 0.176	- 0.205 - 0.069		
12	- 1.063 - 0.789	- 0.622 - 0.348	- 0.912 - 0.639	- 0.744 - 0.470	- 0.572 - 0.298	- 0.547 - 0.273	- 0.692 - 0.419	- 0.448 - 0.174	- 0.380 - 0.106	
18	- 1.360 - 1.086	- 0.919 - 0.645	- 1.209 - 0.936	- 1.041 - 0.767	- 0.869 - 0.595	- 0.844 - 0.570	- 0.989 - 0.716	- 0.745 - 0.471	- 0.679 - 0.403	- 0.434 - 0.160

Table A7: 95% lower and upper confidence intervals for Tukey's pairwise comparison between sampling dates for SQRT transformed CO_2 production data multiplied by 10^6 for pots with small seaweed pieces

Week	0.3	1	2	3	4	5	6	7	8	12
1	- 0.564 - 0.291									
2	- 0.342 - 0.068	0.086 0.359								
3	- 0.432 - 0.158	- 0.005 0.269	- 0.227 0.047							
4	- 0.648 - 0.374	- 0.220 0.054	- 0.443 - 0.169	- 0.353 - 0.079						
5	- 0.677 - 0.403	- 0.249 0.024	- 0.472 - 0.198	- 0.382 - 0.108	- 0.166 0.108					
6	- 0.414 - 0.140	0.014 0.288	- 0.209 0.065	- 0.118 0.155	0.097 0.371	0.126 0.400				
7	- 0.772 - 0.499	- 0.345 - 0.071	- 0.567 - 0.294	- 0.477 - 0.204	- 0.262 0.012	- 0.233 0.041	- 0.496 - 0.222			
8	- 0.836 - 0.562	- 0.409 - 0.135	- 0.631 - 0.357	- 0.541 - 0.267	- 0.325 - 0.052	- 0.296 - 0.023	- 0.559 - 0.286	- 0.201 0.073		
12	- 1.101 - 0.827	- 0.673 - 0.340	- 0.896 - 0.622	- 0.806 - 0.532	- 0.590 - 0.316	- 0.561 - 0.287	- 0.824 - 0.550	- 0.465 - 0.192	- 0.402 - 0.128	
18	- 1.360 - 1.086	- 0.933 - 0.659	- 1.155 - 0.881	- 1.065 - 0.791	- 0.849 - 0.575	- 0.820 - 0.546	- 1.083 - 0.810	- 0.725 - 0.451	- 0.661 - 0.387	- 0.396 - 0.122

B.2 Soluble sugar concentration in soil solution

ANOVA was carried out on the concentration of soluble sugars in soil solution using a GLM with 'sampling date' and 'treatment' as factors. The assumptions of the GLM were checked by drawing a normal probability plot of the residuals and plotting the residuals against the fitted values.

Table A8: ANOVA table for soluble carbohydrate in soil solution

Source	d.f.	SS	MS	*F*	*P*
Treatment	1	9270	9270	1.18	0.287
Sample date	7	217308	31044	3.96	0.005
Treatment*Sample date	7	244569	34938	4.45	0.002
Error	25	196140	7846		
Total	40	772034			

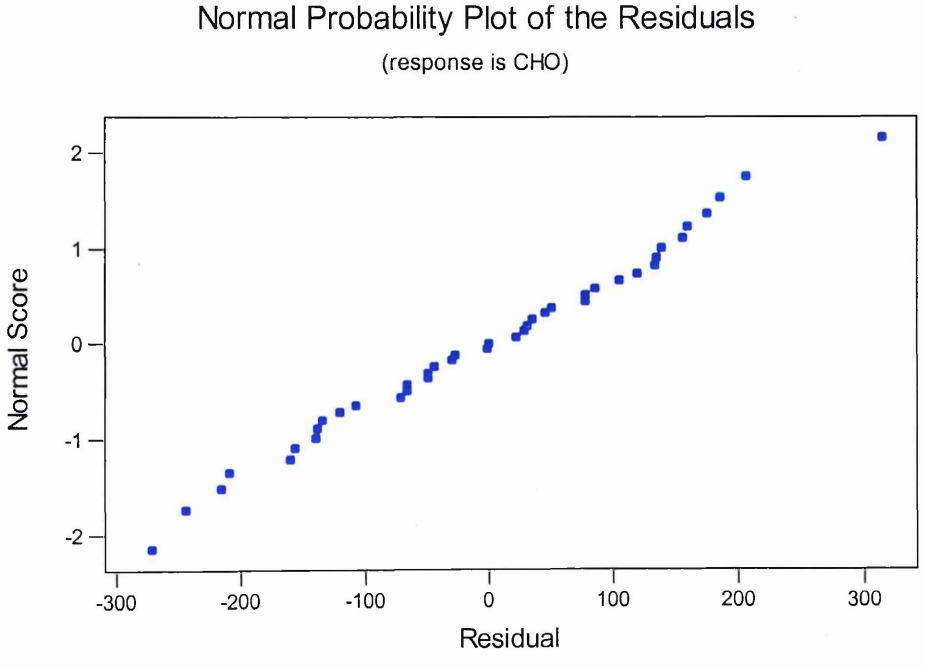

Figure A7: Normal probability plot of residuals for soluble carbohydrate in soil solution

