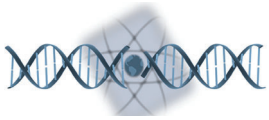
Harnessing the Environment to Identify Nuclear Processes:
I. Biological Markers to Assess Environmental Exposure



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Abstract

The present article serves as a companion piece for "Harnessing the Environment to Identify Nuclear Processes", also published in the 24th edition of the CWMD Journal. There, we presented an overview on the use of natural or engineered biologically-based systems as radiation, biological, and chemical indicators for detection and analysis of nuclear proliferation activities not readily discernible by current methods of monitoring. Biological systems can be leveraged to augment or replace current methods of surveillance through the use of indigenous flora and fauna or those engineered to render specified capabilities. Such systems not only collect but often concentrate materials of interest, thus allowing detection of trace amounts and retention of signal that may otherwise be lost to standard sampling, and, through exploitation of biological signatures, provide a log of activity which allows reconstruction of ephemeral and short-lived events that often challenge conventional monitoring techniques. The approaches described herein focus on the use of naturally-occurring microbial species, multicellular organisms and biologically-derived materials, and ecological networks for collection and analysis or, in some cases, for remote interrogation. Although several of the constructs can be adapted for immediate use, others will require additional research to develop fully mature capabilities for incorporation into the nuclear monitoring toolkit.

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Introduction

The present article serves as a companion piece for "Harnessing the Environment to Identify Nuclear Processes", also published in the 24th edition of the CWMD Journal. There, we presented an overview on the use of natural or engineered biologically-based systems as radiation, biological, and chemical indicators for detection and analysis of nuclear proliferation activities not readily discernible by current methods of monitoring. Current approaches for certain concepts of operation are limited in scope by logistics and instrumentation, and few, if any, near- to mid-field detection schema that meet requirements for covert and persistent environmental monitoring presently exist. We posit that recent advances in the life and physical sciences allow for exploitation and optimization of biological systems to serve as indicators of illicit nuclear activity.

Biological systems can be leveraged to augment or replace current methods of surveillance through the use of indigenous flora and fauna or those engineered to render specified capabilities. Such systems not only collect but often concentrate materials of interest, thus allowing detection of trace amounts and retention of signal that may otherwise be lost to standard sampling, and, through exploitation of biological signatures, provide a log of activity which allows reconstruction of ephemeral and short-lived events that often challenge conventional monitoring techniques. Natural systems require no power, specialized equipment, or complicated emplacement strategies. In cases where organisms do not possess the intrinsic capability to concentrate and/or signal the presence of specific agents, components and pathways within naturally-occurring systems may be engineered to develop the necessary capacity while still operating in accordance with the performance parameters generally described above.

To date, however, limited effort has been applied to investigating the utility of living systems or discrete components and pathways derived from them to assist the development of novel monitoring strategies specific to the identification and characterization of nuclear processes. Most efforts related to environmental monitoring are relevant to either bioremediation (e.g., phytoremediation of contaminated sites) or evaluate endpoints that are not directly useful for the purposes described herein. Recent advances in the fields of biophysics, analytical chemistry, and computational modeling *inter alia* provide unprecedented ability to interrogate and manipulate single-celled as well as multicellular organisms at system and sub-system levels, therefore lending credence to the notion that biological collection and sensing motifs can be successfully developed. The approaches described herein focus on the use of naturally-occurring microbial species, multicellular organisms and biologically-derived materials, and ecological networks for collection and analysis or, in some cases, for remote interrogation. Later articles will explore other applications of biological systems and the enabling technologies that support their use.

Microbial Species

Microorganisms exist in every natural biome, with extremely high population densities per gram of soil, or milliliter of air or water (Figure 1).[1] The term microorganism has broad reach and includes bacteria and archaea, algae, protozoans, fungi, and lichens (symbiosis between fungi and algae). While microbiome communities exist in concert with various higher organisms such as animals and plants, this article focuses on those specific to general environmental settings such as soil, water, and extreme conditions. Most living organisms, including microbes, respond to environmental stimuli such as exposure to ionizing radiation (IR) and chemical compounds. Distinct types, or levels, of radiation result in degrees of physiological, biochemical, and/or genetic outcomes which may be either broad or specific to different microbes.[2] These outcomes can manifest in changes to population density and diversity and alteration of physiological, genetic, or proteomic responses. The tremendous abundance and diversity of microorganisms, and their responses to environmental inputs provides unique opportunities to use them as monitors of the environment, and potentially provide signatures, for example, of nuclear activity.

While microorganisms are traditionally studied individually, a vast majority of environmental microbes are not cultivable or isolatable with current techniques. A new scientific discipline has been established and advanced in recent decades to investigate features and functions of microbes

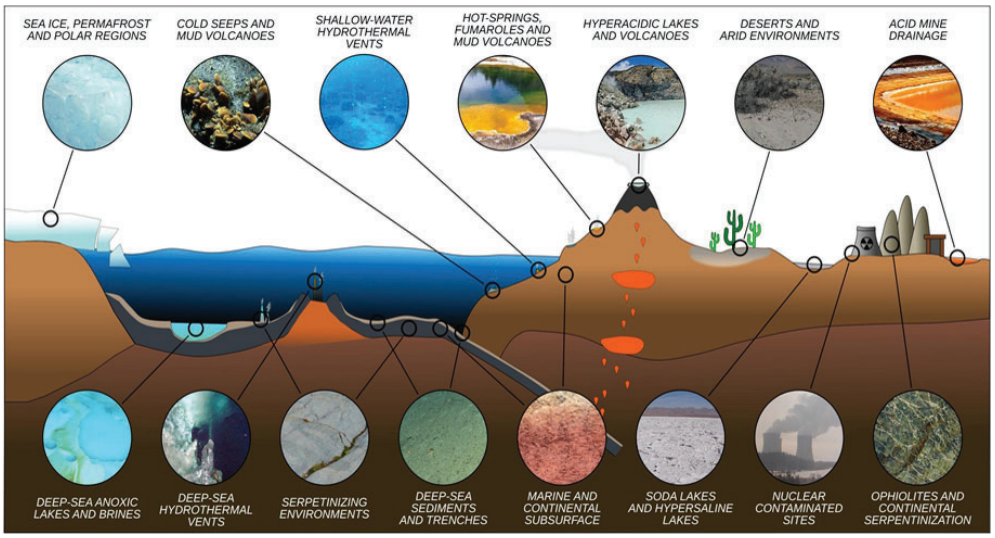

Figure 1. Illustration of the myriad of environmental niches where microbes have been identified. (Taken from Merino et al. 2019)[1]

as a community in their indigenous environment. *Microbiome* is a collective term that describes membership of all the microorganisms living in a loosely defined community. These complex communities often rely on the specific physiological interactions of different species to provide carbon and energy sources for other microbial members. The availability and concentrations of these chemicals play a significant role in determining the dominant type(s) of microbial activity at any given location.

