Bioremediation of Uranium from nuclear waste using cyanobacteria

J Mathuthu

orcid.org 0000-0003-2367-2929

Mini-dissertation accepted in partial fulfilment of the requirements for the degree Masters in Radiation Science at the North-West University

Supervisor: Prof O O Babalola

Co Supervisor: Dr N D Mokhine

Co Supervisor: Dr A Ayangbenro

Graduation: July 2024

Declaration

I, Joseph Mathuthu, a master's student at the Centre of Applied Radiation Science and Technology would like to declare that the project titled, Bioremediation of Uranium using cyanobacteria, is my original work and has never been submitted anywhere.

Student number: 28763734

Signature

Acknowledgements

I would like to acknowledge my supervisory team, Prof. O.O. Babalola and Dr. Ayansina Ayangbenro as well as Dr Naomi Mokhine for their supervision and advice.

List of abbreviations

International Atomic Energy Agency	(IAEA)
Department of Energy	(DOE)
Naturally Occurring Radioactive Material	(NORM)
Economic Co-operation and Development Nuclear Agency	(OECD/NEA)
Low level waste	(LLW)
High level waste	(HLW)

List of figures

TABLE OF CONTENTS

Abstract

Regulations from the International Atomic Energy Agency (IAEA) state that handling radioactive waste is a worldwide undertaking and that methods that have been scientifically confirmed must be used at every stage of the process. The extent of contamination associated with 60 years of global nuclear activity and the inherent high financial and environmental costs associated with invasive physical and chemical clean-up strategies have sparked unprecedented interest in new passive in situ bioremediation processes for nuclear waste-contaminated sites. The purpose of this project is to use cyanobacteria to remove uranium from nuclear waste solution. Cyanobacteria possess the ability to react to harmful metals in order to reduce or eliminate the metal's toxicity. Metal mobility can change as a result of several mechanisms, which alter metal speciation. These methods include the production of metal-binding proteins such as metallothioneins, organic or inorganic precipitation, intracellular compartmentalization, extracellular sequestration, and active transport. Certain detoxifying methods of metal pollutants are facilitated by cyanobacteria. *Anabena dolionum* fared better than the other chemical and biological options that were looked at when it came to high uranium loading values as it reduced uranium with percentages ranging from 32%-80%. This study has revealed that two fresh water cyanobacteria, *Anabena dolionum* and *Anabena cycade*, have a notable ability to sequester uranyl from water. The efficiency of cyanobacteria as metal adsorbents is well-known.

Keywords: nuclear waste, uranium extraction, bioremediation, polluted environment, microorganisms, wastewater treatment

CHAPTER 1: INTRODUCTION AND PROBLEM STATEMENT

Introduction

In an era of steadily increasing reliance on nuclear power, an increase in the formation of radioactive waste from the by-products of such energy generation is unavoidable. Waste radionuclides produced and emitted (intentionally or accidently) from these power plants contribute to the already-existing radioactive contamination of the environment caused by mining and nuclear weapon testing (Chang, 2016). Nuclear industries controlled and unintentional releases, nuclear weapon testing, and nuclear accidents have dispersed radionuclides around the world, including synthetic radionuclides like ^{3}H, ^{90}Sr, ^{131}I, ^{137}Cs, and ^{241}Am. Furthermore, human activities may enhance exposure to naturally occurring radioactive material (NORM), which principally includes ^{238}U, ^{232}Th, and ^{40}K, as well as their decay products (Rogiers et al., 2022).

The accumulation of natural and manmade radionuclides in the environment can be harmful to ecosystems. Radionuclide transfer to vegetation occurs mostly through water bodies, resulting in cytogenetic damage and a reduction in reproductive potential. Furthermore, radionuclides are transferred in large quantities to fish, herbivores, and carnivores when water bodies, such as lakes, are contaminated. When radionuclides build in the food chain, contaminated potable water sources, such as groundwater, or in the atmosphere, humans can be exposed. Plants and other animal life forms readily absorb some of these components once they reach the environment. For example, Sr^{2+} is chemically similar to Ca^{2+} and is absorbed into the body, this is due to Ca^{2+} being a crucial component of mammalian bone structure, which is easily absorbed into bone tissue. Calcium is required for the structural integrity and strength of animal bones and is thus an essential component of their diet. Sr-90 continues to irradiate surrounding bone and muscle tissue after integrating into the bone matrix, causing bone sarcoma and leukaemia (Mtimunye and Chirwa, 2014). Because uranium accumulation has the potential to harm humans and ecosystems, tight control and monitoring are required, as well as prevention and remediation techniques (Rogiers et al., 2022). High amounts of mobile species such as uranium, caesium, strontium, and cobalt are found in wastewater discharged from nuclear fuel processing plants, biochemical research, radioisotope manufacture, and underground nuclear waste sites. The accumulation of these compounds in surface and groundwater systems is caused by improper disposal of waste and wastewater containing these components from diverse activities (Mtimunye and Chirwa, 2014).

Traditionally, radioactive waste from diverse industrial activity has been held for decades in specially designed facilities before being permanently disposed of. The main worry in subsurface repositories is the increased likelihood of radionuclides escaping into neighbouring groundwater systems, leaving the groundwater unfit for drinking. Due to the aforementioned worry, multidisciplinary research has been conducted to evaluate the effects of radioactive waste on underground habitats (Mtimunye and Chirwa, 2014). The disposal of radioactive waste has become a severe environmental issue. Some countries in the twenty-first century are attempting to grow its power by constructing nuclear power facilities, testing nuclear weapons, and reprocessing nuclear weapons. As a result, as a by-product of such power generation, radioactive wastes are produced (Vandana et al., 2021). The release of radioactive wastes into the environment by atomic power plants or other sources, whether intentionally or unintentionally, adds to the already existing wastes. The half-life of some of these radioactive wastes varies between hundreds and thousands of years, implying that it takes longer to reduce the radioactivity of a compound by half. The disposal of such waste materials has become a dilemma for academics, policymakers, and power generation organizations all over the world due to the long half-life periods of those waste elements (Vandana et al., 2021).

The emission of radionuclides from nuclear sites and their subsequent mobility in the environment is a source of widespread public concern, prompting a flurry of new study into important radionuclide environmental fates. Indeed, our nuclear legacy is vast, encompassing 120 Department of Energy (DOE) sites in the United States alone, as well as other facilities in Europe and the former Soviet Union (Lloyd and Renshaw, 2005). Storage has been compromised in some situations, resulting in the poisoning of trillions of gallons of groundwater and millions of cubic meters of contaminated soil and debris. Cleaning up these sites is anticipated to cost more than a trillion dollars in the United States and 50 billion pounds sterling in the United Kingdom (Lloyd and Renshaw, 2005). Given the high costs and technical limitations of current chemical-based approaches, there has been a surge in interest in the interaction of microorganisms with key radionuclides in the hope of developing cost-effective bioremediation approaches for decontamination of nuclear waste-affected sediments and waters (Lloyd and Renshaw, 2005).

The list of radionuclides produced by running fission reactors over the last 60 years is extensive, and includes ^{237}Np, Pu isotopes, Am, ^{3}H, ^{14}C, ^{85}Kr, ^{90}Sr, ^{99}Tc, ^{129}I, and ^{137}Cs, as well as uranium e,g ^{235}U from nuclear fuel. Wastes containing some or all of these radionuclides are generated at a variety of stages throughout the nuclear fuel cycle, ranging from low-level, high-volume radioactive effluents produced during uranium mining to highly radioactive plant, fuel, and liquid wastes generated during reactor operation and fuel reprocessing.

Direct release in geological storage sites is currently the most frequent method of disposing of radioactive waste. However, this process necessitates a high level of maintenance, and as the amount of by-product radioactive waste generated grows, the management and storage of such a large volume of radioactive wastes becomes a major challenge. For the cleanup of radioactive wastes, some physical and chemical approaches have been developed, such as the 'delay and decay' technique (used for short-lived, unsealed radionuclides), export as a means of disposal (for long-lived sealed sources), disposal in the sewage (for radionuclides that are temporarily open. Despite the fact that these technologies have produced few spectacular results, they have yet to achieve popular acceptability due to their high cost, time consuming nature, and potential for environmental damage (Vandana et al., 2021). Bioremediation is the process of reducing contamination to a non-toxic or undetectable level using biological agents such as microorganisms, plants, or other living organisms. Bioremediation has gained a lot of attention for cleaning environments because of its cheap cost, eco-friendly, and successful remediation capabilities (Vandana et al., 2021). Microbial interaction with radionuclides has a lot of promise for radionuclide detoxification via mineralization, accumulation, and transformation. For radioactive waste cleanup, microorganisms such as *Deinococcus radiodurans*, *Rhodotorula taiwanensis* MD1149, *Mucor mehei*, *Chlorella vulgaris*, and *Parachlorella* sp. binos have been researched (Vandana et al., 2021).

To degrade and detoxify radioactive wastes, microorganisms use a variety of methods such as biotransformation, biomineralization, and bioaccumulation (Vandana et al., 2021). The need to optimize techniques for long-term, efficient remediation in the presence of troublesome co-pollutants such as competing anions, toxic metals, organics, and chelating agents remains a concern. The evaluation of appropriate bioremediation end points (such as decreased, insoluble, sediment-bound radionuclides) is still a hot subject. The sensitivity of such 'post-reduction' minerals to reoxidation and remobilisation via microbial metabolism or abiotic mechanisms is of relevance, given the extended half-life of many key radionuclides (Kulkarni et al., 2013).

Lloyd and Renshaw stated that several novel species that extract uranium from solution have recently been discovered. At the Midnite mine, heterotrophic bacteria were recovered from an acidic uranium-contaminated substrate. The uranium buildup and resistance to the actinide were investigated in five isolates and one reference strain, *D. radiodurans*. One Gram-positive isolate with a high G+C content, closely linked to *Arthrobacter ilicis*, collected uranium as precipitates coupled with polyphosphate granules inside the cell. This link was proposed by the authors as a possible detoxifying mechanism in this organism (Lloyd and Renshaw, 2005). The most common methods for uranium bioremediation are (a) enzymatic metal bioreduction of soluble U(VI) to sparingly soluble U(IV), (b) biosorption on cell surfaces, biopolymers, or dead biomass, and (c)

bioprecipitation of U(VI) with ligands such as inorganic phosphate. Enzymatic bioprecipitation of heavy metals as metal phosphates employing microbial phosphatases is one of them, and it is thought to be a promising new strategy for uranium bioremediation (Kulkarni et al., 2013). In contrast to reduced uranium minerals like uraninite [U(IV)], which have a tendency to reoxidize back to the more soluble U(VI), uranium phosphate precipitates [U(VI)] are resistant to oxidation state changes and hence constitute a long-term stable sink for uranium in contaminated environments (Kulkarni et al., 2013). Using bacterial acid phosphatases, many investigations have shown that uranium and other heavy metals (such as cadmium) can be bioprecipitated from acidic wastes. The use of naturally occurring bacteria expressing phosphatases, such as *Citrobacter* sp., *Bacillus* sp., *Rahnella* sp., and *Pseudomonas* sp., for bioremediation of heavy metals like cadmium and uranium in acidic to neutral pH ranges has been described (Jacob et al., 2018). Plants, microbes, and other biological agents such as these are regarded as effective alternative tools for metal removal since they provide simple and environmentally acceptable methods for removing metal. Utilizing biological resources, bioremediation entails the adsorption, reduction, or removal of pollutants from the environment (both microorganisms and plants). Microorganisms' abilities to remove heavy metals come from self-defence mechanisms such enzyme production and cellular morphological modifications. In these defence systems, microbial enzymes including oxidoreductases, oxygenases, and others have a direct role in influencing the rate of bioremediation. Additionally, the use of immobilization techniques on an industrial scale is improving (Jacob et al., 2018).

Aim and Objectives

The aim of this project is to investigate the bioremediation of uranium from nuclear waste solution using cyanobacteria .

Objectives are to:

Specific objectives are to:

- Measure the uranium concentration in the nuclear waste solution using UVVIS Spectrophotometry.
- Determine the effects of bacteria removal in different concentrations of uranium solutions.
- Determine the optimum conditions (pH, temperature and moisture level) of uranium removal by cyanobacteria.

Problem statement

In this research, cyanobacteria were isolated from flowing water and used in the removal of uranium from contaminated water. Afterwards, the isolated cyanobacteria were introduced to uranium waste from CARST (Centre for Applied Radiation Science and Technology) for removal of uranium. The study postulates that if cyanobacteria bioremediates the uranium waste the uranium concentration in the waste will be reduced and this is measured by comparing before and after concentrations of uranium through analyses using UV – visible photospectroscopy. If this experiment is a success it will help combat uranium waste contamination in the environment through use of cyanobacteria. This will also help to better understand the interaction between uranium and cyanobacteria and adds to the body of scientific knowledge

Rationale

Aside from occasional nuclear disasters, such as the Chernobyl disaster in 1986 and the Fukushima Daiichi nuclear disaster in 2011, the widespread use of radioactive materials at research and development, biomedical, and industrial sites has resulted in a significant build-up of radioactive waste. Wastewater discharged by nuclear fuel processing plants, biochemical research, radioisotope manufacturing, and underground nuclear waste sites contains high concentrations of mobile species, including uranium (UO^{22+}), caesium ($^{70}Cs^{+}$), strontium ($^{90}Sr^{2+}$), and cobalt ($^{60}Co^{2+}$) (Prakash et al., 2013). The incorrect disposal of waste and wastewater containing these components from various activities leads to the accumulation of these compounds in surface and groundwater systems. Fission products and U(VI) species are characterized by short half-lives and high radiological decay rates. The waste matrix containing uranium and its fission products is extremely radiotoxic and harmful due to the high radioactivity and decay rates of these elements (Prakash et al., 2013).

One of the most significant difficulties facing the worldwide nuclear industry today is the appropriate management of radioactive waste (Al Nuaimi and Williams, 2022). One of the most important aspects of nuclear waste management is waste minimisation, which is described as the process of lowering the volume and activity of radioactive wastes as much as feasible (Xu et al., 2021). The disposal of radioactive contamination is a problem to scientists, governments, and energy businesses throughout the world due to the extended half-lives of many of these dangerous radionuclides (Evelyn Chang 2016). Current attempts to remove and reduce the discharge of radioactive wastes rely heavily on the isolation of harmful compounds in containment tanks or geologic depositories in the hopes of minimizing leakage into human surroundings. This is where bioremediation comes in.