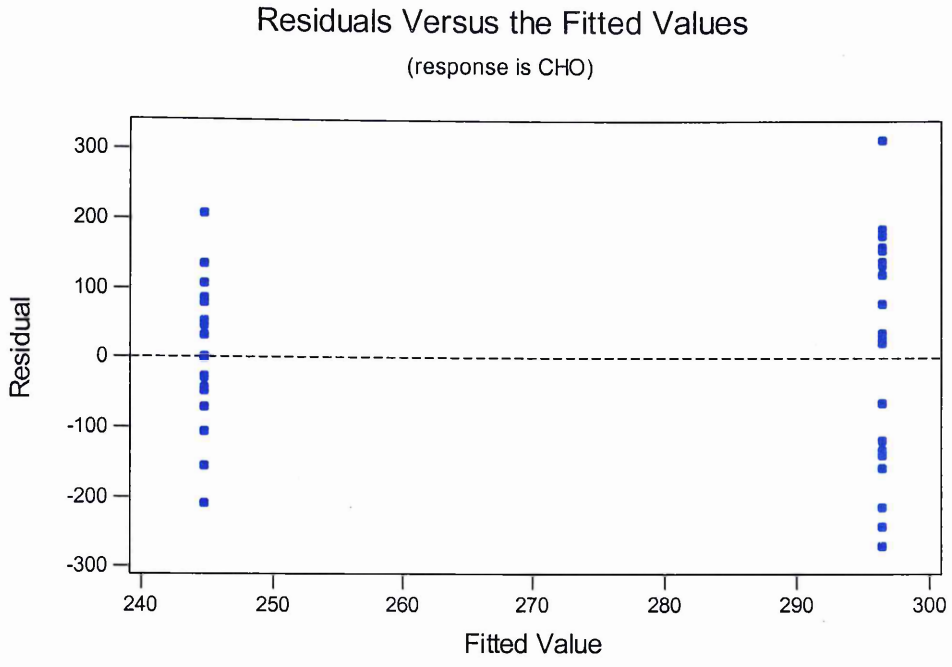

Figure A8: Plot of residuals v fitted values for soluble carbohydrate in soil solution

B.3 ^{99}Tc in soil solution

ANOVA was performed on SQRT transformed ^{99}Tc in soil solution, using a GLM with 'sampling date', 'treatment' and 'treatment nested within pot number' as fixed factors and pot number as a random factor. The assumptions of the GLM were checked by drawing a normal probability plot of the residuals and plotting the residuals against the fitted values.

Table A9: ANOVA table for SQRT transformed ^{99}Tc in soil solution

Source	d.f	SS	MS	*F*	*P*
Treatment	1	138.97	138.97	2.81	0.169
Date	6	1200.04	200.01	31.76	0.000
Treatment*date	6	138.84	23.14	3.67	0.015
Pot(treatment)	4	199.07	49.77	7.90	0.001
Error	18	113.36	6.30		
Total	35	2077.87			

