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Radiation Tolerant Life Forms and Methods Used to Remediate Radioactive Wastes from Soil

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ABSTRACT

The expanding nuclear industry has led to increasing radioactive waste in the environment. Exposure to these wastes causes considerable irreversible damage to the organisms, some of them being even lethal. Conventional methods like incineration, wet oxidation, and acid digestion have been used for radwaste treatment to control this. Apart from them, other organic methods like bioremediation are being widely applied by scientists. Many bacteria, fungi, algae, and plants are observed to possess remediating properties. Hence, these are now used on a large scale to treat the radioactive matter as quickly and effectively as possible. Techniques like bioaccumulation, enzymatic reduction, bioprecipitation, or phytoremediation methods such as phytoextraction and phytostabilization involving such organisms with remedial abilities have successfully removed the radioactive matter to an extent from the contaminated site. Further research is needed to increase the efficiency of the techniques and help remove radionuclides in an environment-friendly manner.

INTRODUCTION

Nuclear energy was used in the 1940s by many military troops and organizations as a new energy source. Seeing the success, it was used widely for military and research purposes such as testing nuclear weaponry, installing weapons for military use, producing nuclear energy, setting up nuclear energy facilities, and mining other radioactive elements. These activities generated huge amounts of waste, almost 90 million gallons (Uzair et al. 2019), including accidental radiation leakages and improper disposal of radioactive wastes. This caused radioactive pollution, exposure to radiation, and other radioactive matter, which had degenerating and lethal effects. To prevent and minimize it, physical methods like barrier construction, solidification, and tilling of fields for radio waste transfer (Ostoich et al. 2022); chemical methods like chemical removal; physical-chemical methods like electrokinetic application, and soil washing have been employed (Yan et al. 2021). Although the above-mentioned techniques have been successful in remediation and are used from time to time, they had limitations such as the high cost of specialized machinery, complex procedures, inefficient for low concentration radio waste removal, risk of perfusion of chemicals used for remediation into the groundwater, permanent biological and physiochemical changes to the soil, causing secondary pollution (Singh et al. 2022). To tackle these limitations, a technique called bioremediation was introduced.

Bioremediation is the method that uses living entities to remove hazardous substances under specified conditions (Dubchak & Bondar 2019) through the organisms' metabolic processes.

In layman's terms, bioremediation is a process to help clean the environment by involving living organisms like plants (called phytoremediation), fungi (mycoremediation), algae (phycoremediation), or enzymes to transform and detoxify the pollutants into less toxic forms (Kumar et al. 2018).

The remediation can be done with different methods, like mycoremediation (Bosco & Mollea 2019), microbe-aided phytoremediation (Dotaniya et al. 2018), nano bioremediation (Cecchin et al. 2017) through omics (Chandran et al. 2020), system biology (Malla et al. 2018) or a combination of inorganic and organic method like electrokinetic bioremediation (Gill et al. 2014).

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Bioremediation is advantageous and is preferred over other conventional methods owing to its low cost, low maintenance (Roh et al. 2015), feasibility, and usage of living entities which reduce the involvement and impact of artificially produced substances on the soil, hence cleaner method (Natarajan et al. 2020). The technique has a lot of potential to be used to remove different types of contaminants, including radwaste, much more efficiently. Intensive research on this technique can help to tap into its potential and develop it further.

EFFECT OF RADIOACTIVE WASTES ON ENVIRONMENT AND LIFE FORMS

Exposure to radionuclides can severely affect the life forms' surroundings and bring detrimental changes to them. Alterations in DNA and lesions formation may occur, eventually leading to DNA degradation by direct and indirect mechanisms (Shukla et al. 2017).

Radwaste bioaccumulating in the plants can enter the food chain and can damage the food chain seriously (Dubchak et al. 2019). Longer-living, larger plant species of an area gradually switch to short-living, smaller plants. All this ultimately leads to losing plant species diversity (Geras'kin 2016).

In the ocean, radioactive wastes stored at great depths can still spread in the water due to high radiation exposure of radwaste or leakage by defective sealing (Natarajan et al. 2020). Exposure to nearby organisms or consumption of such water by the organism can cause grave damage to the health of those organisms. Both terrestrial and aquatic biotas are unsuitable for dumping radioactive wastes.