CHAPTER 2: LITERATURE REVIEW

Bioremediation

Bioremediation is a cutting-edge, environmentally friendly method that uses natural biological processes to entirely remove hazardous pollutants. any procedure that restores the natural environment damaged by contaminants to its original state using microorganisms, fungus, green plants, or their enzymes. In general, there are two types of bioremediation technologies: in situ and ex situ. Ex situ bioremediation entails removing the contaminated material from the site to be treated elsewhere, whereas in situ bioremediation treats the polluted material on the spot. Bioventing, landfarming, bioreactor, compositing, bioaugmentation, rhizofiltration, and bio-stimulaiton are a few examples of bioremediation technology. Bioremediators are microorganisms that carry out the bioremediation process.

Advantages of Bioremediation

• Typically, natural biological processes are used in bioremediation (Vidali

2001).

• This method is inexpensive and requires little in the way of technical support (Vidali 2001).

• Additionally, the public has come to accept it highly because of its environmental friendliness (Vidali 2001).

• Bioremediation techniques are far more cost-effective than conventional incineration techniques (Colberg and Young 1995).

Disadvantages

• Chlorinated organics and high aromatic hydrocarbons are strong contaminants that are shown to be resistant to microbial attack, making them the only kind of contaminants on which bioremediation is effective. Because of this, the process might not be able to detect any degrading reaction or, if it does, it might happen very slowly (Colberg and Young 1995).

• The procedure takes a while—it can take decades at times.

• Experience and knowledge are necessary for the planning and execution of a successful bioremediation program.

• We don't fully understand the components and process of bioremediation (Vidali 2001).

Aims of Bioremediation

The aim of this study was to

The following are the goals of this study that must be completed in order to achieve the objective of ensuring public and environmental safety:

- to achieve the goal of handling the wastes in ways that protect human health and limit the impact on the environment.
- The wastes would need to be segregated or diluted so that the rate or concentration of any radionuclides returned to the environment is safe.
- Combat uranium contamination in the environment.
- Reducing uranium bioavailability in the environment.

Bioremediation being green

One of the main principles of green technology is sustainable development. The ultimate goal of "green technology" is to satisfy societal needs in a way that doesn't deplete the natural resources over time (Yap et al., 2021).

Radioactive waste

The fact that nature has been a significant source of radioactive waste is somewhat unexpected. There is a huge reservoir of primordial radioactivity on the Earth's surface and in the terrestrial crust. Sand mounds, volcanic eruptions, and mineral springs all contain trace amounts of radioactive elements (Lutze and Ewing, 1988).

What is radioactive waste?

Wastes containing radioactive materials are referred to as radioactive wastes. Radioactive materials are made up of unstable atoms that decay and release ionizing radiation. Radioactivity is a natural process in which every atom that is not in its stable state releases its surplus energy in order to become stable (Vandana et al., 2021). Radioactive decay is the name of this process. The process of radioactive decay is atom-specific, and no two types of atom decay at the same rate. Radioactive waste is produced by the nuclear fuel cycle, which is used to create electricity; as well as research, medical, military, and industrial purposes; and accidents (Vandana et al., 2021). Because the biogeochemical complexities at radionuclide and metal-contaminated mixed waste sites make remediation a difficult process, with major economic and technological obstacles, bioremediation

with microorganisms remains the most cost-effective viable cleaning option. It has been shown that uranium-contaminated sites frequently harbour viable and metabolically active microorganisms capable of executing various modes of metal–microbe interaction in order to maintain viability, and that these microorganisms can have a significant impact on the form and distribution of uranium in the environment (Choudhary and Sar, 2011). Radioactive atoms and vast amounts of energy are released during reactions, specifically the fission of nuclei like U and Pu and the fusion of elements like hydrogen. Both processes have the potential to produce enormous, uncontrolled energy releases. To some extent, these reactions can occur in "atom" and "hydrogen" of natural origin or in (thermonuclear) bombs. As in many other businesses, the nuclear industry also produces waste materials that are unusable and unwanted; the leftovers are hazardous (Lutze and Ewing, 1988). Animals and humans alike are at risk from the biological and radiological consequences of uranium isotopes and their progeny. This scenario involves a higher incidence of chemical toxicity than radiotoxicity(Banala et al., 2021). In reducing conditions, the more common and less toxic form of uraninite (UO_2)[s] is insoluble and immobile. Both marine and terrestrial organisms consume uranium that is released into the environment. After entering the circulation, uranium combines with several proteins, including transferrin, human serum albumin, and uranyl bis- and tris-carbonate complexes as well as UO_2-protein complexes. Uranium has a great affinity for phosphate groups and binds to phosphorylated peptides (Banala et al., 2021).

Naturally Occurring Radioactive Material (NORM)

Cosmic radiation and radiation resulting from the decay of naturally existing radionuclides are both examples of natural radiation. Radionuclides believed to be of natural origin were first detected in the oil and gas industry as early as 1904 (Paul et al., 2022). A quantifiable exposure to NORMs occurs in humans. In every region of the earth's mantle and in every living thing's tissue, (NORMs) have always existed in a range of amounts. There is natural radioactivity practically everywhere: in the soil, tap water, oil, and atmosphere (Ali, 2008). The second source of radioactive waste is a by-product of industrial mining, which involves removing radioactive earth material during mineral exploration and exploitation, using just a portion of it, and discarding the remainder as radioactive waste. These are commonly found as residual waste in companies that process fertilizers, iron, thorium, uranium, fossil fuels, cement, etc. They are known as NORMs (Lutze and Ewing, 1988). The term NORM was first used in the late 1980s and has since been adopted by a number of different sectors for monitoring radioactive material. The radionuclides found in oil and gas streams are from the natural decay chains of the primordial radionuclides ^{238}U and ^{232}Th, as well as ^{40}K (IAEA, 2003a, b). Several investigations have revealed the presence of (NORM) in oil and gas fields around the world (Khodashenas et al., 2012). NORM refers to radioisotopes or radioactive

materials that exist naturally in the Earth's crust but have their ionizing radiation exposed to the public domain owing to anthropogenic activities. NORM is produced through the combustion of fossil fuels, as well as mining, fertilizer use, and gas production. Radionuclides of uranium, thorium, radium, radon, lead, and polonium are examples of(NORMs), which can be found in some oil and gas infrastructure (e.g. pipelines). Because of their chemistries and the physical processes of oil and gas extraction, NORMs are found in oil and gas reservoirs all over the world and can cause pollution in subsea infrastructure in the form of scales and sludges (Koppel et al., 2022). The most common cause of NORM exposure is uranium mining. The U-238 and Th-232 decay series are important sources. Another source of NORM is radon gas, which is a decay product of radium but also appears in the intermediate step of radioactive decay of a variety of short-lived radioactive elements. Examples of NORM businesses include the production of oil and gas, the mining and burning of coal, the mining and processing of metals and uranium, the production of geothermal energy, the treatment of groundwater, and the phosphate mining industry, which produces fertilizer (Rogiers et al., 2022). Xhixha and colleagues state that in Albania, as part of the flow back and brine generation, all oil extraction procedures induce Radioactive Materials (NORMs) to rise to the surface (Xhixha et al., 2015). The oil and gas sector produces NORMs, which are residues enriched with radium isotopes originating from uranium and thorium in reservoir rocks. Furthermore, whereas uranium and thorium are both present in hydrocarbon reservoir rocks and are basically insoluble under reducing conditions, their progenies ^{226}Ra and ^{228}Ra concentrate in formation waters. As a result, the long-lived uranium and thorium parent radionuclides do not support the ^{226}Ra and ^{228}Ra , and the ^{226}Ra and ^{228}Ra tend to concentrate in the formation fluids due to half-lives of 1600 and 5.75 years, respectively (Xhixha et al., 2015). Baeza and colleagues also found out that electricity generation from coal-fired thermal power plants (CFPP) is classified as a NORM industry. This is due to the fact that, depending on their origin, the fuels used include varying levels of natural radionuclides, primarily from the uranium and thorium radioactive series. Second, the combustion process produces by-products such as fly ash and slag, which have higher radioactive concentrations than the comparable fuels. Finally, part of these activities may be integrated into the environment as a result of the routine operation of these factories. Indeed, as the fuel is burned in the boilers of these plants, some of the natural radionuclides included in the fuel are volatilized and discharged into the atmosphere (Baeza et al., 2011). At low background activities, NORMs can be found in all situations. They cycle and partition naturally in the environment, reflecting a variety of environmental processes and chemistries (Koppel et al., 2022). Depositional, environmental, and diagenetic lithology geogenic variables influence naturally occurring radioactive elements. As a result, analysing these components serves as a method for determining the ionizing radiation effect in rocky sequences (Salazar et al., 2021). Radioactive material NORMs in different environmental

media with increased human health risks have been identified by the US Department of Health and Human Services (Paul et al., 2022). (NORMs) comprising radionuclides like potassium-40, as well as those in the uranium and thorium family, are found in soil, rocks, and minerals in a wide range of quantities. These minerals scattered throughout the environment, or NORMs, are increasingly exploited as raw materials as global industrial activity develops. Because NORMs have a high degree of radioactivity, there is a risk of exposure to handlers and radiation impacts in the environment during their whole lifecycle, when the NORMs are utilized, products are manufactured, and by-products or trash are formed (Lee et al., 2021).

The possible impact of ionizing radiation from increased exposure to NORM on individuals and the environment is currently not adequately accounted for in Life Cycle. Extending the amount of radionuclides included by ionising radiation life cycle impact assessment (LCIA) models for both human health and biota is a high priority topic, according to the ILCD manual (Goronovski et al., 2018). In huge amounts, naturally occurring alpha-emitting uranium isotopes and their decay products can be found in the world's oceans. NORMs deliver > 98 percent of radiation dose to humans, according to the United Nations Committee on the Effects of Atomic Radiation, with a global average human exposure from NORMs of 2.4 mSv per year (Uddin et al., 2017). Normal quantities NORMs are present in all environmental materials (soils, rocks, water, food) and do not differ significantly from one location to another (Odumo et al., 2011). There is widespread NORMs contamination in oil and gas installations. Maintenance staff and other employees may be exposed to hazardous quantities if any pollution is serious enough. Oil production facilities frequently include radium contamination, but natural gas production and processing facilities are more likely to have radon and its decay products (Gray, 1993).

The radioactive decay of NORMS

The two naturally occurring radioisotopes that initiate the process of radioactive decay are uranium and thorium. These radioactive elements' nuclei emit neutrons, gamma, beta, and alpha particles during their decay process. They also convert into other nuclei until they reach a stable form (Paul et al., 2022).

Uranium

It is estimated that supernovae produced uranium about 6.6 billion years ago. Its gradual radioactivity in the earth's crust, though rare in the solar system, is believed to be the main source of heat that maintains the outer core's liquid state and drives mantle convection, which in turn drives plate tectonics. The decay of potassium 40 and thorium are mixed with this radioactivity. The element was discovered by German chemist Martin Heinrich Klaproth. In 1789, he dissolved

pitchblende in nitric acid and neutralized the solution with sodium hydroxide in his experimental lab in Berlin. This allowed him to precipitate a yellow compound, presumably sodium iduronate. Thinking it was the oxide of some unknown element, he burnt the yellow stuff with charcoal to generate a black powder that he mistakenly believed to be the newly discovered metal (it was an oxide of uranium). The planet Uranus, named after the ancient Greek sky deity, was named by William Herschel eight years before the newly found element was discovered (Awan and Khan, 2015). An environmental risk brought on by the growth of the nuclear sector is uranium (U) pollution. The most radioactive actinide element in the periodic table, uranium is highly chemically poisonous and poses a serious risk to both human and environmental health. Anthropogenic activity has significantly increased uranium pollution as a result of processing uranium, using enriched uranium for electricity generation, conducting nuclear research, and producing nuclear weapons. Uranium is mostly found as hexavalent uranium (U(VI)) and tetravalent uranium (U(IV)) under the majority of naturally occurring redox conditions. U(VI) is more environmentally hazardous than U(IV) due to its substantially higher solubility and mobility, especially if it builds up in the food chain (You et al., 2021). Uranium ore may be found in enormous amounts all over the world, and well-established industrial procedures can be used to extract uranium oxide or metal from it. Natural uranium consists of a mixture of two isotopes, U-235 and U-238, as it occurs in nature. Natural uranium has a low U-235 concentration (Schaper, 2013). Uranium is found in nature as uranium-238 (99.2739 to 99.2752%), uranium-235 (0.7198 to 0.7202%), and very little uranium-234 (0.0050 to 0.0059%). Uranium emits an alpha particle during its gradual disintegration. Since uranium 238 has a half-life of approximately 4.47 billion years and uranium 235 has a half-life of 704 million years, they can be used to calculate the age of the earth. Uranium-235 was found to be the fissile isotope first. Although they are not fissile, other naturally occurring isotopes are fissionable. When uranium-235 is exposed to slow neutrons, it usually splits into two smaller nuclei, releasing two or three extra neutrons as well as nuclear binding energy. A nuclear chain reaction that can result in an explosion or, in certain circumstances, a burst of heat occurs when too many of these neutrons are absorbed by other uranium-235 nuclei(Awan and Khan, 2015). Uranium (U) is a radioactive metallic element that is essential not just as a unique raw material for nuclear power, but also in industrial applications and as a potential environmental threat. Photography, leather, wood, and nuclear energy are just a few of the sectors that use U. Its usage in nuclear energy, in particular, is becoming more crucial. U, on the other hand, has a wide range of harmful effects on living organisms (Ozdemir et al., 2017). Uranium, a metal that is hazardous to living things, is recovered from natural ores in one of two ways: acidic or alkaline extraction. Uranium traces can be found in mill tailings and many types of nuclear waste. At alkaline pH, uranium (VI) forms extremely soluble carbonate complexes that contaminate groundwater and soil, posing health

risks (Appukuttan et al., 2010). Because U(VI) has a far higher solubility and mobility than U(IV), it is more environmentally hazardous, especially if it accumulates in the food chain (You et al., 2021). The two isotopes have differing physical qualities despite being chemically identical: When a neutron strikes a U-235 nucleus, it fissions, producing two fission fragments, 2-3 neutrons, and a substantial amount of energy. The greater the probability of a U^{235} fission, the lower the neutron energy (Schaper, 2013). Uranium (U) is a naturally occurring radioactive element with a specific density of 19 g/cm that can be found in many physical and chemical forms in the earth's crust. Uranium is found in nature as pitchblende, uraninite, carnotite, autunite, uranophane, and torbernite, which are all complex ores. Uraninite, also known as pitchblende, is the principal mineral of uranium, accounting for roughly half to eighty percent of the entire ore. The amount of Uranium in the Earth's crust is around 2.8 mg/kg. Uranium concentrations in river water range from 0.01 to 6.6 g/L, whereas groundwater and seawater have 30 g/L and 3.32 g/L, respectively. However, substantial uranium concentrations have been documented in some areas as a result of numerous anthropogenic activities (Banala et al., 2021). Nevertheless, uranium deposited inside the cells has no recognized biological role, contrary to other metabolically significant metals including Fe, Cu, Zn, Co, and Mn, according to several study studies. Because precipitated uraninite nanoparticles are in the most advantageous thermodynamic state under intracellular redox circumstances, it is safe to deduce that they make up the majority of the cytoplasmic precipitate observed inside cells(Mtimunye and Chirwa, 2014).