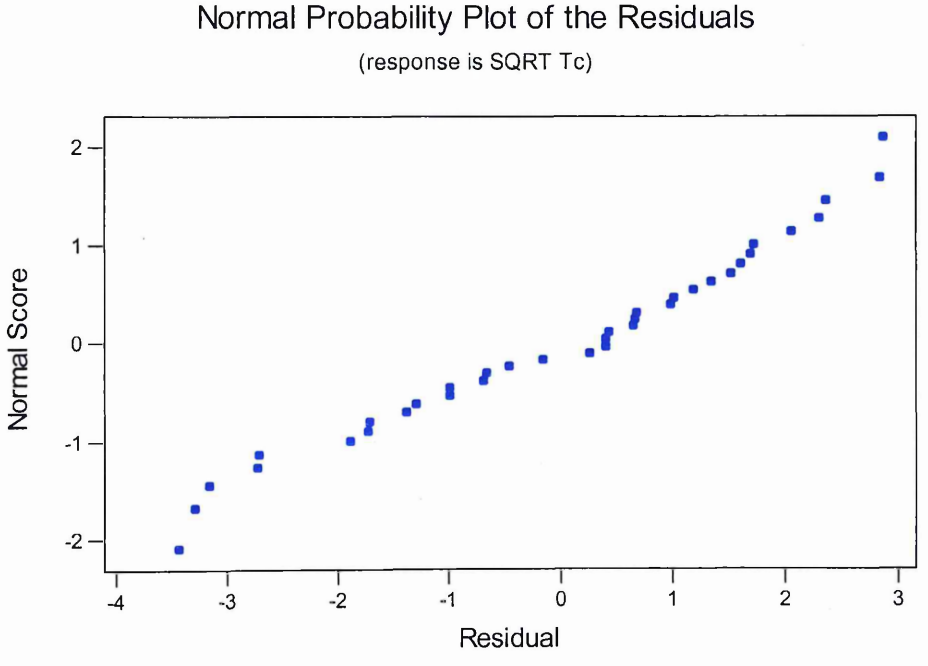

Figure A9: Normal probability plot of SQRT transformed ^{99}Tc in soil solution

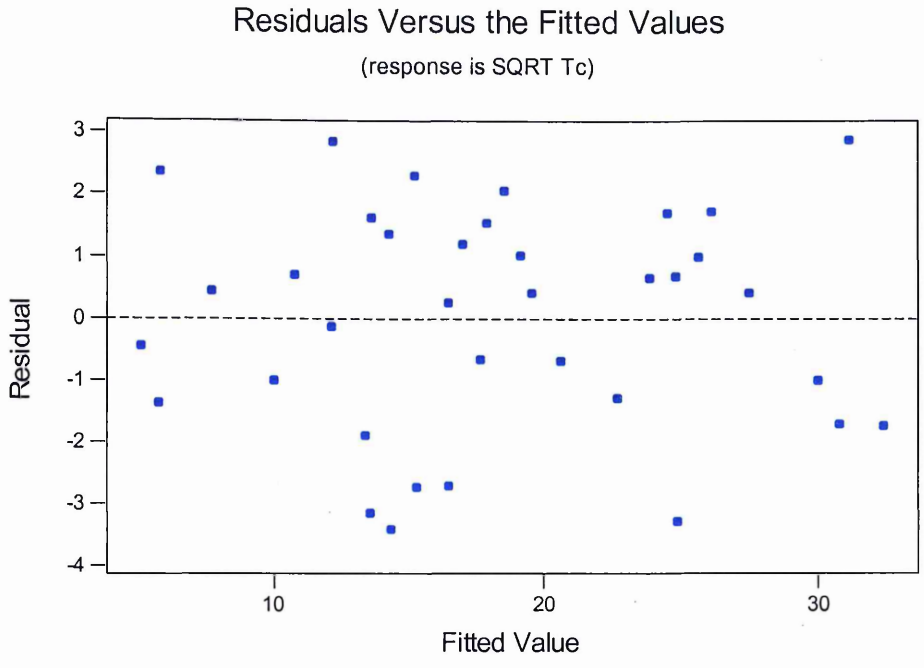

Figure A10: Plot of residuals v fitted values for SQRT transformed ^{99}Tc in soil solution

The data was further analysed using a Tukey's pairwise comparison (Family error rate 0.05) between sampling dates.

Table 10 : 95% lower and upper confidence intervals for Tukey's pairwise comparison between sampling dates for SQRT transformed ^{99}Tc data (large seaweed pieces)

Week	1	2	4	6	8	12
2	-1.446 14.289					
4	- 0.029 15.706	- 6.45 9.285				
6	0.656 16.391	- 5.77 9.970	- 7.18 8.552			
8	1.623 17.360	- 4.80 10.939	- 6.21 - 9.521	-6.90 8.837		
12	5.867 23.764	- 0.55 17.343	- 1.97 15.925	- 2.66 15.241	-3.63 14.272	
18	10.964 28.861	4.54 22.440	3.12 21.022	2.44 20.338	1.47 19.368	- 4.97 15.161

Table A11: 95% lower and upper confidence intervals for Tukey's pairwise comparison between sampling dates for SQRT transformed ^{99}Tc data (small seaweed pieces)

Week	1	2	4	6	8	12
2	- 3.300 14.65					
4	2.435 20.38	-2.132 13.60				
6	10.823 28.77	6.256 21.99	0.521 16.26			
8	2.315 22.79	- 2.149 15.91	- 7.884 10.17	- 16.27 1.787		
12	9.210 29.69	4.746 22.80	- 0.989 17.07	- 9.38 8.682	- 2.741 16.83	
18	10.812 31.29	6.347 24.41	0.612 18.67	- 7.78 10.283	- 1.139 18.13	- 8.034 11.24

C. EXPERIMENT 3

C.1 ^{99}Tc extraction data

A one-way ANOVA was carried out on the cumulative concentration of ^{99}Tc in the shaking water (% of that added with seaweed) with 'sample date' as the factor. The assumptions of the ANOVA were checked by drawing a normal probability plot of the residuals and plotting the residuals v the fitted values.

Table A12: ANOVA table for cumulative concentration of ^{99}Tc in shaking water

Source	d.f.	SS	MS	*F*	*P*
Sample date	4	3404.98	851.24	794.87	0.000
Error	10	10.71	1.07		
Total	14	3415.69			

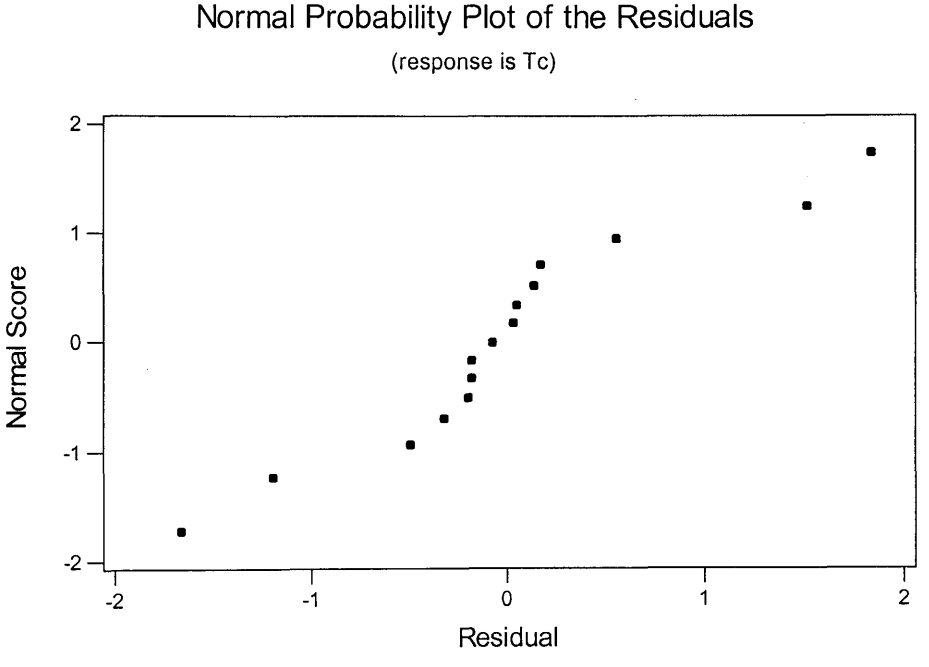

Figure A11: Normal probability plot of residuals for cumulative concentration of ^{99}Tc in shaking water