In humans, low-intensity exposures cause mild skin irritation, but if the exposure continues for a longer time, it can cause hair loss, nausea, dizziness, vomiting, diarrhea, etc. Continuous exposure can lead the person to experience weakness, fatigue, fever, disorientation, low blood pressure, blood in stool, and eventually death (Kaushik et al. 2021).

High-intensity radiation exposure for long durations can cause leucopenia, leukemia, and kidney damage. Skin, lung, and thyroid cancers are some of the diseases also caused by radiation (Kautsky et al. 2013). It also causes irreversible damage like DNA mutations which can pass to future generations. Fetuses are especially susceptible to radiation since contact with radionuclides can cause organ malfunction like poorly formed eyes, smaller brain size or head, mental retardation and abnormal growth, solid childhood cancer, and other congenital disorders (Tang et al. 2018).

The most significant examples are the cases of atomic bomb survivors of Hiroshima and Nagasaki, where nearly 70,000 pregnancies were affected. Some lead to stillborn infants dying within the first 2 weeks or are born deformed with chromosomal aberrations (Brent 2015). The effect can be seen even after 65 years.

RADIONUCLIDES WHEN PRESENT IN SOIL

Radioactive wastes are usually present in minute concentrations in the soil. Depending upon the amount of radwastes, the method and organism are chosen for treatment. For soil with concentrations ranging from 10 μCi of $^{137}Csg^{-1}$ to 20 μCi of $^{137}Csg^{-1}$, microbes like *Rhodococcus*, *Nocardia,* or *Deinococcus radiodurans* are used, whereas concentrations greater than 20 μCi of $^{137}Csg^{-1}$, *Pseudomonas putida*, *Shewanella putrefaciencs* or *Deinococcus radiodurans* are preferred (Shukla et al. 2017). Naturally, radionuclides occur in various forms at different locations around the world. For example, ^{232}Th occurs as monazite rock deposits in Guarapari, Brazil, and Kerela, India, whereas ^{222}Rn is present in the hot springs of Ramsar, Iran (Ostoich et al. 2022).

In India, few regions are exposed to different radionuclides. For example, in South Konkan village, the occurrence of ^{238}U, ^{232}Th, and ^{4}K has caused the soil's radiation level to be 68.08 * 10^{-9} Svh^{-1}. In Gujarat, the presence of U and Th in the groundwater of Thar Desert and Th and Ca from Naredi Cliff has been observed (Sahay et al. 2015). In the soils of Jodhpur and Nagaur regions of Rajasthan, natural radionuclides such as ^{226}Ra, ^{232} Th, and ^{40}K are present (Rani et al. 2015).

In Jharkhand, mining and milling from the Jaduguda uranium mine into the Bay of Bengal has accounted for emitting alpha particles affecting indigenous microbial populations (Patnaik et al. 2018).

TECHNIQUES OF BIOREMEDIATION

In the case of microbial remediation, the metabolic activity of a microorganism determines the degree to which toxic waste is degraded (Natarajan et al. 2020). Effective bioremediation depends on physical, chemical, and biological interaction (Roh et al. 2015, Sengupta et al. 2021). Environmental factors favorable to microbial and plant growth also influence the process, and proper conditions can lead to the remediation process much faster (Dubchak & Bondar 2019). Different methods are performed considering all the above criteria and the organisms engaged. Some of them are discussed below.

Direct and indirect enzymatic reduction: Selecting either method depends on the site's radionuclide presence and soil conditions (Francis & Nancharaiah 2015).

In the direct method, bacteria reduce the organic compounds (substrate) to release electrons which are used to transform oxidized, soluble, and mobile forms of radionuclides (for example, U, Cr, or Tc) into reduced, insoluble, and their respective immobile forms (Shukla et al. 2017). In vitro, Uranium precipitation is exhibited in *Shewanella putrefaciens* on its surface and with hydrogenase combination (as electron donor) in the case of *Desulfovibrio vulgaris* (Jabbar & Wallner 2015). This technique is also applied for reduction of Pu(VI) and Pu(V) to Pu(IV) by *S. putrefaciens*, *G. metallireducens* and *B. subtilis* (Natarajan et al. 2020).

In the Indirect Method, mostly lithotrophic-type bacteria reduce the substance, leading to the reduction of radionuclides. Indirect reduction of soluble contaminants is triggered in belowground and sedimentary environments by sulfate-reducing or metal-reducing microorganisms. An example of a microbe is *Microbacterium flavescens,* used for remediating U-, Th-, and Pu-contaminated soils (Jabbar & Wallner 2015).