Uranium pollution of the environment from phosphate enrichment, radioactive storage site leaks, and uranium mining pose a major risk to human health. In addition to radiation, the variety, structure, and function of ecosystems are seriously threatened by uranium's toxicity to all living things(Choudhary and Sar, 2011). The mobility of uranium in its surroundings is determined by its speciation and redox state (Newsome et al., 2014).

Uses of uranium

Due to its use in both civil and military applications, such as the production of energy, in the medical industry, and for the creation of nuclear weapons, uranium metal and its compounds have attracted a significant deal of interest from physicists and chemists (Awan and Khan, 2015). According to the existing state of affairs, there will not be a major decrease in uranium use in the upcoming years, which suggests that there will be a rising trend in the production of radioactive waste (Deng et al., 2020). Researchers have focused a lot of attention on nuclear power, a wonderful source of clean energy with high efficiency. One of the most important radioactive fuels for nuclear reactors is uranium (VI) (U(VI)). The rising use of nuclear energy may result in the

depletion of current land uranium reserves within a century. The oceans contain over 4.5 billion tons of uranium, which is almost 1000 times more than is found in conventional ore sources and is sufficient to run reactors for 1300 years (Zhu et al., 2022). Throughout much of the 19th century, uranium was not considered to be particularly harmful, which encouraged the development of numerous uses for the element, including the colouring of glass and pottery. Using uranium, Henri Becquerel discovered radioactivity in 1896. He made the finding in Paris by placing a sample of the potassium uranyl sulphate ($K_2UO_2(SO_4)_2$) uranium salt on top of an undeveloped photographic plate and observing that the plate had "fogged." He came to the conclusion that the plate had been exposed by some kind of intangible light or by uranium's radiation (Awan and Khan, 2015).

Radioactive waste from accidents

Nuclear accidents can produce radioactive waste, which is more harmful than waste from other sources. Accidental radioactive wastes result in an uncontrolled amount of emission or direct discharge into the environment (Vandana et al., 2021). Throughout the 45-year history of the nuclear industry, several mishaps have led to environmental radioactive pollution (Eisenbud and Gesell, 1997). The mechanical (physical) protection of the tanks should be improved in order to reduce the risk of accidents, such as the direct impact of objects containing harmful commodities on the boilers. Decompression, the leakage of liquid radioactive waste, and radiation pollution of the local environment are all likely outcomes of boiler damage (Tumanov, 2019). Gas cylinders are potential sources of explosion risk, which can result in the breakdown of equipment delivered, damage of hull structures, and the development of a radiation catastrophe. The following factors may contribute to cylinder explosions. When a gas cylinder's integrity is compromised due to the failure of any node (armature), damage to or corrosion of the casing, or inappropriate operation, the cylinder may explode at its operating pressure. To protect public safety, particularly when transporting trash to disposal facilities, radiological assessments of potential mishaps involving the dispersion of radioactive material are required (Lee et al., 2019). Therefore, protecting the transportation process—especially handling processes, the security of transported commodities, and environmental security—is a crucial concern (Tumanov, 2019).

Examples of Accidental radioactive waste events

In 1986, four RBMK-1000 reactors were employed near the Chernobyl Nuclear Power Plant in Prypyat, Ukraine, 15 km from the proper populated region of Chernobyl, with the goal of providing 1 MW electrical power generation. The RBMK-1000 reactors were graphite-moderated water-

cooled reactors with no containment tank in the western type. The personnel bypassed the safety systems on April 26 to conduct a test, which resulted in a steam explosion. The steam explosion damaged the reactor's upper cover, allowing almost all of the reactor's core water to escape. A suspected second hydrogen explosion occurred as a result of the reaction between steam and graphite or zirconium (Vandana et al., 2021). Three people were killed instantly by the explosion, with another 26 people, including firefighters, dying days later from severe radiation. A total of 238 people survived acute radiation illness (Vandana et al., 2021).

In 1987, two persons from a clinic in Goiania, Brazil, carried two sealed containers home from a radiation unit that where left out and burst the seal. The 1375 curies of cesium-137 chloride salt in the sealed canisters were eventually exposed. They subsequently sold it to someone else, and over the course of a few days, a large area got exposed to it due to its unwitting spread. The incident began on September 12th, and by September 28th 1987, many people had been ill. The government was warned on September 29th, and they began their hunt for contamination and threat removal. In the city's Olympic stadium, the authority set up facilities for injured and contaminated people. A total of 112,800 persons were checked, with 129 of them being found to be radioactively contaminated. This catastrophe resulted in the deaths of five persons and the serious injuries of twenty more (Vandana et al., 2021).

Fukushima accident

Along side the massive Tohoku earthquake and tsunami that struck on March 11, 2011, there was a nuclear disaster at the Fukushima Daiichi nuclear power plant (FDNPP), which resulted in massive radioactive emissions into the atmosphere and the ocean. Air, water, soil, and food can get contaminated by radionuclides, especially iodine-131 (I-131) and cesium-137 (Cs-137), which have a negative impact on human health (Morino et al., 2011).

Radioactive waste from military use

Civil and military nuclear projects are the main sources of radioactive waste (Roberts, 1990). Nuclear weapon testing for military purposes causes massive environmental damage by the emission of radioactive wastes or radionuclides. The testing of nuclear weapons peaked during the Cold War era, following the historic Hiroshima and Nagasaki nuclear bombings during WWII. Between 1945 and 1980, atmospheric tests in the United States totalled 428 megatons, or 29,000 times the magnitude of the Nagasaki nuclear weapon. Although the adoption of the Non-proliferation Treaty and the end of the Cold War era have put a stop to the ongoing competition for

nuclear weapons, it is worth noting that many other countries, aside from the then-global competitors of the West versus the Soviets, have also achieved nuclear capability (Vandana et al., 2021). The main danger with nuclear weapons testing is that radioactive debris becomes trapped in the atmosphere, partitioning in the troposphere and stratosphere, and eventually precipitating for shorter or longer periods of time (Vandana et al., 2021). Military production reactors use low-rated fuel that is discharged after low burn-up, and the amount of waste produced as a result is relatively large when compared to the overall amount of power produced by the reactors. The specifics of the reprocessing chemistry used determine the amounts involved. The scale of defence wastes in the UK is far lower than that of the civil nuclear program. Although the military program generated three times as much low level waste (LLW) as the civil program up until 1979, the volume of military high level waste (HLW) in the USA is only around 25% that of civilian wasted fuel.

Radioactivity in the environment

The international community of nuclear scientists established bars to measure the extent of radiation and its environmental implications. The International Atomic Energy Agency (IAEA) and the Organization for Economic Co-operation and Development Nuclear Agency (OECD/NEA) established the International Nuclear and Radioactive Event Scale (INES) in 1990, which established seven categories of radiological exposure (Vandana et al., 2021).

For level 1 it is an anomaly which occurs when a member of the public is exposed to a radioactive source for longer than the statutory annual limit, a radioactive source is picked up, or minor flaws in safety systems in a facility that contains radioactive chemicals (Vandana et al., 2021). Overexposure of more than 10 mSv (millisievert) to a person or worker operating in a radioactive facility is classified as level 2. A radiation level of more than 50 mSv per hour or contamination in a radioactive facility is also regarded a level 2 risk in the case of a radioactive facility (Vandana et al., 2021). Level 3, often known as a significant occurrence, refers to overexposures to workers of more than 10 times the statutory annual limits, as well as non-lethal burns or radiation inflammation. This level of threat includes exposure of more than 1 Sv/h or unexpected severe contamination in the facility. Any nuclear power plant mishaps, as well as any radioactive items stolen, lost, or misdelivered, fall under this category (Vandana et al., 2021). A level 4 accident, often known as an accident with consequences, involves the death of one person as a result of radiation. It includes little radioactive material exposure that is unlikely to necessitate countermeasures, with the exception of the food industry, which necessitates control measures. This level includes damage to the core structure or fuel melting that results in the leakage of 0.1 percent of the core material or

considerable amounts of radioactivity that can have a severe impact on public health (Vandana et al., 2021). Level 5, or an event with far-reaching implications, involves several radiation-related deaths and necessitates the adoption of countermeasures. This level also addresses larger damage to the reactor core, which could result in a large amount of radiation being released into an installation's grounds. The emission of a considerable volume of radioactive material from a radioactive source or facility is classified as Level 6, often known as a severe accident. Controlling radiotoxicity in the environment necessitates well-thought-out countermeasures. Major accidents, also known as Level 7 accidents, are characterized as a large release of radioactivity from a source or installation that has far-reaching negative repercussions for human health and the environment. It necessitates well-thought-out and long-term countermeasures to reduce its radiotoxicity (Vandana et al., 2021).

Bioremediation of Uranium

Because uranium is non-degradable, the sole option for uranium clean-up is to reduce its mobility and bioavailability. Previous methods for preventing uranium migration into aquifers mainly included pumping out groundwater in heavily polluted areas to treat it on the surface or building an impermeable physical barrier in the under-ground aquifer to prevent uranium diffusion using clay, concrete, or multifunctional materials (You et al., 2021). Although microbial processes are the main forces behind natural pollution attenuation, the accumulation of highly harmful chemicals in the environment reveals that microorganisms alone are insufficient to deal with the pollution flux. Indeed, indigenous microbes are often present in tiny numbers, and degradative metabolisms are primarily inducible rather than constitutive. The activation of native metabolic potentials necessitates correct biogeochemical management, which can be accomplished through the use of bioremediation technology tailored to the ecological site's requirements (i.e. biostimulation, bioaugmentation) (Appukuttan et al., 2010). The most efficient technique is thought to be biological remediation because it is inexpensive and devoid of secondary pollutants. According to the International Atomic Energy Agency's (IAEA) regulations, managing radioactive waste is a global endeavour, and each stage of management calls for the use of techniques that have been scientifically verified (Gonçalves et al., 2022). The bioremediation process is based on bacteria that reduce U(VI). It has been established that the majority of U(VI)-reducing bacteria belong to the phyla Proteobacteria, Firmicutes, and Actinomycetes. Most often, bacteria employ electrons generated from organic substrates to directly decrease U(VI) by converting its oxidized and soluble forms into the reduced U(VI) (IV). Additionally, bacteria can convert substrates into secondary

metabolites, which then mediate the lowering of U(VI), through an indirect process (You et al., 2021).

Models that accurately predict bioremediation outcomes prior to field application should be used to design future bioremediation approaches so that bioremediation strategies can be optimized. To promote efficient U(VI) reduction, for example, it may be better to titrate with the least amount of electron donor necessary while preventing the production of excess biomass that could reduce permeability or promote the growth of organisms, such as acetate-oxidizing sulphate reducers, which consume additional electron donor but do not contribute to U(VI) reduction. Various techniques are used in the creation of models to explain the decrease in U(VI) in subsurface sediments (Williams et al., 2013). To develop completely predictive models, it will be necessary to forecast the responses of key members of the microbial community to changing environmental circumstances during bioremediation. One approach that may be used is called Bottom-Up Genome Scale (BUGS) modeling, in which metabolic models of significant organisms that have an impact on the bioremediation process are combined with hydrological and geochemical models. The benefit of BUGS modeling is that it takes into account significant alterations in microbial physiology (such as growth yields) linked to the shifting environmental conditions that microorganisms encounter during uranium bioremediation; such features are absent from conventional methods for simulating microbial activity. In the relatively simple case of acetate-driven uranium bioremediation, BUGS modelling has proved successful in predicting the course of uranium bioremediation by merging genome-scale Geobacter models with reactive transport models (Williams et al., 2013).

Bioremediation of uranium by *Cyanobacteria*

A global issue is the growing amount of uranium pollution in the environment as a result of its mining and disposal of tailings, the generation of nuclear energy and weapons, nuclear testing, and nuclear accidents. Natural biogeochemical processes involving microbial interactions with metals play a significant role and have significant ramifications for human culture. Therefore, it is essential to increase our knowledge of the interactions between metals and microbes in order to create effective bioremediation methods for sites with metal contamination. Effective metal adsorbents, marine *Cyanobacteria* are a significant sink for metals in aquatic environments (Acharya and Apte, 2013). Numerous methods used by microorganisms have a significant impact on the mobility and speciation of uranium in aquatic environments. The majority of uranium bioremediation research conducted to far has focused on low pH environments and the adsorption of positively charged uranium dioxide onto negatively charged microbial surfaces (Acharya and Apte, 2013). Oxygen-

dependent, gram-negative photosynthetic prokaryotes with a varied morphology, known as cyanobacteria, are found in freshwater, marine, and terrestrial habitats. These organisms are commonly numerous in settings contaminated with metals because they can respond to and adapt to most stressors (Acharya and Apte, 2013).