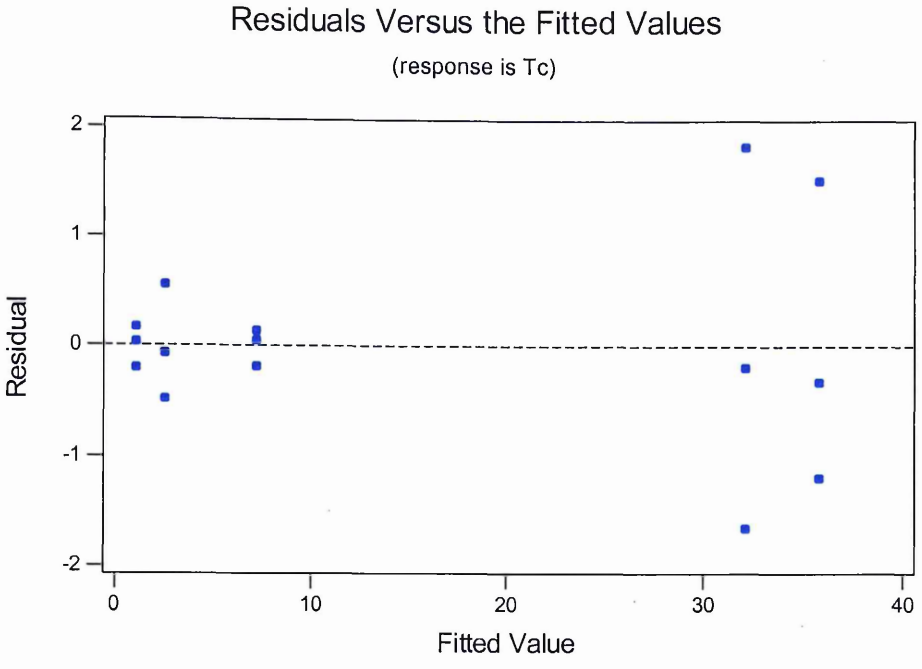

Figure A12: Plot of residuals v fitted values for cumulative concentration of ^{99}Tc in shaking water

The data was further analysed using a Tukey's pairwise comparison (Family error rate 0.05) between sampling dates.

Table A13: 95% lower and upper confidence intervals for Tukey's pairwise comparison between sampling dates for cumulative concentration of ^{99}Tc in shaking water

Day	0.5	1	2	4
1	- 1.388 4.218			
2	3.385 8.942	1.945 7.502		
4	28.262 33.818	26.822 32.373	22.10 27.65	
6	32.002 37.558	30.562 36.118	25.84 31.39	0.962 6.518

C.2 Soluble sugar extraction data

A one-way ANOVA was carried out on the cumulative concentration of soluble sugars present in the shaking water using 'sample date' as the factor. The assumptions of the ANOVA were checked by drawing a normal probability plot of the residuals and plotting the residuals against fitted values.

Table A14: ANOVA table for cumulative concentration of soluble sugars in shaking water

Source	d.f.	SS	MS	*F*	*P*
Sample date	4	102.808	25.702	122.89	0.000
Error	10	2.091	0.209		
Total	14	104.899			

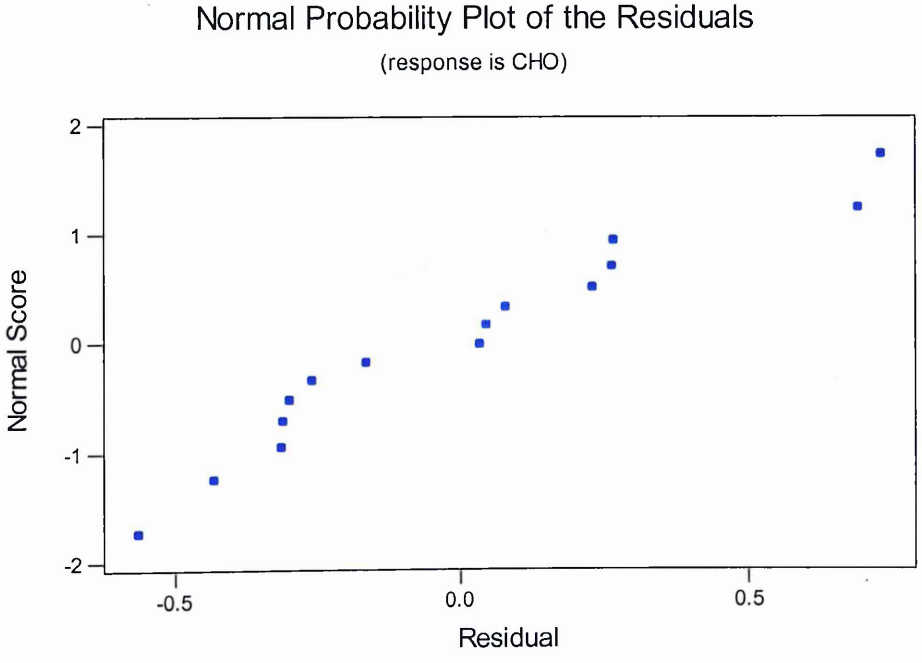

Figure A13: Normal probability plot of residuals for cumulative concentration of soluble sugars in shaking water