The ubiquity and vast abundance of microbes in the environment, and their responses to environmental stimuli and conditions offer advantages as monitors for radiation exposure. Microbes evolve to adapt to their environment and react to long- and short-term environmental changes which are manifested as changes in the composition of the microbial community and/or changes in the genetic makeup of the microorganisms that comprise the community. Nuclear incidents and controlled irradiation experiments on bacterial isolates have shown genetic and metabolic changes in microorganisms in response to radiation. These changes can be transient or persistent.

Most microbial populations reside in environments that are considered suitable for living organisms. However, optimum growth conditions for certain species could occur at extreme temperatures (near freezing or near or above boiling), very acidic or extremely alkaline, low to high salt concentrations (osmotic stress) and degrees of oxidation/reduction (redox) conditions (Table 1). By regarding microbial populations as data-rich environmental sensors and response elements, monitoring scenarios can include sample collections, in-situ sensors, and/or remote sensing. Further, the ability to incorporate microbes into synthetic biology and bioengineering enables the potential for their use as reporters as well.

Factor	Range
Temperature	< 0° to 100° Celcius
Osmotic Pressure	< 1% to 35%
pH	~1 to 10
Water Availability	≥ 60%
Redox Conditions	~400 to 800 millivolts

Table 1. Microbial growth ranges.

IR exposure to a microbial population can be detected as:

- Overall changes in the microbial density and diversity in an area, both chronic and acute
- Physical alterations in the cells, including contaminant sorption, cellular injury/stress
- Changes to metabolic activity (i.e., CO2 release, oxygen or nitrate use, etc.)
- Modification of genetic responses due to radiation.

Microbes can be isolated from the environment through physical removal of small sample sizes of a few milliliters or grams. Filtration from water or air can separate cells based on size through various filter pore sizes. Cell desorption from soil particles can also be accomplished[3] and could lead to filtration and further analysis. Once concentrated, cells can be analyzed for overall changes of taxonomic and functional diversity through molecular techniques that categorize a microbial population based on genus or species, physiological characteristics and even changes in specific genes. The term operational taxonomic unit (OTU) is often used in molecular biology to represent a genus- or species-level relationship.

Molecular techniques are useful in understanding the genetic structure of a species (genomics) or the genomes of a mixed community (metagenomics). Additionally, transcriptomics relates to specific metabolic function at any given time. Proteomics refers to existing proteins and changes due to environmental conditions. Metabolomics is the study of chemical fingerprints that specific cellular processes establish during their activity.[4]

Nuclear materials, and chemicals associated with nuclear activity (e.g., fluorides, nitrates, halogenated organic compounds), can elicit immediate state changes in microbial communities.[2,5-7] Profiling these state changes via nucleotide sequencing of evolutionarily conserved gene sequences or whole genomes, allowing identification of microbial sentinels.[8,9] Popular targets include the 16S rRNA gene for bacteria and archaea, 18s rRNA gene for fungi, inter-transgenic spacers for bacteria, archaea, and fungi, and whole community metagenomics for all. Fluorescent antibodies specific to particular cellular components, and fluorescent probes linked to specific genes are well established methods of analyzing environmental samples. These techniques are often enhanced with flow cytometry[10] and electrochemical techniques as a means of rapid and quantitative detection. In addition to profiling community membership, researchers are looking into functional components of microbiomes. Measurements of small molecules, metabolites, proteins, and gene transcripts aside from, and in conjunction with, membership profiling may provide optimal monitoring solutions.

While this is not meant to be an exhaustive review, the following sections introduce what is known about environmental microbial communities related to common nuclear and radiation sources and exposure levels. Highlighted are gaps in current knowledge and potential utility of microbiota in detection, sensing, and reporting of nuclear sources and sites.

Soil Microbiomes. Microbial communities within soil can be very diverse in composition and geographical sites, depending on whether or not members are motile.[11] This creates an interesting situation whereby organisms can be used as snapshots of single events or changes over time. Studies profiling microbial response to and influence on the local environment are highlighted below.

At the Department of Energy's Oak Ridge site containing uranium (U) contaminated soil, a controlled input of ethanol as an electron donor was introduced to stimulate the resident microbial community to facilitate U(VI) reduction. This prompts immobilization of U and aids in remediation of the soil. When compared to non-treated sites, the ethanol-treated sites had several enriched bacterial genera suspected of performing this unique bioremediation activity.11 Among these enriched genera, *Desulfovibrio* species (spp.) and *Anaeromyxobacter* spp. are known U(VI)-reducers, with the latter previously associated with contaminated subsurface environments. *Rhodopseudomonas* spp. were also enriched and, while not much is known of representative species to reduce metals, the completed genome of *Rhodopseudomonas palustris* revealed the presence of several cytochrome C genes (involved in bioremediation processes), thereby suggesting that other members of this genus possess similar capabilities. Enrichment of unspecified *Pseudomonas* spp. signatures

suggests that some species could metabolize aromatic or chlorinated compounds. In parallel, the researchers investigated the functional responses through the identification of expressed gene signatures to elucidate the potential microbial activities related to U reduction. Using the GeoChip microarray, capable of identifying 2,300 genes, benzoyl-CoA reductase (catalyzes ATP-driven aromatic ring reduction), sulfate reduction, and dissimilatory bisulfite reductase were the predominantly expressed genes found in the treated U sites.[11]

Cesium-137 (Cs-137) remains as the primary source of gamma radiation in the soils surrounding the Chernobyl disaster site[12], where networks of large trenches were constructed to collect the radionuclide waste. Of these, trench 22 (T22) has been used to understand the transfer of radionuclides to the environment. In this study, investigators utilized next-generation sequencing (NGS) platforms to capture as much of the bacterial and archaeal diversity from both the trench site and the surrounding soils. Among the bacterial signatures were *Acidobacteria*, AD3, *Chloroflexi, Proteobacteria, Verrucomicrobia,* and WPS-2. Of the archaeal signatures, Crenarchaeota were dominant. Of interest is that there were no cultured bacterial representatives from the trench site, except for *Bradyrhyzobium, Rhodoplanes, Burkholderia,* and *Sinobacteraceae*. These results highlight the benefit of both culture-based and independent techniques, alone and in concert, for profiling complex communities.