Uranium, other actinides, and fission products are just a few of the priority radionuclides whose solubility could be affected by microbial metabolism. The biostimulation of anaerobic microbial communities to remove redox-sensitive radionuclides such as uranium U(VI) from contaminated groundwaters at nuclear sites has garnered significant interest (Newsome et al., 2014). A large number of microorganisms have the ability to detoxify, convert, or immobilize contaminants such as metallic compounds and other organic pollutants. However, all bacteria are unable to withstand high levels of ionizing radiation and acidic environments (Vandana et al., 2021). The intricate interaction of microorganisms and contaminants is required for microbial bioremediation of radioactive wastes. Actinides and other fission products found in garbage can generate large amounts of alpha radiation and gamma radiation. As a result, using extremophilic bacteria that can withstand high radiation is a must for bioremediation in such extreme environments. Bioremediation of pollutants involves a variety of microbiological processes, but the most essential bioremediation processes for radioactive wastes are biotransformation, biomineralization, biosorption, and bioaccumulation (Vandana et al., 2021).

Bioremediation is based on bacteria that reduce U(VI). It has been determined that U(VI)-reducing bacteria largely belong to three phyla (Table 1): Proteobacteria, Firmicutes, and Actinomycetes, based on 16S rRNA gene PCR amplification, high-throughput sequencing, and phospholipid fatty acid (PLFA) investigations. The phylum Proteobacteria, which contains numerous U(VI)-reducing species and plays a critical role in lowering uranium toxicity and bioavailability, is the most prevalent phylum discovered in U(VI)-contaminated areas (You et al., 2021).

Bioremediation by microbes

Massive amounts of radionuclides and fatal metals-containing wastes are produced by the atomic fuel cycle and nuclear weapon production agencies, medical research institutes, mining, and other sources, posing a severe threat to the environment. The development of new low-cost inventive treatment and remediation advancements, such as bioremediation utilizing microorganisms for adjustment or evacuation and recovery of contaminants, has received a lot of attention because physical and chemical methods of remediation are expensive and produce secondary pollutants (Vandana et al., 2021). Micro bioremediation of U waste by bacteria is a good substitute for physiochemical methods due to its in situ application and excellent efficiency at relatively low

metal ion concentrations (Kulkarni et al., 2013). In order to survive and flourish in environments where uranium is naturally abundant or contaminated, biological organisms including bacteria, fungi, and plants interact with uranium in a variety of ways. Numerous species with the ability to extract or neutralize uranium have been discovered and identified from mining and contaminated sites. These interactions between living things and uranium lead to complexation or sequestration, which reduces the amount of the dangerous metal in the surrounding environment (Banala et al., 2021). It is generally known that microbial systems are adaptable in their ability to remove radionuclides and heavy metals from their immediate environment. By using a variety of physicochemical and biological methods, microbes modify the mobility and speciation of metals, affecting how they behave in the environment. Therefore, microbial bioremediation of radioactive contaminants is currently being extensively investigated (Acharya and Apte, 2013).

Biotransformation by bioreduction

Different electron acceptors can be respired by bacteria in the absence of oxygen to provide energy for metabolism. Following the reduction of nitrate, Mn(IV), Fe(III), and sulphate, followed by the reduction of carbon dioxide to form methane, the most energetically advantageous electron acceptors are employed in order as anoxia proceeds. Although this order normally applies to the natural environment, it should be noted that in some circumstances, such as when there is a surplus of organic matter, nitrate- and metal-reduction or metal- and sulphate-reduction may theoretically take place simultaneously. U(VI) can be reduced to insoluble U(VI) by Fe(III)-reducing bacteria through respiration because they have a comparable redox pair with U(VI) at circumneutral pH. (IV) (Newsome et al., 2014). One of the most significant disadvantages of metals or other radioactive metallic waste components is that, unlike other organic pollutants, they cannot be destroyed, but they can transition or convert from one form to another. The radioactive wastes are initially soluble or insoluble, and the microbiological process may transform the wastes from soluble to insoluble or vice versa after disposal. This microbial process strategy is employed in the field of bioremediation (Vandana et al., 2021). The biotransformation is influenced by the presence of electron acceptors such as oxygen and electron donors such as hydrogen. Bacteria employ nitrates, sulphates, or carbon dioxide as electron acceptors in the absence of oxygen, or anaerobic state. Bacteria can either directly or indirectly convert radionuclides (Vandana et al., 2021).. U(VI) has a redox pair comparable to Fe(III) at circumneutral pH, and Fe(III)-reducing bacteria can respire U(VI) as an alternate electron acceptor, reducing it to insoluble U(IV). Sulphate-reducing bacteria, fermentative bacteria, acid-tolerant bacteria, and myxobacteria are among the other bacteria that can reduce U(VI). Some are storing energy for growth, while others are gaining no

energy. Uranium bioreduction has been proposed as a bioremediation strategy, with the enzymatic reduction of aqueous U(VI) to insoluble U being aided by the addition of an electron source (IV)(Newsome et al., 2014). The transition of U(VI) to sparingly soluble U(IV) species in reducing circumstances (e.g. underground) would drastically reduce its mobility and bioavailability (Zhang et al., 2013).

Concerns about using bioreduction as a remediation strategy include whether reduced U(IV) will be stable over long periods of time, especially if environmental circumstances change, such as to oxidizing conditions (Newsome et al., 2014). The propensity of reduced minerals for reoxidation in the environment is one of the main obstacles for uranium bioreduction. Uraninite and "monomeric" U (IV) are both sensitive to reoxidation, however due to the former's crystalline structure, it is less susceptible than the latter (Sukla et al., 2015).

Direct immobilization of radionuclides

An important environmental concern is the immobilization of long-lived radioactive wastes produced by nuclear power plants (NPPs) during NPP operation, fuel reprocessing, and nuclear reactor decommissioning. For the safe disposal and efficient immobilization of radioactive wastes, appropriate matrices with high durability, stability, and resistivity to various physical and chemical conditions (temperature, pressure, radiation, acidity/alkalinity, etc.) must be designed (Singh et al., 2021). An important environmental concern is the immobilization of long-lived radioactive wastes produced by nuclear power plants (NPPs) during NPP operation, fuel reprocessing, and nuclear reactor decommissioning. For the safe disposal and efficient immobilization of radioactive wastes, appropriate matrices with high durability, stability, and resistivity to various physical and chemical conditions (temperature, pressure, radiation, acidity/alkalinity, etc.) must be designed (Vandana et al., 2021).

Indirect immobilization of radionuclides

Immobilization of primary molecules via bioreduction of a secondary molecule is referred to as indirect immobilization. Microbes can reduce iron Fe(III) and sulphur S(VI) to Fe(II) and S(II) forms, respectively, and oxidation of that bioreduced Fe and S can reduce a primary molecule and turn it from mobile to immobile. Technetium-99 [Tc(VII)] is an example of a radioactive waste with a high danger. Tc(VII) is immobilized by an indirect method in which bioreduced Fe(II) donates an

electron directly to Tc (VII). The decreased Tc (VII) becomes stationary after absorbing an electron from Fe(II) (Vandana et al., 2021).

Biominerelization

Biomineralization is the process of metal precipitation at the microbial cell surface using ligands produced by bacteria such as sulphides, carbonates, phosphates, and hydroxides. Bacteria such as Citrobacter and Serratia were found to be capable of uranium biomineralization (Vandana et al., 2021). This process, called biomineralization or bioprecipitation, produces an insoluble uranium precipitate by complexing with inorganic ligands such phosphates through an enzymatic mechanism. The inorganic phosphate is released when microbial phosphatase enzymes hydrolyze the organic phosphate substrate. This insoluble precipitate is known as metal-phosphate. Broadly speaking, three different uranium biomineralization pathways have been documented: biomineralization triggered by metabolism, extracellular precipitation, and cell surface precipitation brought on by phosphate mineral nucleation (Banala et al., 2021). Under glycerol phosphate conditions, the cell exhibits phosphatase activity and releases inorganic phosphates, which eventually form complexes with uranium at the cell surface in the form of hydrogen uranyl phosphate. When *Pseudomonas* species were given tributylphosphate, they showed similar uranium biomineralization. Bacterial cells clad in uranium phosphates were identified from uranium-contaminated soils, implying that biomineralization is a naturally occurring process (Vandana et al., 2021). Although bacteria can convert soluble U(VI) to comparatively insoluble U(IV), the particle size of tetravalent uranium compounds (such as UO_2) is typically less than 2 nm, allowing for considerable mobility in an aqueous environment. Low molecular weight organic acids and soil humus may also hinder uranium reductive precipitation by reducing U(VI) bioavailability (for example, by imparting steric hindrance) and/or increasing U(VI) solubility (IV). Non-reductive biomineralization produces bigger products, which can help to prevent uranium from migrating into the environment (You et al., 2021). Salome and colleagues explored the competition between U(VI) bioreduction and biomineralization in contaminated sediments, finding that under reducing circumstances and in the presence of glycerol-2-phosphate, U(VI)–phosphate mineral biomineralization outcompeted U(VI) bioreduction (You et al., 2021).

A potential issue with biomineralisation is that fast metal precipitation on the cell surface could theoretically present a barrier to cell metabolism, albeit this has not been observed directly. Other

obstacles could include the expense of the organic phosphate donor, which could limit biomineralisation's economic feasibility as a bioremediation approach (Newsome et al., 2014).

Biosorption

Solubility refers to both adsorption and absorption. Absorption is a three-dimensional process, while adsorption occurs in two dimensions. In treatment technologies, adsorption is the most widely used term, but it includes several other mechanisms. Combining sorption technology with biological material, biosorption is defined as "the uptake of metal species by physicochemical mechanisms and is independent of metabolic process (Vandana et al., 2021). Pre-growth conditions occasionally reduce the efficiency of biosorption, despite the fact that it is a fundamental physical process that is independent of metabolism. Additionally, metabolic activity occasionally affects the process by releasing metal complexing ligands or producing localized pH changes (Banala et al., 2021). Biosorption is the term used to describe the soluble substances that passively deposit onto the surface of cells. Metal ions are deposited on the surface of cells due to electronegative interactions between ionizable groups (such as phosphate, carboxyl, hydroxyl, amine, and sulfhydryl) and metal cations. Because biosorption binds contaminants more quickly than accumulation and makes it easier to recover the biosorbant by removing bound pollutants from the cell surface, it's a viable choice for handling low-concentration metallic wastes (Vandana et al., 2021). Biosoption has a number of drawbacks. When additional non-targeted cations compete and connect with the cell surface, issues can occur in bioremediation, causing the rate of bioremediation to drop dramatically. The cell surface can become saturated, preventing additional cation binding. The bioremediation process may change if the sorbed cell dies because quick cation desorption takes place. Biosorption is a characteristic shared by all biological moieties, including biomass from plants, animals, fungus, bacteria, and algae, as well as by their derived products like chitosan (Banala et al., 2021). Because binding to cell walls is faster than uptake into the cell, and it is easier to remove bound metals from a cell surface to regenerate the biosorbent, biosorption may be best suited to treating effluents with low to medium metal concentrations (Newsome et al., 2014).

Despite the fact that bacteria have the ability to biosorb uranium, it is unlikely to be beneficial in bioremediation. Biosorption has a number of drawbacks, including the fact that desorption from cell surfaces can be as fast as sorption, and other cations compete for binding sites. Additionally, cell surfaces might get saturated fast, inhibiting further biosorption. Although simulated cell breakdown aided the precipitation of uranyl phosphate in one investigation, sorbed material could be released back into solution when cells die and decompose (Newsome et al., 2014).

Bioaccumulation

The accumulation of radioactive wastes inside the cell is referred to as bioaccumulation of radioactive waste. By using "bioaccumulation" methods, microbial organisms may collect a wide spectrum of metal ions (Newsome et al., 2014). Bioaccumulation is responsible for a wide range of metal accumulation. Because certain metal ions have structural similarities to critical components required for bacterial growth and development, they are taken up by chance. Because uranium has no recognized biological purpose, it enters the cell through uranium toxicity-induced membrane permeability (Vandana et al., 2021).

Bioprecipitation

The ability of microorganisms to precipitate uranium includes the following: (a) phosphatase enzyme activity, which releases inorganic phosphate from an organic phosphate donor supplement, (b) hydrolysis or degradation of intracellular polyphosphate granules, which results in phosphate release or efflux, (c) localized alkalization at the cell surface, and (d) sulphides produced by sulphate-reducing bacteria (Sukla et al., 2015).

Bioremediation by fungi

Low pH, high temperature, and strong radiations characterize the environment of radioactive waste contaminated sites, and it appears that no species can survive in such conditions. As a result, searching for bacteria capable of surviving under harsh environmental circumstances is critical for bioremediation. Tkavc et al 2018. identified *Rhodotorula taiwanensis* MD1149, a fungus species capable of surviving under adverse environmental conditions such as pH 2.3, high metal concentrations, and intense radiation. Under severe gamma radiation and low pH, the fungi formed biofilms. Biosorption of uranium from bioleaching uranium ore solutions was demonstrated using the fungus Rhizopus arrhizus and immobilized particles. Uranium biosorption is caused by the amine nitrogen of chitin combined with free radicals (Vandana et al., 2021).

Genetic Engineering: Bioremediation of Radioactive Wastes

Microbes appear to be unable to survive and remediate contaminants due to poor environmental conditions. However, certain bacteria can withstand harsh climatic conditions but are unable to remove pollutants. In this situation, genetic engineering opens up new possibilities in the field of bioremediation, as many microorganisms can be engineered to remove pollutants that natural microbes cannot (Vandana et al., 2021). *Deinococcus radiodurans* is a well-known radioresistant bacteria capable of reducing radioactive wastes such as Cr(VI), U(VI), and Tc (VII). The PhoN gene was expressed in *Deinococcus radiodurans* using rDNA technology, which extended the bacteria's shelf life by 6 months and increased uranium and cobalt bioprecipitation.

Factors Affecting Bioremediation of Radioactive Wastes

The ecologically friendly and cost-effective cleaning of contaminated systems is known as "bioremediation" (Dubey et al., 2011). Microbes have the ability to adapt to changing settings and have been shown to be a potential strategy to bioremediation of radioactive waste. However, some biotic and abiotic stimuli affect microorganisms' biological processes by affecting their behaviour and growth. The rate of bioremediation may be affected by a lack of information about the factors that affect and influence it (Vandana et al., 2021). There are three types of factors that influence microbial processes: Abiotic or physicochemical variables, biological or biotic variables are two types of factors that might affect a person's health and environmental influences. Cyanobacteria have characteristics that enable them to live in and frequently rule a variety of environments (Waterbury, 2006).