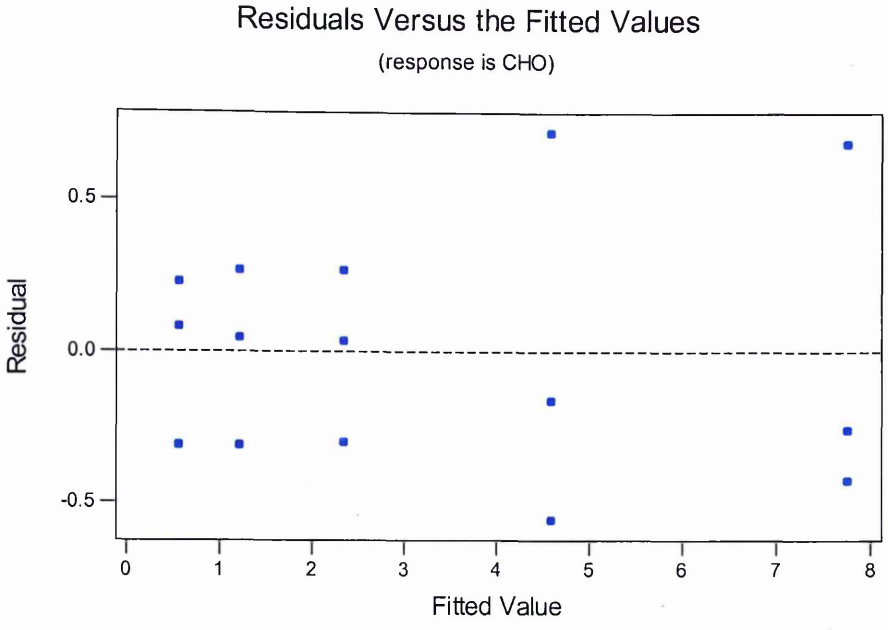

Figure A14: Plot of residuals v fitted values for cumulative concentration of soluble sugars in shaking water

The data was further analysed using a Tukey's pairwise comparison (Family error rate 0.05) between sampling dates.

Table A15: 95% lower and upper confidence intervals for Tukey's pairwise comparison between sampling dates for cumulative concentration of soluble sugars in shaking water

Day	0.5	1	2	4
1	- 0.561 1.894			
2	0.563 3.019	- 0.103 2.352		
4	2.816 5.272	2.150 4.605	1.025 3.481	
6	5.969 8.424	5.302 7.758	4.178 6.633	1.925 4.380

C.3 Ion Extraction Data

C.3.1 Shaking water samples

ANOVA was carried out on the percentage of initial ion concentration, for all ions (Na, K, Ca, Mg and ^{99}Tc), using a GLM with 'sampling date' and 'ion type' as fixed factors and 'sample' as a random factor. The assumptions of the GLM were checked by drawing a normal probability plot of the residuals and plotting the residuals v the fitted values.

Table A16: ANOVA table for ion extraction data from shaking water samples (% of initial)

Source	d.f.	SS	MS	*F*	*P*
Sample	2	21.07	10.53	1.54	0.280
Ion	4	2780.90	695.22	95.00	0.000
Sample date	4	1655.00	413.75	1050.47	0.000
Sample* ion	8	58.54	7.32	8.40	0.000
Ion*Sample date	16	2185.78	136.61	156.84	0.000
Error	32	27.87	0.87		
Total	66	6728.16			

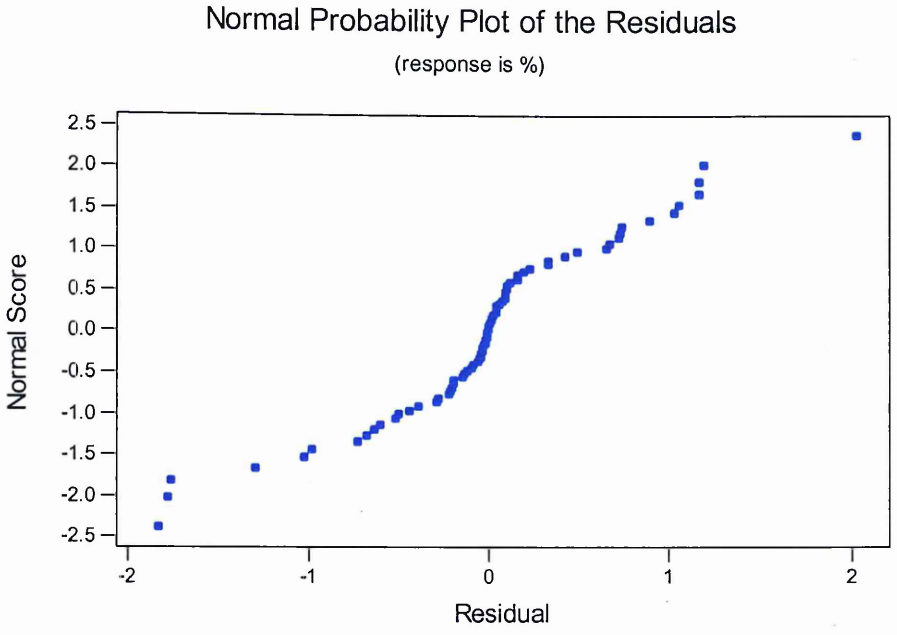

Figure A15: Normal probability plot of residuals for ion extraction data from shaking water samples (% of initial)