In a related study, microbiota were measured and compared at the Chernobyl and Fukushima disaster sites as signatures of Cs-137 and Strontium-90 (Sr-90) exposure.[13] Interestingly, soils in the nearby region of reduced radionuclide contamination had greater bacterial diversity. Microbial sequencing efforts between these two sites revealed 417 shared OTUs, among them being members of *Rhodospirillales, Acetobacteraceae* and C*andidatus Solibacter, Acidimicrobiales, Verrucomicrobia, Bryobacter, Rhizobiales, Proteobacteria*, subgroups 1 and 2 of A*cidobacteria, Ktenodobacterales, Chloroflexi,* and *Thermotogales*, with the most abundant OTUs representing the *phyla Actinobacteria, Acidobacteria*, and *Proteobacteria*. This same study reported certain genes of specific bacteria that could be associated with exposure of specific radionuclides. Some genes and bacteria associated with Sr-90 were IS110 family transposase (T*errriglobus saanensis)*, *amidohydrolase and tetratricopeptide* TPR_1 repeat-containing protein (both from *Gemmatirosa kalamazoonesis*), putative *peptidase* S8 and S53 *subtilisin kexin sedolisin* (*Tetrasphaera japonica* T1-X7), response regulator (*Acidobacteriaceae* sp. KBS89), ABC transporter permease and serine/threonine protein Kinase (both from *Candidatus Koribacter versatilis*), PAS domain S-box protein (*Singulisphaera acidiphila*), among several others. For Cs-137, suspected associated bacterial genes were magnesium-translocating P-type ATPase (*Bryobacter aggregatus*), SAM-dependent methyltransferase (*Candidatus Solibacter usitatus*), peptidase M14 carboxypeptidase A (*Gemmatirosa kalamazoonesis*), and 2-oxoglutarate dehydrogenase E1 component (*Acidobacterium ailaaui*), among others. For both Sr-90 and Cs-137, associated bacterial genes were multidrug efflux RND transporter permease subunit (*Candidatus Koribacter versatilis*), tonB-dependent receptor (*Granulicella tundricola*), and GntR family transcriptional regulator and tetratricopeptide repeat protein (both from *Acidobacteriaceae bacterium* KBS 83), among others.[13] While the influence of radiation on gene expression or the influence of gene expression on radiation response was not characterized, they still provide meaningful targets for developing profiles related to each source.

Methylated iodine-129 (I-129) represents a threat to public health as long-term exposure results in its accumulation in the thyroid. One study sought to explain the biogenic methylation of I-129 released from nuclear facilities and dispersed into the atmosphere and water systems, because global methylated I-129 (up to 4×10^{11} grams per year (g/yr)) could not be explained solely from microalgae activity (up to 10^{10} g/yr).[14] Through microcosm-based cultivation studies with gas chromatography, the researchers were successful in demonstrating and quantifying known soil microbes' methylation of I-129: *Methylobacterium* sp. strain MRCD 18, *Pseudomonas straminae* JCM 2783, *Rhizobium* sp. strain MRCD 19, *Rhodococcus equi* JCM 1311, *Variovorax* sp. strain MRCD 30, and *Zoogloea* sp. strain MRCD 32.[14]

Aquatic Microbiomes. Marine and freshwater environments harbor bacterial communities that, like those of diverse soil systems, contribute to the cycling of nutrients and compounds available to specialized microbial subsets that can metabolically leverage these potential energy sources. Identifying microbial signatures in response to perturbations, such as from radiation, metals, or organic compounds, permits an understanding of biological responses to abiotic influences.

Uranium tailings are usually contained within liners, sealed, and covered in soil after decommissioning. Owing to uranium's toxicity and high solubility in the environment, implications regarding groundwater contamination are a major concern. In the case of the Deilmann Tailings Management Facility, waste tailings are contained in a deposition site covered in nearly 40 m of water to prevent particles from being aerosolized and shield escaping radiation.[15] To profile viable bacteria previously isolated from this site, researchers devised an *in situ* cultivation strategy utilizing polycarbonate coupons submerged at 13 m intervals of depth, up to 41 m. When recovered, replicates of these coupons have shown the presence of biofilms after three months of cultivation. Inductively Coupled Plasma – Mass Spectrometry analysis showed no trends in specific metal accumulation, although dissolved concentrations of vanadium and molybdenum appeared to increase with greater depth, while dissolved concentrations of manganese decreased. Cultivation of viable cells highlighted certain bacterial genera associated with depth and available carbon sources: *Polaromonas* appeared to increase in abundance with greater depth, while *Methylobacterium, Dechloromonas*, and *Aquabacterium* decreased with greater depth. Both *Polaromonas* and *Acidovorax* were found at the 41 m depth, while *Ralstonia*, (some species of this genus are known to reduce iron (Fe)), was found across all depths.[15]

Spent nuclear fuel (SNF) rods are stored in bins with water to help dissipate heat and radioactivity during their decay. Typically, the aluminum coatings on the SNF are well suited to prevent corrosion during this long term storage. However, white precipitates were evident in the storage water at Savannah River storage facility, prompting concern about the potentially rapid degradation of the SNF protective coating, and subsequent chemical and biological investigations.[16] While total organic carbon (TOC) in the storage water was 6.8 mg/l (avg., SD + 12.5), the TOC in the precipitate was 883 µg/g (wet wt.). Major elements associated with the white precipitate were silicon, aluminum, titanium, and Fe. Bacterial 16S rRNA phylotyping revealed the presence of *Aquabacterium, Hyphomicrobium, Pedomicrobium, Rhodoplanes* bacterial genera, and representatives from the *Burkholdariaceae* family.

Methylated I-129 can be transferred from water and ocean systems to the atmosphere for increased dispersion. Two novel strains of *Roseovarius* (closest known relatives to R. *tolerans*) were isolated from seawater and marine mud, and demonstrated to generate forms of methylated I using GC-MS.[17] Motivated by this same public health concern, other researchers have demonstrated and quantified the biogenic methylation of I-129 by specific marine bacteria: *Alteromonas macleodi*i IAM 12920, *Deleya marina* IAM 14107, *Photobacterium phosphoreum* IAM 14401, *Photobacterium leiognathi* NCIMB 2193, *Pseudoalteromonas haloplanktis* IAM 12915, *Shewanella putrefaciens* IAM 12079, and *Vibrio splendidus* NCIMB 1.[14]

Air and Space Microbiomes. In principle, microbiome populations in air can be useful as a tool to detect nuclear or other contaminants, provided we know the patterns of their dynamics. However, among studies on environmental microbiomes, air and space microbiome populations have been least studied. In the following sections, we provide a current view of air and space microbiome research and discuss how it could be applied to the monitoring needs discussed herein.

Atmospheric Microbiomes. Microorganisms in airborne biomes vary in concentration, ranging from approximately $3.9x10^2 – 1.2x10^3$ cells per m^3 in forests and $7.9x10^2 – 7.2x10^3$ cells per m^3 in urban settings, to as high as $1.9x10^7 – 1.0x10^9$ cells per m3 during agricultural activities associated with bailing and combining.[18] About 25% of airborne particulates are biological, including pollen, fungal spores, bacteria, viruses, and so on. Weather conditions control, to a degree, microbial transport with the vertical concentration of bacteria declining much less than fungal spores.[19] Microbial retention in the atmosphere

is extended through contact with water droplets in clouds. About 15% of the volume of the first 6 kilometers of the atmosphere is occupied by clouds[20], thus the atmosphere harbors an enormous transient population of microbes.