Bioaccumulation: Bioaccumulation is the deposition of the radionuclides within the organism (Francis & Nancharaiah 2015) and comprises the phenomenon of bioconcentration and biomagnification (Shukla et al. 2017). It relies on the property of adsorption of radioactive matter on the cell surface of the microbe owing to prevailing electrostatic forces of attraction between the metal cations of radionuclides and the negatively charged cell surface, leading to their binding (Ayansina et al. 2017). This makes removing radionuclides easier and thus prevents leakage (Natarajan et al. 2020). The process can be either active or passive. Active bioaccumulation needs more energy and takes much time. Passive bioaccumulation consumes lesser energy and is relatively faster (Ding et al. 2019). It is best for areas with nutrient limitations. The process was reported for radionuclides like plutonium, cesium-137, americium, strontium-85, radium, Thorium, and cobalt-60. Some of the Gram-positive bacteria, like *Bacillus* sp. (Zhao 2016) And Cyanobacteria like *Arthrospira* (*Spirulina*) *platensis* (Zinicovscaia et al. 2020) indicated the potential for bioremediation by this method. Uranium bioaccumulation in *Pseudomonas* has also been observed (Mahadevan et al. 2017).

Biosorption: The phenomenon of biosorption is described as "The sequestration of positively charged metal ions to the negatively charged cell membranes and polysaccharides secreted on the outer surfaces of bacteria" (Shukla et al. 2017). It immobilizes the radionuclide present and can occur either directly, by nuclide cation interaction with functional groups which have anionic cell walls, or indirectly with EPS, S-layer, or capsule (Chauhan et al. 2021). It is a passive uptake process (Dey et al. 2021). Pu, Np, U, and Th are some radionuclides that can bind onto the cell surface with the help of ligands like amine, carboxyl, phosphate, hydroxyl, and sulfhydryl (Mahadevan et al. 2017). The process is species-specific, i.e., depends on the ligands attached, and is affected by factors such as temperature, aeration, pH, the growth phase of cells, presence of organic or inorganic content and metabolites, secretion or production of exopolymers (Ding et al. 2019). Other factors include the chemical interaction of extracellular biopolymers, functional groups, metal ions, and electrostatic attraction (Dobrowolski et al. 2017). *Pseudomonas* strain is one example that can biosorp U and Th ions through intracellular sequestration (Natarajan et al. 2020). Few bacteria and algal cultures were reported to retain strontium through biosorption (Francis & Nancharaiah 2015). These are shown in Table 1.

Biotransformation/Bioreduction: Biotransformation occurs through various mechanisms: metal oxidation-reduction, changes in pH, solubilization and leaching, volatilization, immobilization, remobilization, or alteration of metal-radionuclide complexes (Francis & Nancharaiah 2015). Bacterial transformations occur through basic chemical processes which direct the formation of co-precipitates, oxides, and organic, inorganic, and ionic complexes of radionuclides (Ding et al. 2019). Different types of bacteria, aerobic or anaerobic (that are actively growing), retain the ability to transform through redox reactions. In most cases, nuclides that are non-sorptive are transformed non-enzymatically or enzymatically (Shukla et al. 2017). It was observed that triheme periplasmic cytochrome type-c has a key role in biotransformation (Jabbar et al. 2015). For U (VI) bioremediation, bacterial groups like acid-tolerant, fermentative, and sulfate-reducing bacteria can act as alternative electron acceptors (Mahadevan et al. 2017). Ecological conditions, electron donors and acceptors, and supplements can affect microbial activity during biotransformation (Uzair et al. 2019).

Some known examples are *Geobacter sulfurreducens* (Vogel et al. 2018) strain PCA, *Shewanella putrefaciencs* strain CN-32, and *Anaeromyxobacter dehalogenans* strain K.

Bioleaching: Also called biomining or bio solubilization involves leaching out of radionuclides from their compact matrices (Qiu et al. 2019). It is not a direct solubilization method, and the energy here is obtained in autotrophic bacteria from reduced Fe or S compounds while simultaneously solubilizing the metals and nuclides. It needs components like moisture, acidic pH, and oxygen to oxidize Fe or S and filter out metals in sulfide form (Shukla et al. 2017). These bacterial types are acidophilic and mesophilic in nature (Srichandan et al. 2019). Scientists have reported

Table 1: Radionuclide-microorganism interaction of certain radioactive elements.