The development and activity of U(VI)-reducing bacteria can be influenced by temperature and pH. The curve for U(VI) reduction rate vs temperature should be bell-shaped, with the maximum rate at the optimal temperature, showing that organisms have only a specified temperature range in which they are optimally active, according to the features of the enzymatic activity (You et al., 2021). The direct influence of pH is similar to that of temperature, although it is difficult to identify the optimal and extreme levels due to the variable tolerance of various U(VI)-reducing microorganisms to acid and alkali. The pH ranges from 4.0 to 4.5 for sulphate-reducing bacteria *Desulfosporosinus* sp. strain GBSRB4.2, acid *Microbacteria* A6, and some *Clostridium* sp. In a culture of sulphate-reducing bacteria and three *Clostridium* species (*Clostridium acetobutylicum, C. pasteurianum,* and *C. sphenoides*), the ideal pH for U(VI) reduction is near neutral. Under strong alkaline circumstances, U(VI) is normally fairly stable, however around pH 10–10.5, the inherent microbial community in the sediments of lime working sites can significantly lower U(VI) (You et al., 2021).

Furthermore, pH can influence U(VI) speciation, redox potential, and biosorption, all of which affect the reduction rate. The conventional redox potentials of U(VI) complexes produced at different pH levels are varied (You et al., 2021).

Physicochemical Factors or Abiotic Factors

PH, solubility, the presence and absence of electron donor and acceptor, and ionic strength are the key physicochemical parameters that affect bioremediation through affecting microbial behaviour and growth. In the microbial biosorption process, pH is critical for the absorption of contaminants such as radionuclides. The rate of bioremediation may be affected by a small change in pH. By modifying the isoelectric points, pH affects cell surface charge. pH directly affects the ionic strength of numerous ligands such as carboxylic groups, phosphate groups, sulphur, and amino groups. Changes in pH affect the ionic strength of these ligands, which affects the rate of biosorption (Vandana et al., 2021). The concentration of *Cyanobacteria* is greatest in environments with neutral to alkaline pH. They dont thrieve in extremely acidic habitats, but they are typical dwellers of hot springs and peat bogs that are acidic and have pH levels above 5 (Waterbury, 2006).

Biological Factors or Biotic Factors

Some biological elements have a significant impact on bioremediation. Microbe specificity for substrates plays an important part in bioremediation, and research has revealed that microorganisms have a wide range of specificity for different types of substrates, which can affect target pollutant remediation. Complete bioremediation is impossible to achieve with a single microbial species; consequently, a microbial consortia is required. The interaction of microbes in microbial consortia is a critical aspect in bioremediation. Individually, all microbial species may be effective remediators, but in groups, they may be allelopathic. As a result, effective microbial consortia design is a critical stage in bioremediation (Vandana et al., 2021).

Climatic Factors

The main causes of global climate change are increased carbon dioxide levels and rising temperatures. Though there is no clear evidence that climate change has an impact on bioremediation, changes in the physicochemical features of the microbial niche may disrupt numerous metabolic processes and, as a result, the bioremediation process. Climate change has a

big impact on microbial extracellular enzyme synthesis, which can aid or hurt the bioremediation process (Vandana et al., 2021). The habitats in which cyanobacteria can be found include a wide variety of temperatures. The majority of cyanobacteria are mesophilic, meaning they can survive in conditions with temperatures as high as 40°C. They normally have maximum growth temperatures below 45°C and growth optimum temperatures between 20 and 35°C. Temperature maxima of isolated cyanobacteria from the open oceans, where temperature ranges are more moderate, frequently hover around 30°C (Waterbury, 2006).

Long thought to create solely insoluble crystalline uranium UO_2 when converting U(VI) to U(IV) by microbial remediation, it was subsequently discovered that non-crystalline U(IV) (NCU(IV)) species could also be produced. Because of its amorphous form and strong reactivity, NCU(IV) is less stable than crystalline UO_2, compromising the remedial effects (You et al., 2021).

Cyanobacteria

The earliest known inhabitants of this planet are *Cyanobacteria*, which have existed for 3.8 billion years. In terms of distribution, *Cyanobacteria* can be found worldwide and in all latitudes. The most ancient photosynthetic prokaryotes, cyanobacteria, are thought to have first colonized the earth during the Precambrian epoch. Because of a variety of general traits, some of which are exclusive to higher plants and bacteria, cyanobacteria are a special assemblage of organisms that inhabit and predominate in a wide range of habitats (Dubey et al., 2011). They are a possible contender for uranium bioremediation due to their capacity to grow in severely polluted environments. The selective, potentially beneficial microorganisms known as cyanobacteria serve humanity in a variety of ways (Karn, 2016). It is common to find cyanobacteria in freshwater, marine, and terrestrial habitats. They are a morphologically diverse group of oxygenic, gram-negative photosynthetic prokaryotes. These organisms are common and can adapt to most stress situations in regions where there is metal contamination. They are prevalent in aquatic environments where they have the capacity to withstand, collect, and detoxify metal contaminants, hence influencing the metals' bioavailability and mobility (Acharya and Apte, 2013).

The cyanobacteria are one of the prokaryote groups with the greatest diversity in terms of morphology and development. They range from straightforward unicellular forms that divide into two cells to complicated filamentous forms with numerous highly differentiated cell types. Due to cellular differentiation and functional specialization between vegetative cells and heterocysts, the sites of oxygenic photosynthesis and dinitrogen fixation, respectively, some filamentous forms are capable of real branching and some are even truly multicellular (Waterbury, 2006).

The fact that many cyanobacteria are noticeable in nature makes it much easier to acquire samples. They stand out as significant elements of the microbial mats in both freshwater and saltwater marshes, in the microbial crusts of the desert, on moist rocks in terrestrial habitats, in the marine intertidal zone, and as epiphytic colonies on land and freshwater plants and marine macroalgae (Waterbury, 2006).

Cyanobacteria can react with dangerous metals in a way that lessens or completely removes the metal's toxicity. These mechanisms alter the metal speciation, which modifies the metals' degree of mobility. Organic or inorganic precipitation, active transport, extracellular sequestration, intracellular compartmentalization, metallothionein synthesis, and other metal-binding protein mechanisms are involved in these activities (Acharya and Apte, 2013). Photosynthetic microorganisms called cyanobacteria, once known as blue-green algae, are prevalent in nature. Humans have always consumed some cyanobacteria, while others are hazardous and have not been consumed by humans for generations (Frigaard, 2018). Cyanobacteria produce a wide range of toxins as well as beneficial chemicals that are interesting from a biological perspective (Sivonen, 2009). Abrupt physical and chemical changes in light, salinity, temperature, and nutritional content can have a significant impact on cyanobacteria. Numerous types of environmental contaminants have been demonstrated to be highly accumulated and degraded by blue greens (Dubey et al., 2011).

They rank among the most successful species in extremely saline settings and are numerically among the most significant organisms on Earth. The availability of P restricts the growth of cyanobacteria in many habitats. It has long been known that at higher pH levels, cyanobacteria in freshwaters and soils tend to be significantly more diverse and plentiful. But there are a lot of records at pH levels lower than that (Mur et al., 1999). The first organisms to colonize bare patches of rock and soil are frequently cyanobacteria. Limnic and marine settings are important cyanobacteria habitats. They thrive in fresh, brackish, salty, and hot spring waters as well as in situations where no other microalgae can live. Cyanobacterial reproduction is exclusively asexual. The capacity of cyanobacteria to store vital nutrients and metabolites within their cytoplasm is amazing. Cyanobacteria have the most basic dietary needs of all living things due to the basic metabolic process of dinitrogen fixation. Cyanobacteria use several environmental cues (such as photic, gravitational, chemical, and thermal) as indicators to optimize their position and so select a niche that is conducive to life and growth (Mur et al., 1999).

Metal-ligand surface complexes are produced by interactions between the metal ions and functional groups on the surface of cyanobacteria, such as carboxyl, phosphoryl, hydroxyl, and amine ligands. This potential remains even in the case of dead cells. By attaching the heavy metals to the cell wall

or extracellular polysaccharides (EPS) present there, these organisms stop the heavy metals from entering the cell (Acharya and Apte, 2013). In order to facilitate metal recovery and biomass regeneration while preserving the biomass's innate capacity to bind metal, the cyanobacterial biomass can be immobilized in solid, inert supports. Loose cells or cell suspensions frequently have smaller particle sizes and lower mechanical strengths. The high pressures required to achieve the appropriate flow rates for metal binding lead to the fragmentation of the free cells. These problems can be successfully solved with immobilized biomass. The most popular method of immobilizing cyanobacteria among the several approaches is entrapping the cells in natural or synthetic polymers (Acharya and Apte, 2013).

Fundamental study on radioactive bioremediation is vital to the development of novel tactics and inventions for earth security. The ability of microorganisms to endure in extremely radioactive environments is crucial for radionuclide bioremediation. However, a few biotic and abiotic variables can change the behaviour and growth of microorganisms, hence changing their biological processes. The rate of bioremediation may change if the elements influencing it are not understood. Therefore, in order to determine the long-term solution, it is essential to comprehend the mechanism by which the components affecting bioremediation work.

CHAPTER 3: METHODOLOGY

In this chapter, the discussion is focused on how the samples were collected and preserved and how they were analysed.

.

Materials and Method

Sample collection

Samples were collected closely following the steps used by (Adekola et al., 2015). Water samples were collected from major water bodies where cyanobacteria were suspected to be in and harvested from a river algae and collected in 500 ml bottles. These were brought back to the lab.

Uranium waste was collected following the method by (Santos and Ladeira, 2011). Uranium ore from CARST (Centre of Applied Radiation Science and Technology) was digested into a liquid using sulphuric acid and diluted using distilled water then collected into 50ml bottles. Some of the uranium waste was collected from waste containers at CARST which contain the remains of uranium after lab work waiting to be disposed. There were 8 samples in total and 3 of those 8 were selected at random.

Water samples were collected from different boreholes in the North West Province. Out of the 35 water samples collected, two were selected for the experiment at random.

Cyanobacteria isolation

In general, all samples received the same care. BG-11 enrichment media was used for isolation of cyanobacteria as described by Jawaharraj et al. (2016) (Jawaharraj et al., 2016). The enrichment media was inoculated with 10 ml of the previously collected water sample to give a final volume of 100 ml. The broth was incubated in an orbital shaker at 180 rpm for 14-21 days at 25°C (until visible cells were observed). Serial dilutions of 1 ml stock solution (growth medium containing visible cyanobacteria cells) with 9 ml sterile saline solutions ensued to give a logarithmic depression of cyanobacteria cells' concentration. The serially diluted solutions were poured onto petri dishes containing BG-11 medium, solidified with 1.5% bacteriological agar. Plates were then incubated for 21 days at 25°C. A series of re-plating was carried out to isolate single and pure colonies.

Anabaena doliolum , a cyanobacterium and other cyanobacteria organisms that were isolated from a river in Mafikeng, was examined for its ability to remove uranium at the ideal pH of 7.0 and temperature of 25˚C. The organisms were exposed to 0.5-1.0 ppm uranium.

Uranium removal by isolated cyanobacteria

Uranium waste from the lab at CARST (was collected into 3 (50 ml) bottles, which were labelled as SL4, 53 and S10K to create a unique identity which makes it easier to locate them among many samples. The remaining 2 samples (water samples) were collected from Mafikeng and labelled M20 and M10 which stands for Mafikeng 20 and Mafikeng 10 respectively. The bioaccumulation experiments were performed in 50 ml falcon tubes under constant agitation (150 rpm) at 25°C. The density of the each cyanobacterial used as inoculum was 1.0 g/L. Centrifugation (10000×g for 5 min) was used to separate the supernatant. A sensitive colorimetric method was used to determine the residual uranium concentration in supernatant and compared with control samples that had no cyanobacteria. This resulted in a total of 96 samples from 6 cyanobacterial isolates with 48 replicates.

Residual uranium concentration was measured using the ultra violet visible spectroscopy (uv-vis v-750)

DNA Sequencing

The genomic DNA from each cyanobacterial isolate was extracted using the Zymo soil microbe's extraction kit (Zymo Research, USA) following the manufacturers instruction. Polymerase chain Reaction (PCR) gene amplification of the 16S rRNA was conducted using a reaction volume of 25 µl containing (0.5 µl each of both forward and reverse primer, 12.5 µl PCR master mix, 1 µl DNA template, 10.5 µl nuclease-free water). Amplification was conducted using universal primer for bacteria (341F) (5′CCTACGGGAGGCAGCAG-3′), and (907 R) (5′-CCCCGTCAATTCCTTTGAGTTT-3′)(Fukuda et al., 2016). PCR was performed using the Bio-Rad C 1000 touch thermal cycler using this condition: 94°C for 2 min for initial denaturation, then 94°C for 30 s, annealing temperature at 59°C for 1 min, extension at 72°C in 2 min for 35 cycles, and a final extension at 72 °C in 8 min. A 1.5% agarose gel containing ethidium bromide was used to check the quality of the PCR products. The gel was observed using a gel documentation system (Gel Doc 2000, Bio-Rad, USA).

Thereafter, PCR products was sequenced at Inqaba Biotechnology Laboratory, South Africa. The sequence from each region of each bacterial isolate was cleaned using Chromas Lite Version 2.1 and edited with Bio Edit Sequence Alignment Editor. The consensus sequence was generated and

Blast on the NCBI database (www.ncbi.nlm.nih.gov/Blast.cgi) using the Basic Alignment Search Tool (Blastn) for identification of the closest representative strains of the isolates. The result of the sequence obtained was compared with other sequences in the NCBI GenBank data. Phylogenetic analysis was conducted using the MEGA X software package.