The potential for the large transient population of microorganisms passing overhead to serve as environmental monitors should be explored more thoroughly. Highly concentrated samples can easily be obtained through conventional air sampling devices and could provide a magnified analytical sample related to covert activities. Sampling can be easily achieved by, e.g., capture of ambient air using conventional air sampling devices and collecting rainwater.[21] In addition, under conditions of sufficient moisture and temperature as well as available carbon and energy[22], evidence has been produced showing microbes can grow[23] and even thrive[24] in cloud water. The most metabolically active members of cloud microbiota were identified as Alpha- (*Sphingomonadales, Rhodospirillales* and *Rhizobiales*), Beta- (*Burkholderiales)* and Gamma-Proteobacteria (*Pseudomonadales*). Also, common isolates from cloud water are the genera *Deinococcus* and *Spirosoma*, known for their high resistance to DNA-damage like that caused by UV light and gamma radiation.[24-26] Given the relative nutrient scarcity of cloud water (compared to groundwater or other terrestrial niches), transcriptomic as well as other analytical approaches targeting metabolic output and function may best highlight changes among these microbiota.

Indoor Microbiomes. Indoor air consists of a variety of solid aerosol particles, including inhalable bioaerosols, which recently have been the focus of scientific research because of their impacts on public health due to COVID-19.[27] In the present section, we cover a broad range of microbiome samples from air in different enclosed spaces such as houses, subways, office buildings, and even in areas with low gravity and high radiation such as found in the International Space Station (ISS).[28]

Microbiome populations sampled in subways have high representation of typical skin microbes, but the dynamics of this microbiome are influenced by levels of carbon dioxide, temperature, and time of day.[29] Microbiome population studies in residences reveal similarities between indoor microbiome compositions and surrounding outdoor environments. The composition slightly differs depending on the frequencies and sources of ventilation, as well as the number of people living in the residence whose skin microbiome contributes to the variation. Additionally, the frequency of vacuuming can increase representation of the floor microbiota in the sampled air.[30] While the fungal portions of air microbiome samples also resemble outdoor populations, they have dispersal limitations due to the increased size and weight compared to bacteria and viruses.[31] As such, they might be a more stable tool for verification of nuclear materials. For instance, the high radiation absorption capacity of gilled fungi (mushrooms), suggests airborne fungi spores would be worthwhile tools30. Interestingly, observations of ISS surface microbiomes revealed they are heavily influenced by astronauts' microbiome and are not easily changed even after the individuals' departure.[32] Such organisms, including those inside of the ISS where they are subject environmental stressors like radiation and microgravity, have been a focus of studies during recent years.[32,33] Determining whether collection of microbiota from surfaces and air represents a useful tool for radiation monitoring merits additional research.

Studies on Radiation Effects: Differences by Distance. Some studies, including those evaluating the impacts of both the Chernobyl and Fukushima accidents, have focused on radiation effects as they relate to distance from exposure sources.[34-38] A notable study focusing on soil microbiome highlighted increased Cs-137 concentration upwards of 1 km from the Chernobyl power plant (10-563,000 Bq Cs-137/kg dry soil). Further, analysis of community diversity showed that distance and contamination were significant influences on the microbiome structure of sampled sites. Community compositional profiling of the most contaminated sites revealed increased abundance of radioresistant *Geodermatophilus bullaregiensis*.[35] For those sites, the vast majority of radioactive contamination results from americium-241 (recently discovered), cesium-137, strontium-90, and iodine-131. Studies on earthworms from the Chernobyl exclusion zone (CEZ) have shown no significant differences in oxidation and other radiation impacts, but the authors recommended that soil microbiome studies may better elucidate differences that depend on ex-

posure to variable radiation levels related to distance from primary sources.37 Although such efforts are very limited and nascent, increased focus will undoubtedly reveal their utility.

Microbiomes in Extreme Environments. "Extremophiles" are microorganisms that inhabit environments where the conditions are marked by extremes of temperature, pH, salinity, pressure, or radiation that are often inhospitable to most life forms. The cellular and metabolic machinery of extremophiles have evolved to withstand challenging environmental conditions, making them attractive candidates for biotechnology applications. A classic example is the discovery of a heat-stable DNA polymerase isolated from *Thermus aquaticus*, a microorganism originating from Yellowstone National Park, USA[39], and its adoption for use in polymerase chain reactions to assist the replication of DNA.[40]

Isolation of extremophile microorganisms can be complicated, as the environment itself might be dangerous or logistically challenging for sampling and/or the extreme conditions or mixture of substrates for cultivation might be difficult to achieve. Nonetheless, they are a significant part of the "microbial dark matter" that has yet to be discovered[41] and undoubtedly will offer unique insights into new metabolic pathways and survival strategies for future research.

Certain microorganisms such as *Deinococcus*, *Spirosoma*, *Rufibacter,* and *Hymenobactertibetensis*[42] have self-repair mechanisms making them resistant to denaturing effects and oxidative damage outcomes related to radiation exposure.[43] For instance, *Deinococcus radiodurans* is an extremophile capable of surviving ionizing and ultraviolet radiation that are lethal to humans[44], it was originally isolated from canned meat that was exposed to X-irradiation.[45] Additionally, *Chroococcidiopsis*, a cyanobacterium, employs quick and efficient DNA repair mechanisms to resist damaging effects due to IR.[46]

Microalgae have been found to successfully fractionate uranium isotopes.[47] Moreover, fungi are capable of immobilizing radionuclides.[48] For example, fungi such as *Cladosporium cladosporioides* and *Penicillium roseopurpureum* decomposed radioactive debris caused by the Chernobyl reactor within 50 to 150 days.[49] Melanin, a natural pigment produced by some fungi, may have helped to mitigate the deleterious effects of radiation.[50] Melanized fungal species colonized the walls of the Chernobyl reactor and exhibited growth toward radiation, perhaps using it as a nutritional source.[51]

It is common to find microorganisms, termed "*polyextremophiles*", capable of withstanding multiple harsh conditions in water and soil matrices contaminated by effluent from nuclear processes. Culturing techniques that replicate a combination of extremes (e.g., presence of heavy metals and concomitant low pH) prove helpful to isolate them.[52] Polyextremophiles and their metabolic products have been evaluated for their potential to act as microfactories for metal and radionuclide remediation of contaminated soil and water.[53] They could be used as natural sentinels or as sensor elements in hybrid detection devices to provide indication of anthropogenic activity.