Radionuclide	Microorganism	Type of Interaction	Reference
Hydrogen Uranyl Phosphate (HUP)	*Serratia* sp.	Precipitation via the activity of a high radio-stable phosphatase enzyme.	Lopez-Fernandez et al. (2021)
Strontium ^{90}Sr	*Bacillus* sp	Accumulation into biogenic carbonate minerals.	
	Cyanobacteria	Accumulation into calcium carbonates.	
Technetium ^{99}Tc	*Geobacter sulfurreducens* and *Shewanella oneidensis*	Bacterial accumulation.	
Thorium ^{232}Th	*Streptomyces sporoverrucosus*	pH and ionic strength-dependent biosorption in living and dead cells of the organism.	
Neptunium ^{237}Np	*Shewanella* sp.	Biosorption by whole cells, the cell wall, and extracellular polymeric substances of algae.	
Uranium ^{238}U ^{235}U	*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Gallionella*, *Bacillus* and *Sphingomonas*	Biomineralization by passive sorption on cell wall extracellular polymers and secretion of phosphate groups.	
	Stenotrophomonas sp.	Immobilization using phosphatase enzymes under changing environmental conditions.	
Plutonium ^{239}Pu	*Pseudomonas* sp.	Influencing the redox cycling and mobility of Pu in the environment as a reductant and sorbent.	
	Saccharomyces cerevisiae	Sorption from aqueous radionuclide solutions at pH 1-2 by immobilized algae.	
Americium ^{241}Am	*Saccharomyces cerevisiae*		
Cerium ^{140}Ce	*Saccharomyces cerevisiae*		
Curium ^{242}Cm	*Rhodotorula mucilaginosa*	Reversible and pH-dependent biosorption.	

Acidithiobacillus ferrooxidans (Mao et al. 2015), *Sulfolobus* (Reitz et al. 2015), and *Acetobacter* sp. (Qu et al. 2019) as microbes that can solubilize metals. The process is affected by microbial activity, physical factors like pH surrounding the bacteria, moisture, oxidation state of the nuclide, and inorganic content as substrate needed for the bacteria (Kaksonen et al. 2017). A vital bacterial metabolite, the presence of citrate also enhances the solubility of nuclides.

Bioprecipitation: This occurs after converting a nuclide from soluble to insoluble (Sahinkaya et al. 2017). It is achieved by carrying out oxidative and reductive reactions leading to precipitation. Precipitation of radionuclides and metals happens largely in carbonates or hydroxides form (Shukla et al. 2017). The site where precipitation occurs in a microbial cell is the 'nucleation site,' and the precipitation process in it depends on the ligand concentration produced by the cell (Shukla et al. 2017). Microbial ligand production, biogenic mineral formation (Jabbar & Wallner 2015), valence, and oxidation state of the radionuclide are important factors of bio precipitation. Secretions from bacteria and metabolism can cause changes in pH in its immediate surroundings, hence, changing the pH of the area adjoining the metal in the process. Co-precipitation is a phenomenon related to it where elements amalgamate in minerals of metal oxide during precipitation. The method has been investigated for removing Strontium (Francis & Nancharaiah 2015), Uranium (Xu 2018). *Shewanella putrefaciens* is known for successful U(VI) bioprecipitation (Huang et al. 2017).

Biomineralization: The method uses living organisms like fungi, microalgae, bacteria, protozoa, or cyanobacteria to form minerals (Ding et al. 2019). It can be of two types: biologically controlled biomineralization (BCM) or biologically induced biomineralization (BIM) (Singh et al. 2021). This depends on temperature, pH, ions, enzyme activity, and humic substances (Jiang et al. 2020). It often leads to stiffening and hardening of the mineralized contaminants, which are later removed separately, so it lessens soil contamination. In the case of fungi, many microbial biomineralization formations are supplemented by sorptive interactions and fungal mycelium branching for a strong metal removal system (Gadd & Pan 2016). The method is attempted to remove toxic radioisotopes like Tc by flow through biostimulated sediment column bioreactors at even minute concentrations (Thorpe et al. 2016). Biomineralization for U(VI) is possible with the help of *Kocuria* sp. (Wang et al. 2019) and *Saccharomyces* *cerevisiae* (Zheng et al. 2017). *Serratia* sp. relies on the synthesis of crystalline hydroxyapatite to be used later to recover Eu and Sr (Gangappa et al. 2016).