CHAPTER 4: RESULTS AND DISCUSSION

Several cyanobacterial isolates were isolated form the water samples and ten (10) that showed different morphological appearance on BG-11 solid medium were identified using DNA sequencing as show in the Table 1. The majority of the isolates (80%) belong to the genus *Anabaena*. Similarly, the evolutionary relationship of the isolated organisms was inferred by using the Maximum Likelihood method and Tamura-Nei model(Tamura and Nei, 1993). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

Table 1: cyanobacterial isolates and their corresponding similar match from the NCBI genbank database

Name			
Consensus_J1	Anabaena cycadae PKGSAK6	JQ964322	95.96%
Consensus_J2	Anabaena cycadae PKGSAK6	JQ964322	95.93%
Consensus_J3	Anabaena cycadae PKGSAK6	JQ964322	95.94%
Consensus_J4	Anabaena doliolum GSPKAK3	JX075259	90.52%
Consensus_J5	Anabaena cycadae PKGSAK6	KF157402	88.58%
Consensus_J6	Anabaena doliolum GSPKAK3	JQ964322	90.71%
Consensus_J7	Anabaena doliolum GSPKAK3	JQ964322	91.11%
Consensus_J8	Anabaena cycadae PKGSAK6	JQ964322	95.97%
Consensus_J9	Anabaena cycadae PKGSAK6	JQ964322	95.97%
Consensus_J11	Anabaena cycadae	JQ964322	95.87%

	PKGSAK6		

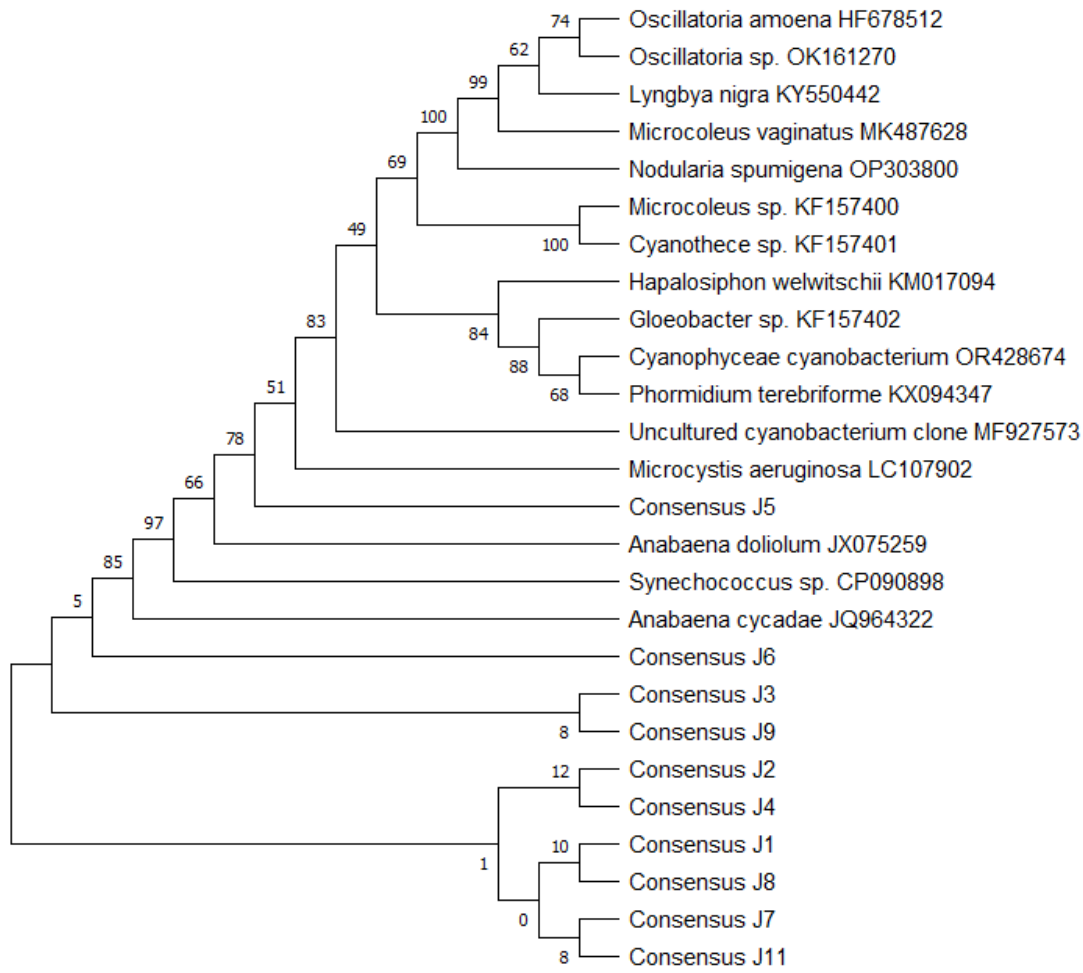

Figure 1: Phylogenetic tree constructed using neighbour joining method, showing relation of the isolated species with other homologous species collected from Mafikeng.

Isolates J6, J3, J9, J2, J4, J1, J8, J7, and J11 were phylogenetically similar to *Anabaena cicadae.* Almost all organisms belonged to *Anabaena cycadae* except for J5, which belonged to *Microcystis aeruginosa*. See Figure 1.

Twelve (12) samples were analysed for residual uranium after introducing the bacteria. Six samples (J6, 3J, J10, J6J10 J10J15, J15) of bacteria were introduced to the same sample.

As seen in Figure 3 above J6 bacteria had the greatest effect and reduced the most uranium from the sample as it varies from the original with an average percentage reduction of 32%.

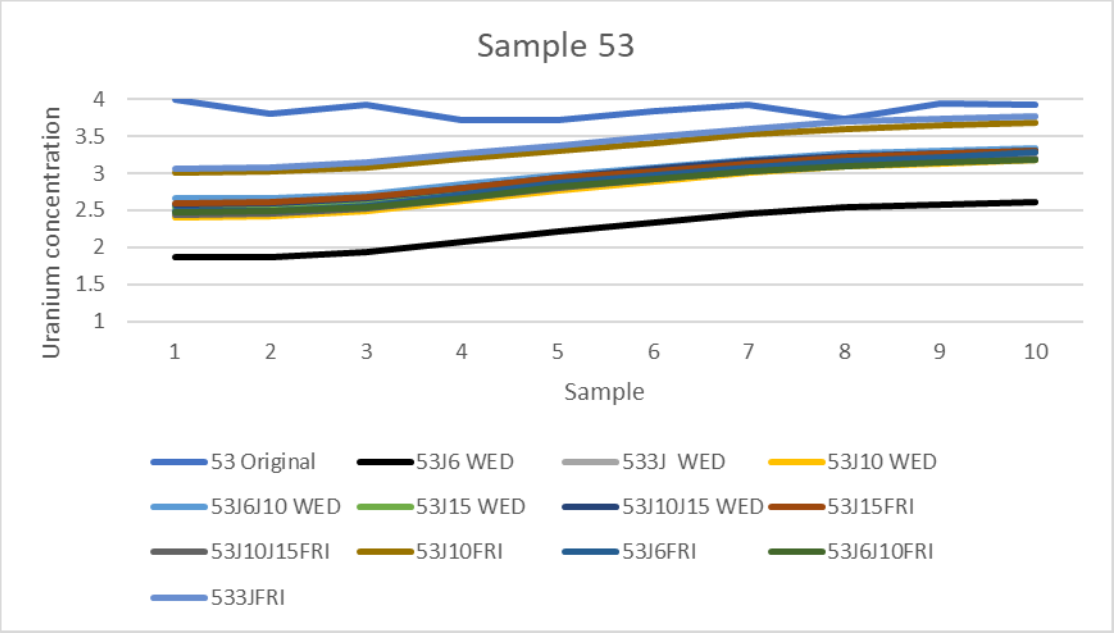

Figure 2: SAMPLE 53 Day 1 and Day 2 of 48 hr and 96 hrs respectively

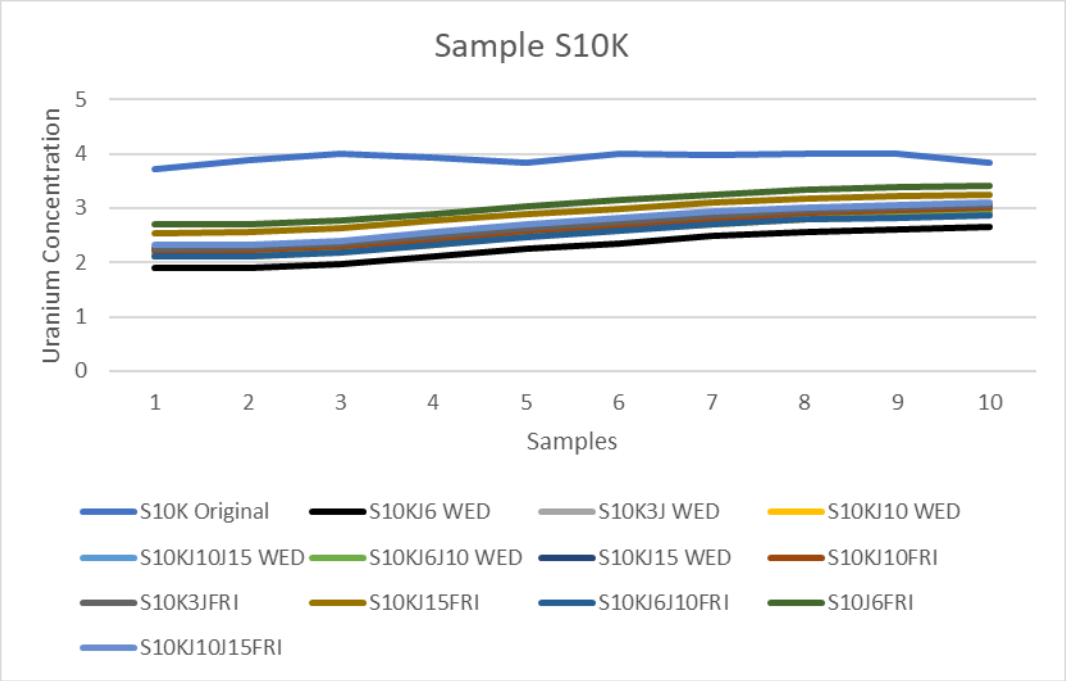

Figure 3: Sample S10K Day 1 and Day 2 of 48 hr and 96 hrs respectively

In Figure 2 & 3, J6 bacteria on the Day 1 had the greatest effect and reduced the most uranium from the sample as it varies from the original with an average percentage reduction of 41%. Also, J6 had the least average of 21% on the Day 2 sample.

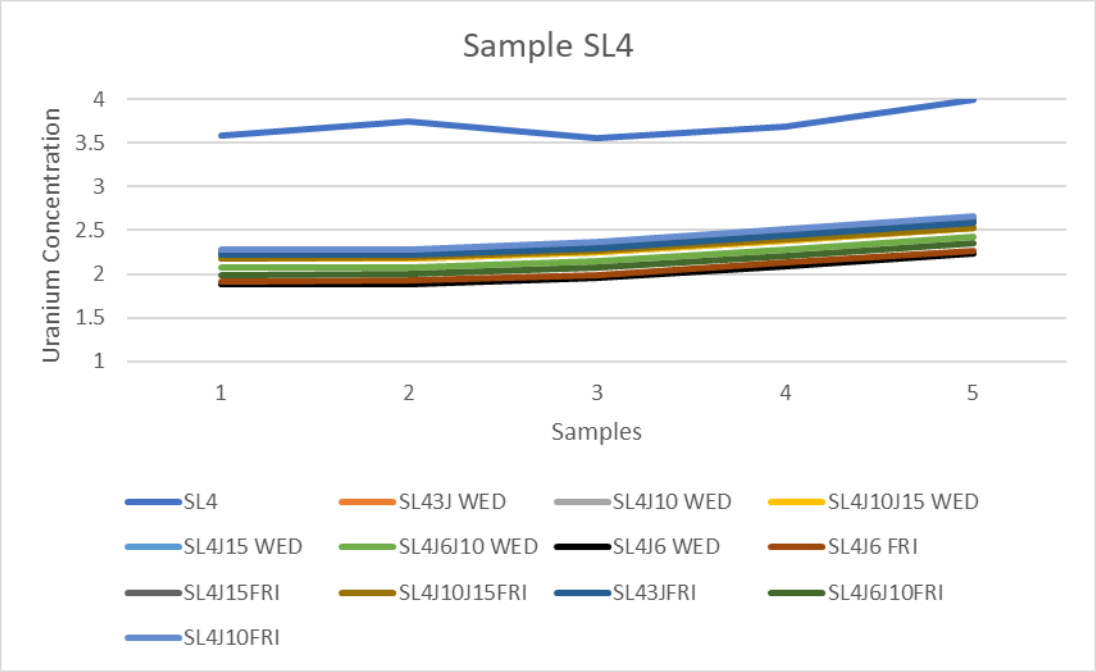

Figure 4: Sample SL4 Day 1 and Day 2 of 48 hr and 96 hrs respectively

In Figure 4, Sample SL4J10 FRI was reduced the least it had average percentage reduction of 34% . J6 bacteria for Day 1 and Day 2 reduced uranium the most with averages of 46% and 44% respectively. The other samples were reduced with slightly more than 35%.

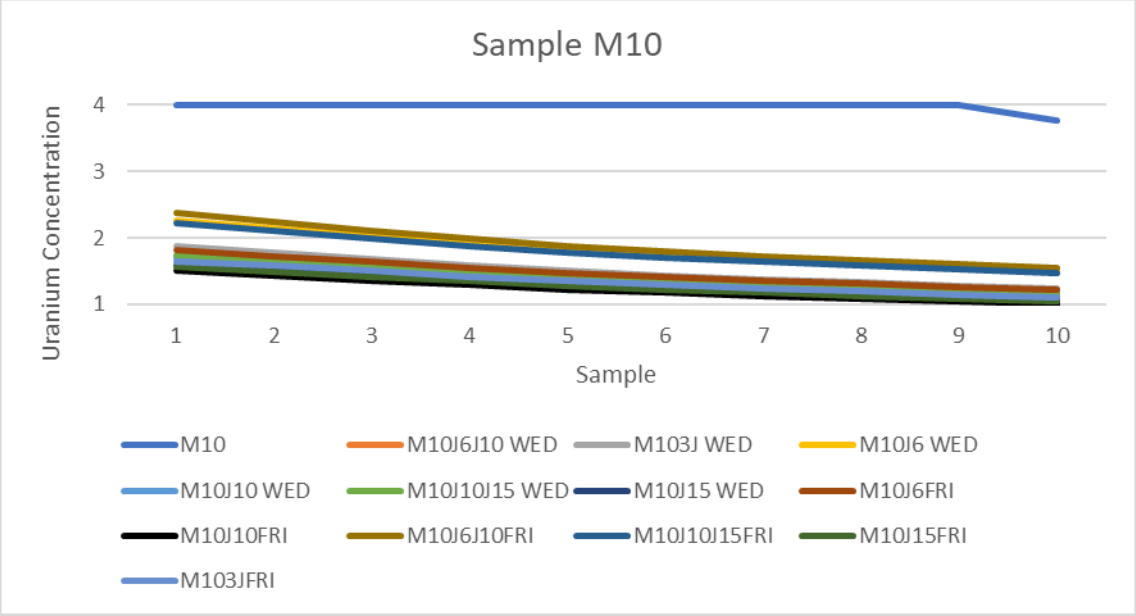

Figure 5: Sample M10 Day 1 and Day 2 of 48 hr and 96 hrs respectively

In Figure 5 Sample M10J10 FRI was reduced the most, it had average percentage reduction of 65.9% . Sample M10J6J10 FRI was reduced the least.