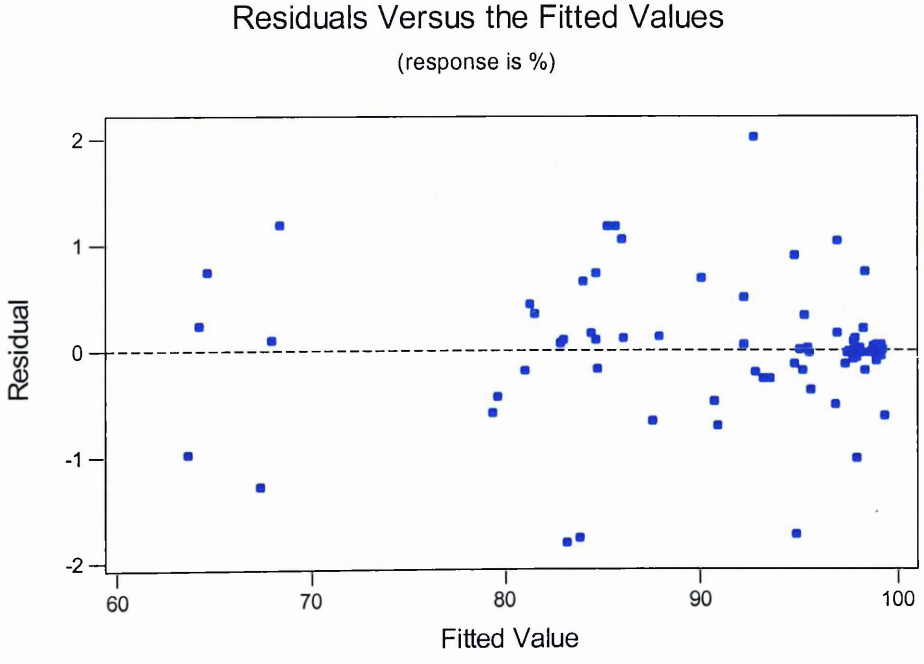

Figure A16: Plot of residuals v fitted values for ion extraction data from shaking water samples (% of initial)

The results were further analysed using a Tukey's pairwise comparison (Family error rate 0.05) between sampling dates and ion type.

Table A17: 95% lower and upper confidence intervals for Tukey's pairwise comparison between sampling dates for potassium extraction

Day	0.5	1	2	4
1	- 5.06 1.02			
2	- 7.72 - 1.64	- 5.70 0.38		
4	-13.94 - 7.86	- 11.92 - 5.84	- 9.26 - 3.18	
6	- 14.62 - 8.54	- 12.60 - 6.52	- 9.94 -3.86	-3.72 2.36

Table A18: 95% lower and upper confidence intervals for Tukey's pairwise comparison between sampling dates for sodium extraction

Day	0.5	1	2	4
1	- 5.87 0.21			
2	- 7.66 - 1.58	- 4.83 1.25		
4	- 9.11 - 3.03	- 6.28 - 0.20	- 4.48 1.60	
6	- 9.39 - 3.31	- 6.56 - 0.48	- 4.77 1.31	- 3.32 2.76

Table A19: 95% lower and upper confidence intervals and P-values (bold figure) for Tukey's pairwise comparison between sampling dates for calcium extraction

Day	0.5	1	2	4
1	- 3.25 2.83 **1.0000**			
2	- 3.55 2.23 **1.0000**	- 3.34 2.74 **1.0000**		
4	- 4.09 1.99 **0.9990**	- 3.89 2.19 **1.0000**	- 3.58 2.50 **1.0000**	
6	- 4.40 1.68 **0.9772**	- 4.19 1.89 **0.9967**	- 3.89 2.19 **1.0000**	- 3.34 2.74 **1.0000**

Table A20: 95% lower and upper confidence intervals and P-values (bold figure) for Tukey's pairwise comparison between sampling dates for magnesium extraction

Day	0.5	1	2	4
1	- 3.42 2.66 **1.0000**			
2	- 3.96 2.12 **0.9999**	- 3.58 2.50 **1.0000**		
4	- 5.82 0.26 **0.1071**	- 5.44 0.64 **0.2854**	- 4.90 1.18 **0.7199**	
6	- 6.08 0.000 **0.0506**	- 5.69 0.39 **0.1529**	- 5.16 0.92 **0.5019**	- 3.29 2.79 **1.0000**

Table A21: 95% lower and upper confidence intervals for Tukey's pairwise comparison between ^{99}Tc and all other ions for shaking water samples on all sampling dates

Day	K	Na	Mg	Ca
0.5	- 5.97	- 13.71	- 3.57	- 2.52
	- 0.32	- 8.06	2.08	3.13
1	- 4.53	- 15.10	- 2.51	- 1.29
	1.12	- 9.45	3.13	4.36
2	- 4.49	- 12.17	1.67	3.13
	1.16	- 6.52	7.32	8.78
4	14.171	11.268	24.688	27.468
	19.815	16.912	30.332	33.112
6	17.23	14.72	28.17	30.90
	22.87	20.37	33.82	36.55

Table A22: 95% upper and lower confidence intervals for Tukey's pairwise comparison between sodium and other ions over all sampling dates for shaking water samples

Day	K	Mg	Ca
0.5	4.91 10.56 **0.0001**	7.31 12.96 **0.0001**	8.36 14.01 **0.0001**
1	5.72 11.37 **0.0001**	9.76 15.41 **0.0001**	10.99 16.63 **0.0001**
2	4.86 10.50 **0.0001**	11.02 16.66 **0.0001**	12.48 18.12 **0.0001**
4	0.08 5.73 **0.0374**	10.60 16.24 **0.0001**	13.38 19.02 **0.0001**
6	- 0.32 5.33 **0.1412**	10.63 16.27 **0.0001**	13.36 19.00 **0.0001**

Table A23: 95% upper and lower confidence intervals for Tukey's pairwise comparison between potassium, and magnesium and calcium ions over all sampling dates for shaking water samples

Day	Mg	Ca
0.5	- 0.42 5.22 **0.1908**	0.63 6.27 **0.0046**
1	1.22 6.86 **0.0005**	2.44 8.09 **0.0001**
2	3.34 8.98 **0.0001**	4.80 10.44 **0.0001**
4	7.69 13.34 **0.0001**	10.47 16.12 **0.0001**
6	8.12 13.77 **0.0001**	10.85 16.50 **0.0001**

Table A24: 95% upper and lower confidence intervals for Tukey's pairwise comparison between calcium and magnesium ions over all sampling dates for shaking water samples

Day	Mg
0.5	- 4.09 1.09 0.9990
1	- 4.27 1.81 **0.9926**
2	- 4.50 1.58 **0.9530**
4	-5.82 0.26 **0.1081**
6	-5.77 0.31 **0.1243**

C.3.3 Ion concentration of seaweed data

ANOVA was carried out on the percentage of initial ion concentration, for all ions (Na, K, Ca, and Mg), using a GLM with 'sampling date' and 'ion type' as fixed factors and 'sample' as a random factor. The assumptions of the GLM were checked by drawing a normal probability plot of the residuals and plotting the residuals v the fitted values.