Genetically modified organisms: Recombinant DNA technology and genetic engineering are employed to generate tailor-made organisms, which increase their biodegradation potential and therefore help in the successful remediation of radwaste (Kumar et al. 2018). This method generates different protein constructs with genes with desired traits and properties for remediation. These genes of interest are then combined in a single bacterial cell with improved metal binding properties and high adsorption capacity (Omran 2021). Finally, they accumulate metal ions by sorption. One example is *Deinococcus radiodurans*, a microorganism observed to tolerate ionizing radiations up to $10*10^3$ Sv (Shukla et al. 2017) and is currently known as the most radiation-tolerant organism. It is an extremophilic bacterium that can thrive under high temperatures, low nutrients, and high radiation exposure (Manobala et al. 2019) by producing several copies of its genome and performing DNA repair mechanisms when required (Natarajan et al. 2020). This microbe is genetically engineered and then used for remediation purposes. It converts volatile and highly toxic metals into less mobile and toxic forms. It remediates the radionuclides through biofilm formation (Shukla et al. 2017). Genetically engineered *Pseudomonas aeruginosa* (Tapadar et al. 2021) and *E. coli* strain with genes from *Serratia marcescens* and *Helicobacter pylori* (Uzair et al. 2019) have also been experimented with to successfully remove uranium through precipitation and sorption, respectively.

Omics-Implemented bioremediation: It takes into account the genomic structure of the remediating organisms. Data regarding catabolic genes, enzymes, or proteins with bioremediating capabilities are taken from proteomics, metabolomics, transcriptomics, metagenomics, and functional genomics (Upadhyay et al. 2019). These are then identified and isolated for further bioremediation processes. Metagenomics is the study of genetic matter taken from the environment, which has the potential for bioremediation (Sengupta et al. 2021). Proteomics is the study of proteins through biochemical means (Dey et al. 2021). The combination of the above studies helps to obtain efficient strains of microbes and increase the metabolism of the contaminants (Malla et al. 2018). Many microorganisms' genome sequencing and profiling have been conducted. For example, transcriptional profiling of *Shewanella oneidensis* (known to contain co-metabolic pathways) was performed during U(VI) reduction (Wang et al. 2017a, 2017b). A biomarker of *G. sulfurreducens* activity was also developed through proteogenomic analysis for Uranium bioremediation (Marques 2018).

With improving technology, more progress can be made in this direction. Next-generation sequencing allows enhanced expression of desirable genes and proteins (Fonti et al. 2015). Genome-wide transcriptome methods lead to better analysis of metabolic pathways and physiology of the microbes (Lourenço et al. 2019). Integrating all the information gathered related to the properties and functions of microbes helps in their improved selection during the bioremediation process.

PROCESSES SIMILAR TO BIOREMEDIATION

Biostimulation: Here environmental conditions are optimized to encourage the growth of existing bioremediating microbial populations. It is done by adding rate-limiting nutrients or electron acceptors like oxygen, nitrogen, carbon, or phosphorous (Tribedi et al. 2018), modifying physical factors like pH, temperature, aeration, etc. (Mallavarapu et al. 2020) to stimulate the growth of present microscopic assemblage for degradation of radionuclides. These microorganisms then help in bioremediation of toxicants. A biostimulation experiment by UMTRA, Colorado, confirmed the precipitation of U(IV) by adding acetate as an electron donor (Roh et al. 2015). Since the method accelerates the development of indigenous or non-indigenous microbes for bioremediation purposes, it comes under 'enhanced bioremediation' (Kumar et al. 2018). The method is advantageous for low-cost and native microbial population exploitation without adding allochthonous species (Bosco & Mollea 2019). Care must be taken when adding nutrients since they should be evenly distributed and readily available to the subsurface microbes. Also, the surface should be permeable with no cracks or fractures (Jayaprakash et al. 2019). *Arthrobacter ilicis* and *Geobacter* have been identified to remove radionuclides like U(VI), Pu(IV), Tc(VII), and Np(V) through biostimulation (Shukla et al. 2017).