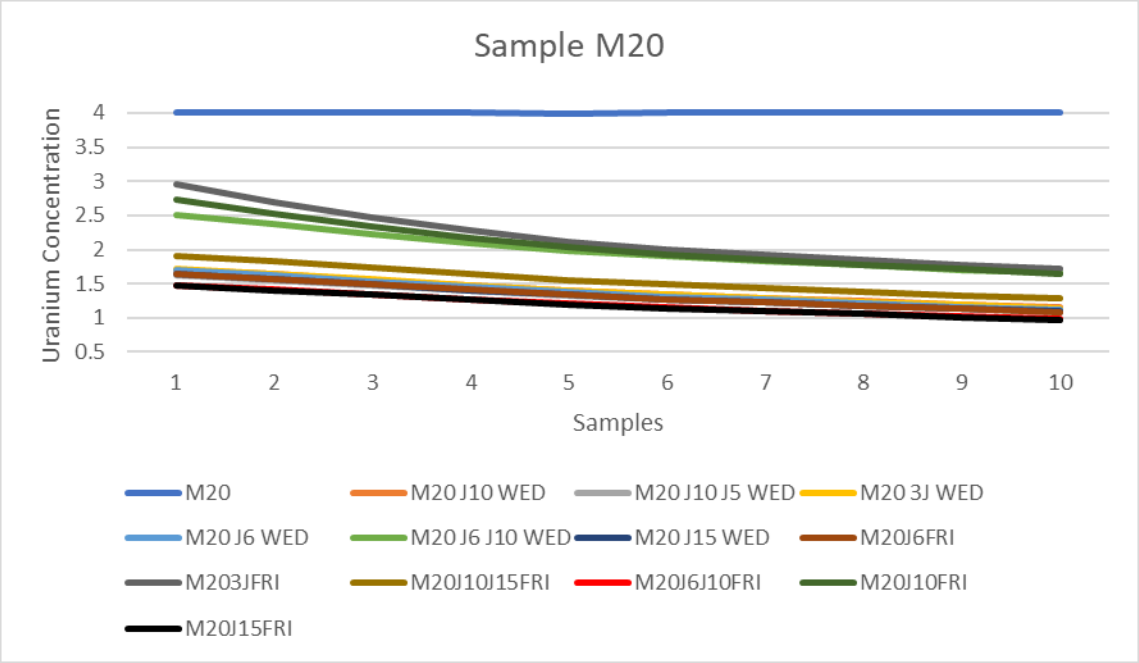

Figure 6: Sample M20 Day 1 and Day 2 of 48 hr and 96 hrs respectively

In Figure 6, Sample M20J15 FRI was reduced the most, it had average percentage reduction of 70% . Sample M203J FRI was reduced the least.

Discussions

Concerns over the fate of uranium contamination in surface, ground, or natural waters (ponds, lakes, and saltwater) resulting from the mining, production of nuclear energy, and storage of radioactive waste have been raised by the public. Since uranium has no biological function, its toxicity to humans is well known. Numerous microorganisms that are available in pure culture can attain U(VI) reduction. The vast majority are anaerobes that may reduce other metals, especially Fe(III), which is often the most common electron acceptor in underground environments.(Waseem et al., 2015). It is now known that spores can occasionally catalyze U(VI) decrease in addition to vegetative cells (Grecz et al., 2018). Of the 5 samples used in this study, S10K, SL4 and 53 are uranium waste samples while samples M20, M10 are water samples. Bacteria J6 reduced the uranium the most in the uranium samples but not necessarily in the water samples. For water samples M20 and M10, bacteria J10 and J15 were very close as they reduced the uranium the most compared to other bacteria. Uranium in water samples was reduced more than the uranium in uranium waste samples. This might be due to the harsh environment of the uranium waste samples, causing the bacteria in water samples to survive better and not die off (Banerjee et al., 2022). Although other studies marine bacteria was studied, uranium was reduced with up to percentages of

90% (Banerjee et al., 2022) and this agrees with our study even though in our study fresh water bacteria was used and the reduction was just below 85% with an average of 70 %.

When it came to high uranium loading values, *Anabaena dolionum* outperformed the other chemical and biological alternatives that were examined. As the results show it produced the highest levels of reduction on the samples compared to other organisms and this ranged from 40% reduction to 70% reduction.
Toxicity of the nuclear waste may have prevented the cyanobacterial species used in this study from growing at optimum condition and as a result their ability to utilize uranium was reduced (Waterbury, 2006).

Overall, it is evident that microbial cycling processes have a major influence on the behaviour of radionuclides in a variety of environments. These processes will be crucial to the management of contaminated land sites and geological disposal scenarios where biogeochemical processes are anticipated to take place, and they should be taken into account when developing safety cases. Microbes do not appear to have uranium transporters, and passive diffusion accounts for the majority of intracellular uranium build-up. Because of its strong positive charge, UO_2^{2+}, the major aqueous uranium species under low pH circumstances, is thought to be extremely hazardous. Several important microbial interactions with uranium include binding to various cell surface ligands at low to neutral pH, chelation by extracellular polysaccharides (EPS) and intracellular polyphosphates, binding to S-layer proteins and siderophores, precipitation as inorganic mineral phase or reduction to insoluble U(IV).

Given the relative simplicity of the microbial community in the context of uranium bioremediation at the incident site, it is now feasible to model and evaluate the in-situ activities of major subsurface populations and develop instruments that could eventually be applied to more complex bioremediation sites. In an ideal future, models that can accurately predict the outcomes of bioremediation before field application will be used to construct bioremediation procedures, enabling the optimization of bioremediation approaches (Williams et al., 2013). In the near term, uranium can be effectively removed from contaminated groundwater by promoting microbial U(VI) reduction, which stops its mobility. The long-term viability of this strategy is still highly unknown, and there are still a lot of alternatives that could improve the procedure that need to be considered. An important outcome of the significant investment made in uranium bioremediation research over the past ten years has been the development of techniques for assessing and modelling the activity of subsurface microbial communities. These techniques are expected to be applicable to other forms

of subsurface bioremediation as well as to the study of diverse microbial processes in a wide range of soils and sediments.

It has been demonstrated that the cyanobacterial cells concentrate many metals via channels known as porins, which are created by either active or passive processes, the metal ions are able to penetrate the cyanobacterial membranes (Mahana et al., 2021). Cyanobacteria's surface functional groups, such as carboxyl, phosphoryl, hydroxyl, and amine ligands, bind to metal ions to create metal–ligand surface complexes. This potential is still available even when the cells are dead. An additional aspect of cyanobacteria's internal metal accumulation process is the creation of metal-binding proteins, or MTs. These are low molecular weight, cysteine-rich proteins that bind metal ions in metal thiolate clusters. Their synthesis has been shown to increase in response to increased concentrations of metals. For the detoxification of effluents, cyanobacteria are being used more frequently than conventional wastewater treatment facilities because of their strong metal adsorption capabilities (Al-Amin et al., 2021).
Anabaena doliolum in this study might be producing the metal-binding proteins mentioned above in order to bioremediate the uranium waste.

It is proposed that, at pH values close to neutral, carboxyl groups serve as the primary metal ion sink (Adusei-Gyamfi et al., 2019). It has been demonstrated that siderophores can sequester thorium, uranium, gallium, chromium, and nickel. The organism J6 is phylogenetically similar to *Anabae cicadae*, which belongs to the family **Nostocaceae.** *Anabaena doliolum* , a cyanobacterium that was isolated from a river in Mafikeng, was examined for its ability to remove uranium at the ideal pH of 7.0 and temperature of 25˚C. The organism exposed to 0.5-1.0 ppm uranium within seven days showed a high percentage of metal elimination (65–70%). The main method of removing metals from cells was biosorption onto the cell surface. The main functional groups on the cell surface involved in cadmium binding are thought to include hydroxyl, amides, carboxyl, sulphate, and carbonyl groups. According to the International Atomic Energy Agency's (IAEA) regulations, managing radioactive waste is a global endeavour, and each stage of management calls for the use of techniques that have been scientifically verified. The identification of several microorganisms capable of decreasing U(VI) provides a foundation for further research into this pollutant's bioremediation. U(VI) reduction takes place primarily outside the cell, either through c-type cytochrome reduction on the outer membrane surface or in the matrix via pili or electron shuttle molecules. U(VI) molecules can also be carried over the membrane into the cell, where they are reduced in the periplasm by c-type cytochromes and in the cytoplasm by thioredoxin. The total

number of electrons transferred in enzymatic processes, as well as the whole electron transfer pathways, are unknown (Shi et al., 2016).

CONCLUSION

As everyone knows, cyanobacteria are powerful metal adsorbents. One of the cyanobacteria-based bio adsorbents was successfully commercialized and used to treat wastewater after extensive research. A basic understanding of the mechanisms by which cyanobacterial cells resist or mitigate uranium toxicity will be useful in the development of tools for either restoration of damaged aquatic environments or recovery of uranium from lean sources like saltwater. Examining if cyanobacterial strains that are genetically modifiable possess such a capability could shed light on the genetic underpinnings of the phenomenon and pave the way for future genetic engineering efforts to enhance such traits. Since the isolated cyanobacteria have a great potential for metal removal, it is possible to investigate their use in the removal of uranium waste.

To avoid any negative consequences on their health and to take preventative action, it is vital to monitor the water quality that both humans and animal's use. A basic understanding of the mechanisms by which cyanobacterial cells resist or attenuate uranium toxicity will be useful in developing strategies for uranium recovery from lean sources such as saltwater, contaminated wastewater, or remediation from aquatic habitats.

Bioremediation of radionuclides research is essential for the development of new tactics and technologies to protect the environment. Radionuclide bioremediation is largely dependent on the microorganisms' capacity to live in extremely radioactive environments. However, some biotic and abiotic stimuli affect microorganisms' biological processes by affecting their behaviour and growth. The rate of bioremediation may be affected by a lack of information about the factors that affect and influence it. As a result, it is critical to comprehend the mechanism by which the elements impacting bioremediation contribute to the discovery of a long-term solution. Uranium (U) pollution is a threat to the environment that has arisen as a result of the nuclear power industry's growth.

Microbial reduction of hexavalent uranium (U(VI)) to tetravalent uranium (U(IV)) lowers U solubility and mobility, and has been presented as a viable technique for uranium contamination remediation. As a result, uranium pollution must be remedied, with a focus on lowering U(VI) concentrations, for greater environmental conservation and human health. Environmental uranium pollution has been remedied using a variety of physical, chemical, and biological means. Biological remediation is the most promising option since it is both cost-effective and free of secondary

pollution. It is primarily accomplished by surface adsorption, internal accumulation, mineralization, and reduction techniques. Numerous factors, some complex and even contradictory, affect the pace at which microorganisms reduce U(VI). For instance, different pH values are preferred by different microorganisms, and different environments favour different electron suppliers. Additionally, although slowing down the rate of breakdown, bicarbonate can increase U(VI) bioavailability. Future studies should thus concentrate on analysing the collective effect of numerous influencing elements in order to compare and evaluate the most beneficial lowering settings. To select the optimal electron donor and reduction situation, a fuller understanding of the geochemical parameters and microbial ecology at a contaminated site is also required (Jiang et al., 2019).

Microbes are now used in the bioremediation of nuclear waste in a limited capacity. Even yet, the idea of using natural bacterial chemistry to treat and recover radioactive species appears to be a very promising approach of eliminating radionuclides from the environment. As our reliance on nuclear energy grows in tandem with rising energy demand, it becomes increasingly important that we devise practical, cost-effective ways to minimize hazardous radioactive leakage into our daily environment. We are presented with a sustainable alternative to current chemical treatments of radionuclides through further engineering of radiation-resistant microorganisms and possibly some fast-growing plants. Two fresh water cyanobacteria, J6 and J20J15, have been found in this investigation as having a significant capacity for uranyl sequestration from water.

REFERENCES

ACHARYA, C. & APTE, S. K. 2013. Insights into the interactions of cyanobacteria with uranium. *Photosynthesis Research,* 118**,** 83-94.

ADEKOLA, O., BASHIR, A. & KASIMU, A.-M. 2015. Physico-chemical characteristics of borehole water quality in Gassol Taraba State, Nigeria. *African Journal of environmental science and technology,* 9**,** 143-154.

ADUSEI-GYAMFI, J., OUDDANE, B., RIETVELD, L., CORNARD, J.-P. & CRIQUET, J. 2019. Natural organic matter-cations complexation and its impact on water treatment: A critical review. *Water research,* 160**,** 130-147.

AL-AMIN, A., PARVIN, F., CHAKRABORTY, J. & KIM, Y.-I. 2021. Cyanobacteria mediated heavy metal removal: A review on mechanism, biosynthesis, and removal capability. *Environmental Technology Reviews,* 10**,** 44-57.

AL NUAIMI, A. H. & WILLIAMS, L. G. 2022. Radioactive waste management in the UAE: Proposal for an inventory management system. *Progress in Nuclear Energy,* 146**,** 104140.

ALI, F. A. 2008. Measurements of naturally occurring radioactive materials (NORMs) in environmental samples. *Faculty of Engineering and Physical Sciences, University of Surrey*.