Table A25: ANOVA table for seaweed ion concentration data

Source	d.f.	SS	MS	*F*	*P*
Sample	2	1233.3	616.6	0.92	0.430
Ion	3	14968.9	4989.6	23.81	0.001
Sample date	5	6015.2	1203.0	1.98	0.168
Sample* ion	6	1257.2	209.5	1.42	0.239
Ion*Sample date	15	1334.3	89.0	0.60	0.848
Error	30	27.87	0.87		
Total	61	6728.16			

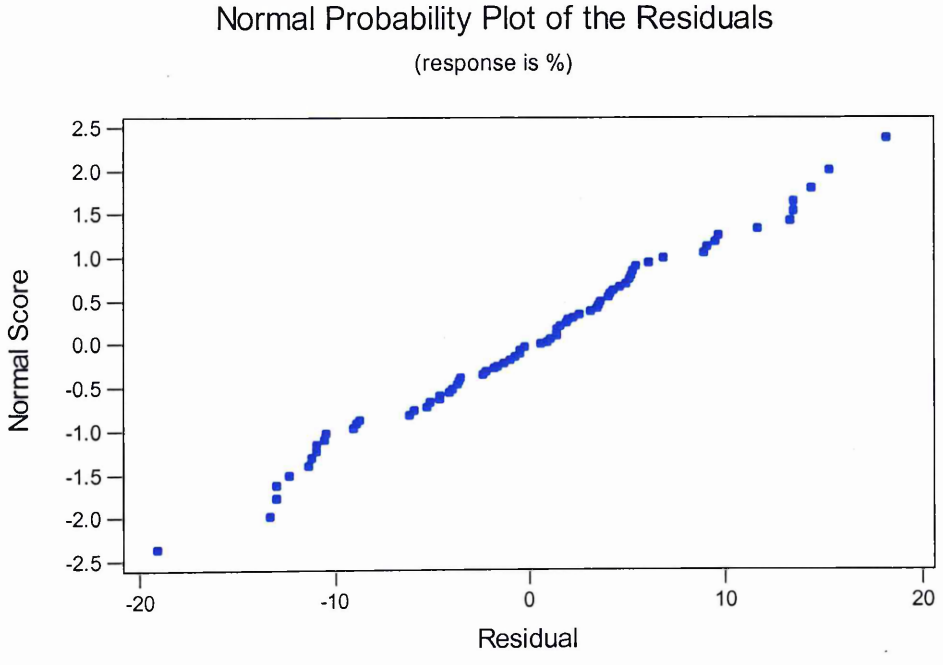

Figure A17: Normal probability plot for residuals for ion concentration of seaweed data

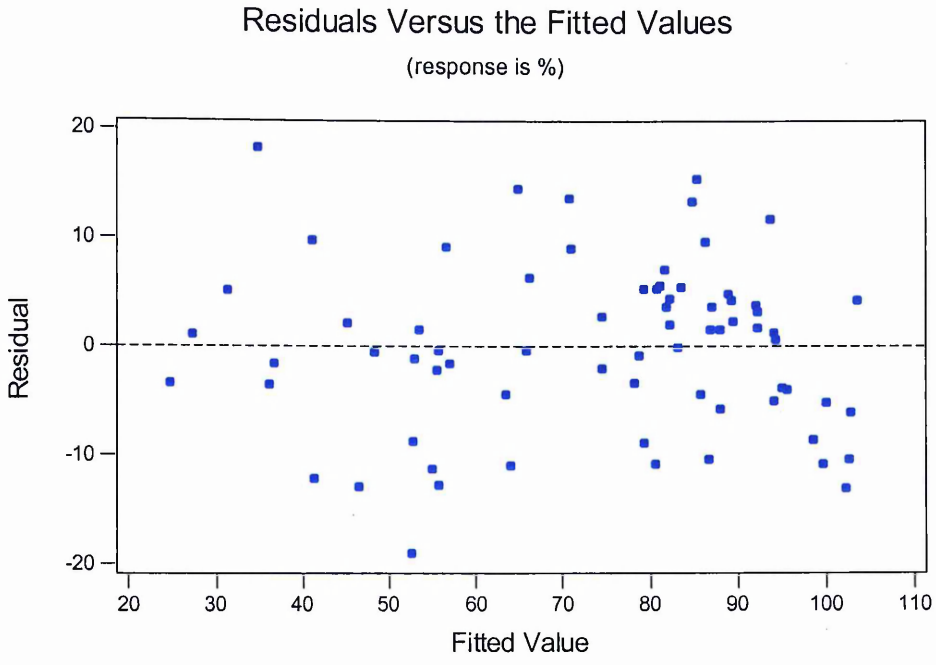

Figure A18: Plot of residuals v fitted values for ion concentration of seaweed data

The data was further analysed using Tukey's pairwise comparison (Family error rate 0.05) between sampling dates and ion type.