Bioaugmentation: The method is executed when the native microbial population present at the contamination site is unable to degrade the pollutants (Mallavarapu et al. 2020). In this method, microorganisms are added to enhance and speed up the degradation process of pollutants (Xu 2018). Microbes with high catabolic potential are generally added (Agnello et al. 2016). This is done by (i) adding pre-modified bacteria, (ii) adding pre-modified consortium, (iii) adding relevant genes in microbes for biodegradation (iv) introducing genetically modified bacteria (Upadhyay et al. 2019). The microbes introduced should retain genetic stability and viability during storage, withstand harsh conditions, and adapt to a foreign environment. Nutrient content, moisture, aeration, pH, and soil type can affect the efficiency of bioaugmentation (Jayaprakash et al. 2019). It is applied with biostimulation and comes under 'enhanced bioremediation' (Kumar et al. 2018).

Through the above processes of crystallization and precipitation of immobile and insoluble compounds by micro (or macro) organisms, metal biorecovery is possible.

Phytoremediation: Bioremediation done by plants is phytoremediation. It is a subcategory that includes plants, accompanied by rhizospheric and endophytic microbes, to remove the contamination in soil, sediments, sludge, and ground or surface water and clean the environment (Kumar et al. 2018). It considers plants' natural ability to uptake or absorb radioactive contaminants through roots and translocation to the upper part of the plant (Sharma et al. 2015). It thus uses this as an advantage to reduce its toxicity. These plants range from hyperaccumulators (e.g., *Helianthus*) to bio-accumulators (Dubchak & Bondar 2019).

It is a cost-effective practice since the expenditure is less than that of conventional methods, and it is environmentally friendly, as it preserves the environment in its natural state. The recovery and reusability rate of valuable metals is higher. Also, the plants can be easily monitored, and the progress can be tracked down (Eskander & Saleh 2017). Its extensive use was started in the 1990s by researchers and US Environment Protection Agency (Shmaefsky 2020). Since then, it has been employed in the sites contaminated by U, Th, and Ra (Natarajan et al. 2020).

Phytoextraction: Also called Phytosequestration, Phytoaccumulation, or Phyto absorption, this technique utilizes the plant's ability to pick up contaminants from the soil and transfer them to the harvestable parts of the plant (Natarajan et al. 2020), which can be obtained later by harvesting the incinerating or composting the particular plant (Kumar et al. 2018). It removes the toxins from the soil by not disturbing the soil structure and impacting little on soil fertility. For this method, fast growing plants are used that (i) can produce large quantities of plant biomass (ii) have capacity to tolerate and extract radionuclides at high concentrations (iii) are able to translocate the radionuclides to the plant biomass (Sheoran & Sheoran 2017). These plants are called hyperaccumulators and are known to accumulate toxicants at a concentration 100 times greater than what a normal plant would accumulate (Sheoran et al. 2016). The contaminants extracted are much smaller than the initial quantity in the soil or sediment. Hence, it is best suitable for areas of low-level contamination (Dubchak & Bondar 2019). The efficiency of the process also depends upon the bioavailability of the radioactive pollutants present (Khan et al. 2020). It is popularly employed for ^{137}Cs, ^{90}Sr, and ^{235,238}U (Dijoo et al. 2020). Research has been done on Catharanthus (for ^{137}Cs), Cannabis (for ^{90}Sr), Festuca, and Zea (for ^{222}Rn and ^{226}Ra) (Filippis 2015).

Rhizofiltration: It is specified for wastewater where the roots of plants are used to concentrate and precipitate radionuclides from that wastewater (Kumar et al. 2018). This can be done ex-situ or in situ, where plants (preferably hydrophytes) are grown hydroponically and, after their growth, relocated to a polluted water stream (Sharma et al. 2015) or grown straight into the water body polluted by radioactive effluent. For this technique, plants with rapidly growing root systems are chosen (Natarajan et al. 2020). Scientists thought of using several ponds in the sequence where the water flow rate is set to be slow to clean water contaminated by radionuclides (Dubchak & Bondar 2019). This permits relatively cheaper procedures with low capital costs. Water, sludge, and plant samples were taken regularly from all the parts of that system created to calculate the complete mass balance of radioactivity. It was later calculated that such a system removed 99.3% of the radioactivity. This approach was used for ^{90}Sr and ^{137}Cs and U removal from water (Filippis 2015) and is most effective in U removal. Nowadays, seedlings (blastofiltration) or excised plant shoots (caulofiltration) are used to remove contaminants from streams (Rezania et al. 2020). *Helianthus annuus L.* is a suitable plant that can remove 80% of the U within 24 hours from the contaminated water (Tonelli et al. 2020). *Phragmites australis* (Wang & Dudel and *Phleum pratense* (Mikheev et al. 2017) are also known for U and Cs remediation, respectively. One limitation of this process is that it can't extract the contaminant below the rooting depth. Also, proper care and maintenance are required since the plants can become a potential radiation source while extracting the contaminants from the soil.