APPUKUTTAN, D., NILGIRIWALA, K. S., MISRA, C. S. & APTE, S. K. 2010. Natural and Recombinant Bacteria for Bioremediation of Uranium from Acidic/Alkaline Aqueous Solutions in High Radiation Environment. *Journal of Biotechnology,* 150**,** 53-53.

AWAN, I. Z. & KHAN, A. Q. 2015. Uranium-The Element: Its Occurrence and Uses. *Journal of the Chemical Society of Pakistan,* 37**,** 1056-1080.

BAEZA, A., CORBACHO, J. A., GUILLÉN, J., SALAS, A. & MORA, J. C. 2011. Analysis of the different source terms of natural radionuclides in a river affected by NORM (Naturally Occurring Radioactive Materials) activities. *Chemosphere,* 83**,** 933-940.

BANALA, U. K., DAS, N. P. I. & TOLETI, S. R. 2021. Microbial interactions with uranium: Towards an effective bioremediation approach. *Environmental Technology & Innovation,* 21**,** 101254.

BANERJEE, S., KUNDU, A. & DHAK, P. 2022. Bioremediation of uranium from waste effluents using novel biosorbents: a review. *Journal of Radioanalytical and Nuclear Chemistry,* 331**,** 2409-2435.

CHANG, E. 2016. Nuclear waste bioremediation. Stanford University, UK.

CHOUDHARY, S. & SAR, P. 2011. Uranium biomineralization by a metal resistant Pseudomonas aeruginosa strain isolated from contaminated mine waste. *J Hazard Mater,* 186**,** 336-43.

DENG, D., ZHANG, L., DONG, M., SAMUEL, R. E., OFORI-BOADU, A. & LAMSSALI, M. 2020. Radioactive waste: A review. *Water Environment Research,* 92**,** 1818-1825.

DUBEY, S. K., DUBEY, J., MEHRA, S., TIWARI, P. & BISHWAS, A. 2011. Potential use of cyanobacterial species in bioremediation of industrial effluents. *African journal of Biotechnology,* 10**,** 1125-1132.

EISENBUD, M. & GESELL, T. F. 1997. *Environmental radioactivity from natural, industrial and military sources: from natural, industrial and military sources,* Elsevier.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *evolution,* 39**,** 783-791.

FRIGAARD, N.-U. 2018. Chapter 2 - Sugar and Sugar Alcohol Production in Genetically Modified Cyanobacteria. *In:* HOLBAN, A. M. & GRUMEZESCU, A. M. (eds.) *Genetically Engineered Foods.* Academic Press.

FUKUDA, K., OGAWA, M., TANIGUCHI, H. & SAITO, M. 2016. Molecular approaches to studying microbial communities: targeting the 16S ribosomal RNA gene. *Journal of UOEH,* 38**,** 223-232.

GONÇALVES, M. F. S., PETRACONI FILHO, G., COUTO, A. A., SILVA SOBRINHO, A. S. D., MIRANDA, F. S. & MASSI, M. 2022. Evaluation of thermal plasma process for treatment disposal of solid radioactive waste. *Journal of Environmental Management,* 311**,** 114895.

GORONOVSKI, A., JOYCE, P. J., BJÖRKLUND, A., FINNVEDEN, G. & TKACZYK, A. H. 2018. Impact assessment of enhanced exposure from Naturally Occurring Radioactive Materials (NORM) within LCA. *Journal of Cleaner Production,* 172**,** 2824-2839.

GRAY, P. R. 1993. NORM Contamination in the Petroleum Industry. *Journal of Petroleum Technology,* 45**,** 12-16.

GRECZ, N., ROWLEY, D. B. & MATSUYAMA, A. 2018. The Action of Radition on Bacteria and Viruses. *Preservation of food by ionizing radiation.* CRC Press.

JACOB, J. M., KARTHIK, C., SARATALE, R. G., KUMAR, S. S., PRABAKAR, D., KADIRVELU, K. & PUGAZHENDHI, A. 2018. Biological approaches to tackle heavy metal pollution: A survey of literature. *Journal of Environmental Management,* 217**,** 56-70.

JAWAHARRAJ, K., KARPAGAM, R., ASHOKKUMAR, B., PRATHEEBA, C. N. & VARALAKSHMI, P. 2016. Enhancement of biodiesel potential in cyanobacteria: using agro-industrial wastes for fuel production, properties and acetyl CoA carboxylase D (accD) gene expression of Synechocystis sp. NN. *Renewable Energy,* 98**,** 72-77.

JIANG, B., GONG, Y., GAO, J., SUN, T., LIU, Y., OTURAN, N. & OTURAN, M. A. 2019. The reduction of Cr (VI) to Cr (III) mediated by environmentally relevant carboxylic acids: State-of-the-art and perspectives. *Journal of Hazardous Materials,* 365**,** 205-226.

KARN, S. K. 2016. Application of cyanobacteria for bioremediation of wastewaters.

KHODASHENAS, A., ROAYAEI, E., ABTAHI, S. M. & ARDALANI, E. 2012. Evaluation of naturally occurring radioactive materials (NORM) in the South Western oil wells of Iran. *J Environ Radioact,* 109**,** 71-5.

KOPPEL, D. J., KHO, F., HASTINGS, A., CROUCH, D., MACINTOSH, A., CRESSWELL, T. & HIGGINS, S. 2022. Current understanding and research needs for ecological risk assessments of naturally occurring radioactive materials (NORM) in subsea oil and gas pipelines. *Journal of Environmental Radioactivity,* 241**,** 106774.

KULKARNI, S., BALLAL, A. & APTE, S. K. 2013. Bioprecipitation of uranium from alkaline waste solutions using recombinant Deinococcus radiodurans. *J Hazard Mater,* 262**,** 853-61.

KUMAR, S., STECHER, G., LI, M., KNYAZ, C. & TAMURA, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution,* 35**,** 1547.

LEE, J., KIM, Y.-J., CHAE, J.-S., OH, J. S., KWON, E., LIM, J.-M., LEE, H., HAN, J. H., PHAM, M. K., NOUR, S., LA ROSA, J., GACA, P. & DANIEL, B. 2021. Preparation and evaluation of new reference materials for naturally occurring radioactive materials (NORM): Zirconium silicate, bauxite, and phosphogypsum. *Applied Radiation and Isotopes,* 168**,** 109525.

LEE, U., KANG, K. J., MIN, J. & KIM, H. R. 2019. Consequence analysis of a transportation accident of radioactive spent resin waste from a heavy water-cooled reactor to a the Gyeongju radioactive waste disposal facility. *Progress in Nuclear Energy,* 110**,** 34-40.

LLOYD, J. R. & RENSHAW, J. C. 2005. Bioremediation of radioactive waste: radionuclide-microbe interactions in laboratory and field-scale studies. *Curr Opin Biotechnol,* 16**,** 254-60.

LUTZE, W. & EWING, R. C. 1988. Radioactive waste forms for the future.

MAHANA, A., GULIY, O. I. & MEHTA, S. K. 2021. Accumulation and cellular toxicity of engineered metallic nanoparticle in freshwater microalgae: Current status and future challenges. *Ecotoxicology and Environmental Safety,* 208**,** 111662.

MORINO, Y., OHARA, T. & NISHIZAWA, M. 2011. Atmospheric behavior, deposition, and budget of radioactive materials from the Fukushima Daiichi nuclear power plant in March 2011. *Geophysical research letters,* 38.

MTIMUNYE, P. J. & CHIRWA, E. M. N. 2014. Characterization of the biochemical-pathway of uranium (VI) reduction in facultative anaerobic bacteria. *Chemosphere,* 113**,** 22-29.

MUR, R., SKULBERG, O. M. & UTKILEN, H. 1999. CYANOBACTERIA IN THE ENVIRONMENT.

NEWSOME, L., MORRIS, K. & LLOYD, J. R. 2014. The biogeochemistry and bioremediation of uranium and other priority radionuclides. *Chemical Geology,* 363**,** 164-184.

ODUMO, B., MUSTAPHA, A., PATEL, J. P. & ANGEYO, H. K. 2011. Radiological survey and assessment of associated activity concentration of the naturally occurring radioactive materials (NORM) in the Migori artisanal gold mining belt of southern Nyanza, Kenya. *Applied radiation and isotopes : including data, instrumentation and methods for use in agriculture, industry and medicine,* 69**,** 912-6.

OZDEMIR, S., ODUNCU, M. K., KILINC, E. & SOYLAK, M. 2017. Resistance, bioaccumulation and solid phase extraction of uranium (VI) by Bacillus vallismortis and its UV–vis spectrophotometric determination. *Journal of environmental radioactivity,* 171**,** 217-225.

PAUL, S. N., FRAZZOLI, C., SIKOKI, F. D., BABATUNDE, B. B. & ORISAKWE, O. E. 2022. Natural occurring radioactive materials (NORMs) from mining sites in Nigeria: A systematic review of geographical distribution and public health concern. *Journal of Environmental Radioactivity,* 249**,** 106889.

PRAKASH, D., GABANI, P., CHANDEL, A. K., RONEN, Z. & SINGH, O. V. 2013. Bioremediation: a genuine technology to remediate radionuclides from the environment. *Microbial Biotechnology,* 6**,** 349-360.

ROBERTS, L. E. 1990. Radioactive waste management. *Annual Review of Nuclear and Particle Science,* 40**,** 79-112.

ROGIERS, T., VAN HOUDT, R., WILLIAMSON, A., LEYS, N., BOON, N. & MIJNENDONCKX, K. 2022. Molecular Mechanisms Underlying Bacterial Uranium Resistance. *Frontiers in Microbiology,* 13.

SALAZAR, S., CASTILLO, L. A. & MONTES, L. 2021. Evaluation of naturally occurring radioactive materials (NORM) in the soil, in a potential area for unconventional reservoirs in the Rancheria Sub-Basin. *Chemosphere,* 283**,** 131098.

SANTOS, E. A. & LADEIRA, A. C. Q. 2011. Recovery of Uranium from Mine Waste by Leaching with Carbonate-Based Reagents. *Environmental Science & Technology,* 45**,** 3591-3597.

SCHAPER, A. 2013. Uranium

Technical Background and Definitions. *Highly Enriched Uranium, a Dangerous Substance that Should Be Eliminated*. Peace Research Institute Frankfurt.

SHI, L., DONG, H., REGUERA, G., BEYENAL, H., LU, A., LIU, J., YU, H.-Q. & FREDRICKSON, J. K. 2016. Extracellular electron transfer mechanisms between microorganisms and minerals. *Nature Reviews Microbiology,* 14**,** 651-662.

SINGH, B. K., HAFEEZ, M. A., KIM, H., HONG, S., KANG, J. & UM, W. 2021. Inorganic waste forms for efficient immobilization of radionuclides. *ACS ES&T Engineering,* 1**,** 1149-1170.

SIVONEN, K. 2009. Cyanobacterial Toxins. *In:* SCHAECHTER, M. (ed.) *Encyclopedia of Microbiology (Third Edition).* Oxford: Academic Press.

SUKLA, L. B., PRADHAN, N., PANDA, S. & MISHRA, B. K. 2015. *Environmental Microbial Biotechnology*, Springer.

TAMURA, K. & NEI, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and evolution,* 10**,** 512-526.

TUMANOV, A. Risk assessment of accidents during the transportation of liquid radioactive waste in multimodal transport. IOP Conference Series: Earth and Environmental Science, 2019. IOP Publishing, 032078.

UDDIN, S., BEHBEHANI, M., ABA, A. & AL GHADBAN, A. N. 2017. Naturally Occurring Radioactive Material (NORM) in seawater of the northern Arabian Gulf - Baseline measurements. *Mar Pollut Bull,* 123**,** 365-372.

VANDANA, U. K., GULZAR, A. B. M., LASKAR, I. H., MEITEI, L. R. & MAZUMDER, P. B. 2021. Role of Microbes in Bioremediation of Radioactive Waste. *In:* PANPATTE, D. G. & JHALA, Y. K. (eds.) *Microbial Rejuvenation of Polluted Environment: Volume 1*. Singapore: Springer Singapore.

WASEEM, A., ULLAH, H., RAUF, M. K. & AHMAD, I. 2015. Distribution of natural uranium in surface and groundwater resources: a review. *Critical reviews in environmental science and technology,* 45**,** 2391-2423.

WATERBURY, J. B. 2006. The cyanobacteria—isolation, purification and identification. *The prokaryotes,* 4**,** 1053-1073.

WILLIAMS, K. H., BARGAR, J. R., LLOYD, J. R. & LOVLEY, D. R. 2013. Bioremediation of uranium-contaminated groundwater: a systems approach to subsurface biogeochemistry. *Current Opinion in Biotechnology,* 24**,** 489-497.

XHIXHA, G., BALDONCINI, M., CALLEGARI, I., COLONNA, T., HASANI, F., MANTOVANI, F., SHALA, F., STRATI, V. & XHIXHA KAÇELI, M. 2015. A century of oil and gas exploration in Albania: Assessment of Naturally Occurring Radioactive Materials (NORMs). *Chemosphere,* 139**,** 30-39.

XU, T., WANG, S., LI, Y., LI, J., CAI, J., ZHANG, Y., XU, D. & ZHANG, J. 2021. Review of the destruction of organic radioactive wastes by supercritical water oxidation. *Science of The Total Environment,* 799**,** 149396.

YAP, J. K., SANKARAN, R., CHEW, K. W., HALIMATUL MUNAWAROH, H. S., HO, S.-H., RAJESH BANU, J. & SHOW, P. L. 2021. Advancement of green technologies: A comprehensive review on the potential application of microalgae biomass. *Chemosphere,* 281**,** 130886.

YOU, W., PENG, W., TIAN, Z. & ZHENG, M. 2021. Uranium bioremediation with U(VI)-reducing bacteria. *Science of The Total Environment,* 798**,** 149107.

ZHANG, C., DODGE, C. J., MALHOTRA, S. V. & FRANCIS, A. J. 2013. Bioreduction and precipitation of uranium in ionic liquid aqueous solution by Clostridium sp. *Bioresour Technol,* 136**,** 752-6.

ZHU, J., ZHAO, L., SONG, D., YU, J., LIU, Q., LIU, J., CHEN, R., SUN, G. & WANG, J. 2022. Functionalized GO-doped double network antibacterial hydrogels for efficient uranium extraction from seawater. *Desalination,* 540**,** 115993.