Tertiary (Non-Radioactive) Compounds as Microbial Influencers. Previous sections provided evidence that microbial communities can sense and respond to the presence of radionuclides and that the signal is potentially retained after the radioactivity is no longer detectable or the radionuclides have been removed. It is plausible to posit that microbial communities will also respond to other materials, including industrial chemicals, which may be present as a consequence of nuclear activity. Although there are numerous compounds used for diverse types of nuclear materials extraction, featured here is a smaller list of compounds that are commonly used as part of well-established protocols like plutonium uranium reduction extraction (PUREX).[54]

One of the main solvents used in the PUREX process is tributyl phosphate (TBP), an industrial solvent with toxic, corrosive, and carcinogenic properties. A number of bacteria from the genera *Alcaligenes, Providencia, Delftia, Ralstonia*, and *Bacillus* have been isolated that can grow on TBP as the sole carbon (C) and phosphorus (P) source[55], showing degradation in laboratory cultures of >50% from 5 mM TBP after 4 days. Soil samples from a uranium mine, containing complex microbial communities, were incubated in the laboratory with 1,000 ppm TBP and other carbon sources. Upwards of 60% of the TBP was removed in 4 days and 80% in 10 days.[56] However, TBP is not a commonly utilizable source of

plutonium (Pu), particularly for marine bacteria. A recent study evaluating bacterial growth on 22 organic Pu pollutants found that none of the 17 tested strains could grow on this compound.[57] Research examining the influence of TBP on microbial community structure are presently lacking. As such, field contamination studies to monitor microbial communities in plots (for soil and sediments) or mesocosms (for aquatic systems) amended with different concentrations of TBP are necessary to determine whether TBP contamination yields observable and distinctive changes.

Another compound commonly used in nuclear processing is nitric acid (HNO_3), which results in high concentrations of nitrate and eventually nitrite in effluents. An early study documented microbial changes, including reduced community diversity and alterations of taxa, at sites contaminated with nitrate, nickel, aluminum, and uranium.[58] A subsequent study examined a similarly contaminated groundwater community with metagenomics[59], finding genes specific to resistance for nitrate, heavy metals, and acetone. Due to the presence of multiple contaminants (nitrate, nitrite, heavy metals, other organics), it was not possible to attribute observed microbial responses to a specific compound. However, a more recent study found that members from genus *Bacillus* dominated in soil community following direct additions of HNO_3[60]. With regard to nitrate contamination and its impact on microbial communities, substantial literature exists, and includes references to the nitrogen cycling genes required for the biological removal of heavy metals, including radionuclides, implicated in nitrate and nitrite reduction.[61] Nitrate contamination can result from other processes and practices (e.g., agricultural practices), but identification of microbial community changes combined with orthogonal detection schema to characterize any additional contaminating agents could yield information indicative of nuclear reprocessing and associated with the specific process that is being used.

Hyrdrofluoric acid (HF) is likewise used in the PUREX process. Unlike HNO_3, HF dissolution does not result in the production of a macronutrient like nitrate, thus HF microbial signatures may be more specific to industrial contamination like that resulting from reprocessing. One study used cultivation-dependent methods to examine soil bacteria at different distances (3, 7, and 20 km) from a source of HF and found decreasing soil respiration, biomass, and culturable bacteria as the samples got closer to the pollution source.[62] A subsequent study evaluated soil samples incubated with increasing HF concentrations in the laboratory for up to 10 days and found, not unexpectedly, that certain taxa increased in relative abundance, and others decreased in response to HF treatment.[63] Recent work also examined the impact of HF contamination on soil microbial communities60, finding that the genus *Bacillus* and other acidophilic (acid-loving) microbes became numerically dominant.

Kerosene and oxalate used in the PUREX process may also produce changes to microbial communities. Both are organic compounds that can be used by microbes as a source of carbon for growth and/or respiration.[64,65] Oxalate-degrading bacteria are more common, since this compound is released into the soil by plants and into the gut by animals[66], whereas kerosene degradation is a less common phenotype associated with degradation of hydrocarbons. A significant amount of literature exists regarding the effects of kerosene on microbial community structure because it is a common hydrocarbon pollutant, and the genes involved in kerosene degradation are well-characterized.[67] Conversely, oxalate pollution is uncommon, and literature surveys reveal no relevant literature with respect to the impacts, if any, environmental oxalate contamination may have on microbial communities.

Although numerous articles detail microbial response to TBP, HF, HNO_3, kerosene, and oxalate, most of the work, with a few exceptions, has interrogated naturally polluted sites or evaluated changes associated with laboratory enrichment experiments. A fully factorial ecotoxicology experiment where different toxic components, including radionuclides, are added individually and in combination to soil plots and aquatic mesocosms would be of high value. To develop a more comprehensive understanding that extends to multiple ecosystems, studies that replicate the work in different soil and water matrices with different geochemistries would be required.

Section Summary. It is logical to speculate that microbes and microbiome communities can serve as unique, sensitive natural sensing systems based on a number of qualities:

- Microbiomes exist in nearly all environments.
- Microbes, either as individual species or as diverse communities, respond to environmental perturbation with physiological, biochemical, genetic, and epigenetic as well as community composition and functional changes.
- Changes can be leveraged as signatures for detection and monitoring applications.
- Unique microbial features such as the ability to live in extreme conditions can be exploited for developing novel sensing motifs.

The studies delineated here represent various environmental systems affected by radiation, chemical, and metal sources and established use cases for monitoring scenarios. Additional work will bolster previous results in addition to providing new data that support deeper and more precise inferences. For example, whole genome assemblies would produce more taxonomic resolution (e.g., strain level variation). The combination of other 'omics technologies (i.e., proteomics, metabolomics, transcriptomics, etc.) with next generation sequencing (NGS) may offer resolution at the species, strain, and functional levels; however, these combinations are still fairly new, and good reference databases that allow adequate annotation are not fully formulated. Whereas such challenges are daunting, the collective body of work to date laid the foundation for the next phase of microbiome research that seeks application rather than phenomenology. The sections above, while not exhaustive or systematic, provide a glimpse into these efforts and offer potential targets for more focused approaches to address the questions surrounding environmental microbiomes and their potential to act as sentinels for ecosystem change.