Phytovolatilization: The method uses the plants to convert the toxicants into volatile forms to be discharged into the atmosphere (Kumar et al. 2018). It can be direct (through stems and leaves) or indirect (through roots) (Limmer & Burken 2016). It is used for ^{3}H, i.e., Tritium remediation, which is a radioactive isotope of Hydrogen with a half-life of 12 years approx., decaying into stable helium. Experiments conducted showed that reduction in radioactive Tritium (up to 40%) could be accomplished by releasing the titrated water into the atmosphere in water vapor form since it gets easily isolated by air and emits almost no exposure externally instead of flowing it in surface water streams near the sites (Dubchak & Bondar 2019). Commonly phreatophytes that are deep-rooted and have high transpiration capacity are deployed for this type of remediation (Khan et al. 2020), providing a system with enhanced evapo – transpiration and hydraulic control. *Typha latifolia* is one of the few plants apt for Selenium decontamination (Tonelli et al. 2020). The plant enzymes convert the inorganic Se to different volatile forms, like dimethyl selenide and dimethyl selenone (Sharma et al. 2015).

Phytostabilization: It focuses on the stabilization and storage of radionuclides for longer durations. It is based on radionuclides sequestration in the soil near the area of roots (Tonelli et al. 2020) but not in the tissues of the plants. Since the contaminants are stored in the root area, they become less available to livestock, wildlife, and humans, and the exposure is greatly reduced (Natarajan et al. 2020). Additionally, the phytostabilizing plants can reduce soil water and wind erosion and thus prevent radwaste's dispersal into dust particles, runoff, or leachate (Filippis 2015). The technique requires a dense root system to stabilize the soil and minimize water percolation, preventing soil erosion and radionuclide leaching (Dubchak & Bondar 2019). Green plants which are deep-rooted and fast-growing (e.g., *Cyprus*) are preferred since they reduce the stabilization process to large amounts (Sharma et al. 2015). This method has been used to stabilize U mine tailings (Wetle et al. 2020). *Cannabis sativa* L. and *Vetiveria (Chrysopogon) zizanioides* are a few plants used at mine tailings for phytostabilization of U and Cs, respectively (Khan 2020). Some of the plants known for phytoremediation of certain radionuclides are listed in Table 2.

Mycoremediation: Remediation by fungi is known as mycoremediation. It was first observed in Chernobyl Nuclear Power Station, where few fungi could generate spores. It was degrading and feeding on the soil contaminated by high Co, Pu, and C concentrations. Many species of fungi are observed to be able to remediate radionuclides from soil. These were later called radiotrophic fungi (Júnior et al. 2020).

The fungi remediate in the form of arbuscular mycorrhizae by forming associations like ectomycorrhizae or in any other way to immobilize the radionuclides, which are then taken up by plants (Sharma et al. 2015). The physicochemical properties of fungal cell walls play a key factor in radionuclide immobilization (Dighton 2019). Other factors include temperature, moisture, assembly, and activity of the microbial population, soil conditions like type, organic matter amount, and water availability (Kapahi & Sachdeva 2017). The cost-effectiveness, low maintenance, and ubiquitous nature of most fungi species allow their widespread use for bioremediation (Jain et al. 2017). *Aspergillus niger* and *Rhizopus arrhizus* can remove Thorium through mycoremediation (Francis & Nancharaiah 2015). Oyster mushrooms are also known to be bioremediated Plutonium-239 and Americium-241 (Dubchak

Table 2: Higher plant species and algal species-appropriate in phyto/phycoremediation of radionuclide contaminated sites.

Algae/Higher plants	Species Name	Radionuclide	References
Algae	*Oedogonium* sp.	^{90}Sr	Iwamoto and Minoda (2018)
	Ophiocytium sp.	^{125}I	Iwamoto and Minoda (2018)
	Vacuoliviride crystalliferum, *Galdieria sulphuraria*	^{137}Cs	Iwamoto and Minoda (2018)
	Cladophora, Oedogonium, Rhizoclonium	^{238}U	Kumar and Kundu (2020)
Higher plants	*Egeria densa, Euphorbia macroclada, Astragalus gummifer, Verbascum cheiranthifolium, Phaseolus acutifolius*	^{90}Sr	Iwamoto and Minoda (2018)
	Pinus radiata, Pinus ponderosa	^{90}Sr	Dighton et al. 2019
	Amaranthus retroflexus, Beta vulgaris, Brassica napus, Chenopodium quinoa	^{137}Cs	Iwamoto and Minoda (2018)

Table 3: List of certain fungal species known to remediate radionuclides from contaminated sites.