Table A26: 95% lower and upper confidence intervals and P-values (bold figure) for Tukey's pairwise comparison between sodium and all other ions in seaweed samples over all dates

Week	K	Mg	Ca
0.7	-11.17 67.86 **0.4350**	-12.16 66.87 **0.4979**	-9.02 70.01 **0.3122**
1.7	- 13.98 65.05 **0.6182**	- 11.95 67.08 **0.4843**	- 50.9 73.94 **0.1513**
3	- 10.93 68.10 **0.4203**	0.22 79.26 **0.0474**	13.67 92.70 **0.0016**
8	-17.17 61.87 **0.8133**	- 8.84 70.20 **0.3026**	4.06 83.10 **0.0187**
12	- 34.16 44.87 **1.0000**	- 16.79 62.24 **0.7928**	- 5.58 73.45 **0.1669**
15	- 13.49 65.54 **0.5855**	- 4.98 74.05 **0.1480**	-2.78 76.25 **0.0933**

Table A27: 95% lower and upper confidence intervals and P-values (bold figure) for Tukey's pairwise comparison between potassium, and magnesium and calcium in seaweed samples over all dates

Week	Mg	Ca
0.7	-40.51 38.53 **1.0000**	-43.69 35.34 **1.0000**
1.7	-37.49 41.546 **1.0000**	- 30.63 48.41 **0.7829**
3	- 28.36 50.67 **0.9999**	-14.92 64.12 **0.6794**
8	- 31.19 47.85 **1.0000**	-18.286 60.75 **0.8678**
12	-22.14 56.89 **0.9765**	- 10.936 68.10 **0.4205**
15	-31.01 48.023 **1.0000**	-28.81 50.23 **1.0000**

Table A28:95% lower and upper confidence intervals and P-values (bold figure) for Tukey's pairwise comparison between calcium and magnesium in seaweed samples over all dates

Week	Mg
0.7	-42.66 36.38 **1.0000**
1.7	- 43.38 32.66 **1.0000**
3	-52.96 26.07 **0.9989**
8	- 52.42 26.62 **0.9994**
12	- 50.72 28.31 **0.9999**
15	- 41.72 37.31 **1.0000**

Table A29: 95% lower and upper confidence intervals and P-values (bold figure) for Tukey's pairwise comparison for calcium concentration in seaweed samples over all sampling dates

Week	0.7	1.7	3	8	12
1.7	- 45.84 33.19 **1.0000**				
3	- 44.15 34.89 **1.0000**	- 37.82 41.21 **1.0000**			
6	- 51.65 27.18 **0.9997**	-45.52 33.51 **1.0000**	-47.22 31.81 **1.0000**		
12	-54.63 24.40 **0.9950**	-48.30 30.73 **1.0000**	- 50.00 29.03 **1.0000**	- 42.30 36.74 **1.0000**	
15	-41.10 37.93 **1.0000**	- 34.77 44.26 **1.0000**	- 36.47 42.58 **1.0000**	- 28.77 36.74 **1.0000**	- 25.99 53.05 **1.0000**

Table A30: 95% lower and upper confidence intervals and P-values (bold figure) for Tukey's pairwise comparison for potassium concentration in seaweed samples over all sampling dates

Week	0.7	1.7	3	8	12
1.7	- 52.58 26.45 **0.9993**				
3	-66.60 12.44 **0.5157**	- 53.53 25.50 **0.9981**			
6	- 70.93 8.10 **0.2669**	- 57.86 21.17 **0.9601**	- 43.85 35.18 **1.0000**		
12	- 81.06 - 2.03 **0.0309**	- 67.99 11.04 **0.4268**	- 53.98 25.05 **0.9971**	- 49.65 29.39 **1.0000**	
15	-49.66 29.23 **1.0000**	- 36.59 42.44 **1.0000**	- 22.58 56.45 **0.9819**	- 18.25 60.79 **0.8660**	- 8.12 70.92 **0.2675**

Table A31: 95% lower and upper confidence intervals and P-values (bold figure) for Tukey's pairwise comparison for magnesium concentration in seaweed samples over all sampling dates

Week	0.7	1.7	3	8	12
1.7	- 49.56 29.47 **1.0000**				
3	- 54.45 24.58 **0.9957**	- 44.40 34.63 **1.0000**			
6	- 61.61 17.42 **0.8266**	- 51.56 27.47 **0.9998**	- 46.68 32.36 **1.0000**		
12	- 62.70 16.34 **0.7670**	- 52.65 26.38 **0.9992**	- 47.76 31.27 **1.0000**	- 40.60 38.43 **1.0000**	
15	- 40.16 38.87 **1.0000**	- 30.12 48.38 **1.0000**	- 25.23 53.80 **0.9975**	- 18.07 60.96 **0.8580**	- 16.98 62.05 **0.8034**

Table A32: 95% lower and upper confidence intervals and P-values (bold figure) for Tukey's pairwise comparison for sodium concentration in seaweed samples over all sampling dates

Week	0.7	1.7	3	8	12
1.7	- 19.77 29.26 **1.0000**				
3	- 66.84 12.20 **0.5001**	- 56.58 22.45 **0.9805**			
6	- 64.94 14.10 **0.6258**	- 54.68 24.35 **0.9948**	- 37.62 41.42 **1.0000**		
12	- 58.07 20.96 **0.9557**	- 47.81 31.22 **1.0000**	- 30.75 48.28 **1.0000**	- 32.65 46.38 **1.0000**	
15	- 47.34 31.69 **1.0000**	- 37.09 41.95 **1.0000**	- 20.02 59.01 **0.9317**	- 21.92 57.11 **0.9733**	- 38.79 50.24 **1.0000**