Multicellular Organisms and Biologically-Derived Materials

Plants as Sentinels for Detecting Nuclear Processes

Plants have a number of features that make them promising candidates to serve as sentinels for detection of illicit nuclear activity, or more generally, unexpected IR exposures. By virtue of their generally sessile life-cycle, plants must respond to environmental threats *in situ*. As a result, many plant species have evolved robust responses to a wide range of biological or abiotic stressors.[68,69] Characterizing and understanding the sensitivity and specificity of these responses could facilitate harnessing them as indicators of radiation or nuclear material exposure. Chemicals, radiation, or thermal energy released into the environment by nuclear activities may alter the plant species distribution within the local area or lead to spectral changes in vegetation.[70,71] Plants display a range of lifespans allowing historical sampling, potentially over long periods of time. During these lifespans, they are constantly sampling the air and groundwater as well as interacting with microbial communities present on leaves and roots. Thus, they have the potential to serve as bioaccumulators of compounds in the environment. Furthermore, certain plant species have been identified that hyperaccumulate metals and radionuclides, thereby serving as natural amplifiers and collection devices.[72,73] Plants have been shown to have long-lasting biological changes, known collectively as 'plant memory', which may provide signatures of chemical or radiation exposure when no direct chemical or physical signal remains in the environment.[74] Plant memory includes protein, transcript, and epigenetic changes. Detection technologies leveraging plants as described above will require physical collection and analysis of plant tissue. However, some plant responses, such as changes in plant distribution and spectral signatures, may be monitored using remote sensing techniques. The present section provides a review of current data on the ability of plants to sense, signal, and respond to IR, nuclear, and process materials that reside in the environment and means by which such information might be used.

Changes in Plant Species Distribution as Indicators of Radiation Exposure. Ecological studies have documented significant changes in species distribution within the natural environments surrounding the sites of several nuclear power plant accidents, including Kyshtym, Chernobyl, and

Fukushima, as well as contaminated processing sites like the Oak Ridge National Laboratory site and the Mayak plutonium plant area.[75-79] The Kyshtym and Chernobyl accidents both resulted in vegetation death at areas receiving high doses of radiation. At the highest radiation doses (60 – 200 Gy), tree death was observed, with coniferous trees showing greater radiosensitivity than deciduous trees.[75,76,78] Moreover, specific tissues such as the buds and needles were more susceptible to radiation damage. As a result, forest growth and recovery was also impacted, with birch replacing pine in the areas around Kyshytm and Chernobyl.[75,78] At high radiation doses (30-50 Gy), herbaceous plant death was observed, with species death corresponding to radiosensitivity, phase of life cycle at the time of exposure, and the effective dose rate based on exposure route, including gaseous deposition through the air, soil surface exposure, or transport of the radionuclide through the roots.[75,80] At moderate radiation doses (5-10 Gy), plant death did not occur; however, there were visible signs of stress, like abscission of needles and damage to reproductive buds.78 Furthermore, suppression of vegetation growth was observed at lower radiation doses (0.5 – 1 Gy)[75,78,] and an overall reduction in biodiversity was observed in all ecosystems surrounding the sites of nuclear power plant accidents.[75,77]

The release of radionuclides from small-scale or clandestine nuclear processes is likely to be significantly lower than that released by the aforementioned nuclear power plant accidents[81] At these radiation doses, ecological succession resulting in presence, absence, or changes in abundance of particular species within the surrounding environment is unlikely. However, more sensitive and subtle plant changes, including reduced growth rates as well as spectral, protein, transcript, and epigenetic changes, may serve as indicators of anthropogenic activity. If there is a significant release of radionuclides into the local environment, changes to native flora based on radiosensitivity may serve as a remote indicator, as described above. A list of radiation-resistant plant species is shown in Table 2.[79] Radiation-resistant plant species were generally shown to have smaller genomes and concomitant high tolerance to heavy metals. It is worthwhile to note that changes in plant species within the environment will be complicated by other factors including the composition of the soil, which will affect the bioavailability of the radionuclide, and the availability of nutrients, which will impact plant stress response and survival. Therefore, environmental modeling and machine learning will likely play important roles in using plant species as indicators of nuclear activity.

Plant Taxa	Genome size (Mb)	Location of Study
Willow trees (genus *Salix*)	~425-429	Chernobyl, Oak Ridge, Fukushima, Canada
Birch trees (genus *Betula*)	~430-600	Chernobyl, Kyshtym
Alder trees (genus *Alnus*)	513-983	Chernobyl, Canada
Aspen trees (genus *Populus*)	440-593	Canada, Chernobyl
Sedges (genus *Carex*)	~150-300	Chernobyl, Mayak, Oak Ridge, Fukushima
Raspberries and related species (genus *Rubus*)	291-308	Fukushima, Canada, Chernobyl
Sorrels and related species (genus *Rumex*)	3200-3700	Chernobyl, Fukushima
Common reed (*Phragmites australis*)	~470-560	Mayak, Chernobyl

Table 2 . Radiation-resistant plant species identified through prior environmental monitoring studies.[79]

Optical Spectroscopy for the Detection of Nuclear Chemical Effluents in Plant Sentinels. The U.S. Government investment in next-generation hyperspectral imaging (HSI) systems has expanded the application of spectral signatures to determine process activity. HSI data provides high spectral content and is spatially mapped to show the distribution of vegetation exposed to source emissions from nuclear processing facilities. The advantage of using plant sentinels from an optical remote sensing perspective is that they are stationary and provide a persistent signal. Remote sensing and data collections over denied areas are usually episodic events and the ability to time source re-

leases to collection periods is a major shortcoming. Once vegetation uptake response from known process source emissions can be related to a reflective spectral response, plant sentinels could be used as *in situ* indicators for detection and monitoring using non-contact, passive remote sensing optical techniques.

A key gaseous phase source emission, HF, is transported through the atmosphere to expose vegetation species through leaf structure absorption.[82] Taylor et al. published a seven-year study (1972-1978) that evaluated fluoride air emissions and the potential vegetation impacts at the Paducah Gaseous Diffusion Plant (PGDP).[83] Continuously operating air sampling stations provided seven day averaged air samples of HF, and fluoride concentrations were measured in *Festuca arundinacea Schreb* sample collected from the surrounding grass areas. While air concentrations of HF varied from 0.01 to 24.5 µg (HF)/m³, fluoride concentrations in the grass measured as high as 1,000 µg/g near the PGDP and approximately 100 µg/g at distant locations. Similar relationships between F air concentrations, plant uptake and distance (>10 km) from emission source were found in studies of aluminum smelters.[84]

The use of spectral reflectance to study photosynthesis and related vegetative processes has been ongoing for decades[85,86], and numerous vegetation indices and algorithms have been developed to estimate plant stress factors and physiological conditions. An example of relevant research is the Combined Vegetation Index (CVI) developed in the context of a South Korean HF explosion accident.[87] In this study, remote sensing HSI data were utilized to interrogate foliage damage caused by the sudden and accidental release of HF. The deployed HSI system had a spectral range from 360 to 1,047 nm. Detailed spectral analysis of the data indicated that fluctuations occurred between 786 nm and 801 nm in the HF-affected vegetation. The study demonstrates that HF exposed vegetation detection is possible using HSI techniques; however, it also illustrates that more work is needed with higher resolution spectrometers that have a wider spectral range in order to deal with potential confounding stress factors so that the F-signal is definitive.