Fungi species	Radionuclides	References
Penicillium sp.	^{90}Sr, ^{238}U, ^{232}Th	Lopez-Fernandez et al. 2021
Pleurotus eringii	^{34}Cs, ^{137}Cs, ^{85}Sr	
Hebeloma cylindrocarpon	^{90}Sr	
Cortinaraiacea sp.	^{137}Cs	
Serratia sp.	^{137}Cs, ^{90}Sr, ^{60}Co	
Boletus, Paxillus, Tylopilus, Lactarius rufus, Leccinum, Amanita, Cortinarius, Suillus variagatus	^{137}Cs	
Aspergillus niger and *Paecilomyces javanicus*	^{238}U	
Rhizopus sp.	^{241}Am, ^{144}Ce, ^{147}Pm, $^{152+154}Eu$, ^{233}U, ^{230}Pu	

& Bondar 2019). A few of the fungal species known for mycoremediation are given in Table 3.

LIMITATIONS OF BIOREMEDIATION AND PHYTOREMEDIATION

Although the above-discussed methods show many prospects for their uses, they still face some challenges. Bioremediation has high specificity, i.e., we can't use every plant for any given remediation method (Dubchak & Bondar 2019). These are based on the properties and compatibility of both organisms and toxicants. Since naturally occurring life forms are involved, the procedure will take comparatively longer (Butnariu & Butu 2020). Also, no method can 100% remediate the soil; some minute amount of radwaste can still be left in the soil (Kumar et al. 2018).

In the case of phytoremediation, the area and depth covered by the roots of the plant pose a limitation to the remediation process (Khan et al. 2020). Again, due to less biomass and slower growth of plants, more time will be taken (Sheoran & Sheoran 2017). The remediation can continue as long as the plant survives in the soil, i.e., proper maintenance and cultivation of plants are essential (Filippis 2015). Successful lab phytoremediation experiments do not guarantee the same success rate at the practical field level (Yadav et al. 2018). Extreme caution is required to handle and dispose of contaminated plants (Farraji et al. 2016).

FUTURE PROSPECTS

Many aspects of bioremediation are explored by the continuous efforts of researchers and scientists, such as electrokinetic remediation (Cameselle 2015), algal remediation (Iwamoto & Minoda 2018), etc. These methods will be used for bioremediation purposes in the future. Numerous organisms with potential bioremediating properties are now discovered, which will be applied to the process in the coming days. These would be either used naturally or may be genetically transformed (called transgenic plants) into better radio-tolerant forms which can perform the procedure effectively. Various branches of science are participating to improve the chances of bioremediation. Geophysics is one of them, which uses geophysical monitoring to supervise the contaminated soils and analyze the changes occurring so. This is started for in situ bioremediation projects for consistent data collection which helps in real-time monitoring (Nivorlis 2019). Within the next few years, it can become essential for bioremediation monitoring.

CONCLUSION

Bioremediation and Phytoremediation methods are fast-growing and popularly used for radioactive waste removal or treatment. Being organic methods, which does not produce any side effect while performing the process and are cost-effective simultaneously, gives them an advantage. Hence their popularity is increasing. With advancing time, scientists are searching for more organisms (microbes, fungi, or plants) that can be used naturally or by genetic modifications. They can successfully remediate radioactive wastes by any means. The existing biotechnological methods are also enhanced with improving technology for better remediation results. In phytoremediation, plants native to the contaminated area are looked for as they will have the least external input. After remediation, they should be removed, or they might decompose into the contaminated soil. The most used way is to incinerate the ground and use ashes for disposal. Microbial remediation has enormous potential to control the activity and solubility of radioactive matter. New tolerant microbes are discovered that can withstand the wastes of extreme radioactive toxicity. These microbes can be employed in the future, boosting the remediation process and radioactive waste removal rate. Research on this field should be more to find out more ways of effective remediation. New and improvised techniques will be developed only when different science disciplines collaborate and work together.

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