Other source emissions from industrial facilities are in the form of solids, including heavy metals, and liquids that exit through waste streams and accumulate in local vegetation. Metal-vegetation interactions, as measurable through optical remote sensing techniques, have been researched

Key Features/ Index*	Formula	Metal(s)	Vegetation Type	Ref.
CR_{1730}	--	General HM	Floodplain	91
DVI	$2.4(MSS7 - MSS5)$	Ni, Cd, Cu, Pb, Zn	Floodplain, ryegrass	92
EGFN	$\frac{Max(R650' - R750')}{Max(R500' - R550')}$	Zn	Conifer	93
NDVI	$\frac{R800 - R670}{R800 + R670}$	Cr, Pb, Zn, V	Gray birch	94
		Ni, Cd, Cu, Pb, Zn	Rice	95
		Hg	Mustard spinach	96
NPCI	$\frac{R680 - R430}{R680 + R430}$	General HM	Stinging nettles, Reed canary grass, Meadow foxtail	91
PRI	$\frac{R531 - R570}{R531 + R570}$	General HM	Floodplain	91
		As	Ferns	97,98
		General HM	Stinging nettles, Reed canary grass, Meadow foxtail	91
R_{850}	--	Cd, Cu, Pb, Zn, As	Peas	99
R_{1650}	--	Cd, Cu, Pb, Zn, As	Peas	99
R_{2200}	--	Cd, Cu, Pb, Zn, As	Peas	99
REP	$Max(R690' - R740')$	Ni, Cd, Cu, Pb, Zn	Floodplain, ryegrass	92
		Pb	Rice	100
		Cu	Peas, maize	101
		Zn	Sunflower	101
		General HM	Floodplain Bluegrass, ryegrass	102,103
		Hg	Mustard spinach	96
		General HM	Stinging nettles, Reed canary grass, Meadow foxtail	91
RGI	$\frac{R695}{R554}$	Cr, Pb, Zn, V	Gray birch	94
RVI	$\frac{Red}{Near\ Infrared}$	Hg	Mustard spinach	96

*CR = Continuum Removed; DVI = Difference Vegetation Index; EGFN = Edge-Green First Derivative Ratio; NDVI = Normalized Difference Vegetation Index; NPCI = Normalized Pigment Chlorophyll Index; PRI = Photochemical Reflectance Index; R = Reflectance; REP = Red Edge Position; RGI = Red Green Index; RVI = Ratio Vegetation Index

Table 3: Key spectral features and vegetation indices related to metal stress in the literature.[104]

primarily in the agricultural and ecological sciences. Notable spectral features and vegetation indices related to metal stress found in literature are summarized in Table 3. Hexavalent chromium (Cr(VI)), a heavy metal associated with the nuclear industry, has been widely researched. Cr(VI) species are mobile in the environment and readily taken up by plant roots[88,89] Chromium induces decreases in photosynthetic pigments chlorophyll-a, chlorophyll-b, and carotenoids.[89] Reflectance spectroscopy was applied to study the effects of chromium on the Chinese brake fern (*Pteris vittata*), and a unique ratio index (R_{1110}/R_{810}) was identified to differentiate Cr(VI)-exposed ferns from arsenic-stressed ferns and a control.[90]

In addition to identifying specific wavelengths and spectral indices, other analytics and machine learning approaches may further enable the detection of plant spectral features as indicators of radiation or chemical exposure. For example, hyperspectral reflectance imaging and multivariate curve resolution alternating least squares analysis was applied to *Arabidopsis* to identify unique spectral features to differentiate Cs stress from two other stress phenotypes resulting from exposure to salt and copper.[105]

A central point, and one that is reiterated multiple times throughout the present article regardless of the system or method of interrogation, is the need to isolate features associated with the uptake of the source emission versus stress indicators caused by naturally occurring environmental factors. Research should be guided by robust experimental protocols in order to verify the relationship between source emission, plant uptake, and spectral reflective response. In this regard, useful work includes co-stressor experiments, which combine source emission exposures and environmental stress factors, for different vegetation species, and application of advanced microscopy methods to assess plant physiological changes to quantify spectral reflective response.

Plant Accumulators for Collection of Radionuclides. Plants have been extensively studied for their ability to uptake and sequester heavy metals, including radionuclides.[71,73,80,89,106-132] While many studies have focused on the potential of plants for bioremediation of contaminated sites, these plant species may also be used as natural accumulators for detection of nearby nuclear processes. Examples of plant accumulators for certain chemicals associated with nuclear processes are listed in Table 3. It is important to note that the accumulation levels reported in Table 3 are dependent on the exposure dosage, so accumulation measurements are not directly comparable to source terms. However, the list nonetheless provides proof-of-principle that plants can be used as monitoring systems which have particular utility for tipping and cuing as well as broad area surveillance. In addition to the examples provided in the table, plant species such as *Sebertia acuminata, Arabidopsis halleri, Thlaspi caerulescens, Thlaspi praecox,* and *Solanum nigrum*, have been shown to hyperaccumulate other metals like nickel.[73,122] Manipulating the mechanisms of metal hyperaccumulation to enable radionuclide accumulation in species like these could be a fruitful area of research. As discussed in the previous section on *Changes in Plant Species*, environmental factors and soil chemistry play a key role in uptake and transport of chemical species. For example, the addition of organic acids like citrate to soil have improved uranium uptake in several Brassica species by more than 1000-fold.[117]

Chemical accumulation in plants has been studied extensively for environments surrounding nuclear accidents as well as nuclear power plants, providing data for a wide range of plant species and exposure levels. However, only a limited number of plant species were analyzed in each study, and crop species were a primary focus due to potential human health effects. Furthermore, the high number of environmental variables in field studies makes it challenging to predict chemical accumulation in plant species for a specific environmental scenario. Future research efforts would benefit from focusing specifically on indigenous plant species relevant to regions of interest and developing high-throughput laboratory techniques combined with machine learning to understand the influence of environmental variables as well as to identify plant species best suited for accumulating signatures of interest.

Chemical	Accumulating plant species	Level of measured accumulation	Ref.
Americium	*Elodea canadensis*	Up to 3,280 Bq/g dry weight (^{241}Am)	106
Cesium	*Petasites japonicus*	Up to 78 Bq/kg dry soil of ^{137}C	129
	Gypsophia paniculate	7339.49 mg/kg dry weight	132
	Helianthus annuus L. (sunflower)	Up to ~2,700 Bq/mg dry weight	125
Chromium	*Dicoma niccolifera*	1.5 mg/g dry weight	116
	Suteria fodina	2.4 mg/g dry weight; 48,000 ppm	116
	Leptospermum scoparium	2,470 ppm	116
Neptunium	*Fontinalis antipyretica* (moss)	Up to 1490 Bq/kg	108
	Alfalfa	Concentration ratio of 4.2 with 2.6 x 10^{-6} mg/g Np	111
Plutonium	*Fontinalis antipyretica* (moss)	Up to 4.1 Bq/kg	108
	Brassica juncea (Indian mustard)	Up to 1699.1 Bq/g in shoots; up to 24,785.3 Bq/g in roots (^{239}Pu)	118
	Onion moss	24.27 Bq/kg (^{238}Pu), 52.78 Bq/kb (^{240}Pu)	110
Strongtium	*Picea excelsa*	Up to 328.42 ppm in needles	121
	Parthenocissus quinquefolia	262.2 µg/g	119
	Helianthus annuus L. (sunflower)	Up to ~4,500 Bq/mg dry weight	125
Tritium	Apple	Up to 96.1 Bq/kg	128
	Potato	Up to 208.0 Bq/kg	128
	Parmelia sulcate	Up to 4460 Bq/kg	131
	Evernia prunastri	Up to 6020 Bq/kg	131
Uranium	*Picea excelsa* (spruce tree)	Up to 310.86 ppm in roots	121
	Cyperus iria	38.83 µg/g	119
	Juncellus serotinus	37.7 µg/g	119
	Water lily	1538 mg/kg in root; 3446 mg/kg in aerial tissue	112
	Mustard	7145 mg/kg in root; ~380 mg/kg in aerial tissue	112
	Ryegrass	~980 mg/kg in root; ~600 mg/kg in aerial tissue	112
	Bidens pilosa	721.46 mg/kg in root; 661.36 mg/kg in aerial tissue	112
	Wild stonecrop	~800 mg/kg in root; ~1600 mg/kg in aerial tissue	112
	Purple sweet potato	5712 mg/kg in root; 3.48 mg/kg in aerial tissue	112
	Brassica juncea and *Brassica chinesis*	> 5,000 mg/kg in shoots	117

Table 4. Plant accumulators of chemicals associated with nuclear processes.

Harnessing Transcriptional Effects of Ionizing Radiation on Plants. IR-induced changes in gene expression offer a promising potential tool for detection. Gene expression, including variation in transcript or exon usage, may be identified by RNA sequencing methods that can be applied either in the laboratory or in the field. The large numbers of genes in a typical plant transcriptome provide multiple detection opportunities either focused on specific genes or via broad, multigene fingerprints or profiles. Plants are known to modulate gene expression in response to a wide variety of biotic and abiotic stressors, whether chronic or acute in nature. While there is a long history of experimental and observational studies of the effects of IR on gene expression in plants, consistent and widely applicable conclusions are difficult to draw from the literature.[133-136] Difficulty arises from the diversity of plant species that have been studied, differing developmental stages at which experimental radiation has been applied, and variability in the populations and sites at which they were grown. There is also considerable variation in the quality, dose, and duration of radiation exposure in experimental systems as well as in the timing of sampling post-exposure. Much of the available data derive from less well controlled, non-experimental systems such as sites such as Chernobyl[137], overt attempts to generate new varieties for agribusiness[138,139], and food sterilization applications.[140] Finally, many studies have relied on older, array-based systems or targeted studies of specific genes in assessing IR effects on gene expression in plants.

At relatively high acute doses of external gamma radiation exposure (e.g. ≥100 Gy), a wide variety of genes active in abiotic and biological stress responses or metabolism are reported to display altered expression.[141,142] These include genes involved in DNA damage and oxidative stress responses, among others.[143,144] Kovalchuk et al. also observed upregulation of DNA damage and oxidative stress response genes in *A. thaliana* when exposed to lower doses of gamma radiation (e.g. 1.0 Gy) but only for acute doses.[145] Sugimoto *et al*. compared the transcriptomes of *B. rapa* plants (Mizuna) grown on the International Space Station to those grown on the ground.[146] They observed significant differences in the expression of genes that are responsive to reactive oxygen species, as might be induced by space irradiation, including gene expression changes that were unique to the space-grown plants. In our own unpublished studies (Zhou *et al.* (in preparation)),

we have examined transcriptional responses at lower doses of gamma radiation (from 1.4 cGy to 1.0 Gy) in multiple plant species and observed that the number of genes with significantly altered expression upon exposure increases with decreasing dose, suggesting that there may be opportunities for detecting IR exposure at low doses through transcriptomic assays.

RNA sequencing (RNA-seq) approaches provide the opportunity to generate highly quantitative data on gene and transcript expression in response to stressors such as IR and with minimal tissue requirements. The ability to "fingerprint" transcriptional responses offers the potential to disentangle gene expression changes that may be specific to IR exposure from other confounding and more generalized stress responses. However, before transcriptional data can be used to reliably detect radiation exposures or to provide dosimetry, further characterization of transcriptional responses to IR in plants needs to be performed. This would include surveying of ubiquitous plant varieties that could serve as broadly distributed sentinels. Plants in the genus *Brassica* are one potential candidate. There are more than 30 species of *Brassica*, some of which are cultivated (mustards, cabbages), but most of which grow wild on every continent except Antarctica. Alternatively, conserved pathways or genes that respond to IR across species would need to be identified and characterized. Regardless of the plant species or gene targets chosen as potential sensors, transcriptional responses need to be characterized at different doses and dose rates, as well as in different tissues and for different types of exposures.

Epigenetic Responses to Ionizing Radiation in Plants. An alternative to quantifying transcripts produced by genes in response to IR exposure is to examine the actions of factors that regulate the expression of these genes. Such factors could include alterations in DNA methylation, chromatin accessibility, histone modifications, or altered expression of non-coding RNAs.[147] Among these different factors, DNA methylation is of particular interest because it has the potential to be maintained over long periods of time and, indeed, may be maintained transgenerationally.[148,149] Plants, in general, tend to have higher overall levels of DNA methylation than other organisms but with considerable variation between species.[150] Prior studies of the effects of IR on DNA methylation in plants have primarily utilized older approaches that survey only limited portions of the genome, such as digestion of genomic DNA with methylation-sensitive restriction enzymes, and methods of detection such as Southern blotting or radioactive nucleotide incorporation[137,151], that have modest sensitivity to detect changes, particularly when those changes occur in only a limited number of cells in a given tissue. In contrast, current genomic technologies, such as sequencing of bisulfite or enzyme modified genomic DNA or direct detection of modified nucleotides, allow comprehensive detection of methylated sites across the genome, while the ability to sequence at depth allows the detection of changes occurring in relatively small numbers of cells in a population.

Only a few published studies have examined variation in DNA methylation in response to IR in plants. Kim *et al*. noted decreasing methylation, primarily at CHH and CHG sites, with increasing gamma radiation doses from 5-200 Gy.[152] Caplin et al. noted reductions in global DNA methylation in *A. thaliana* in response to chronic exposure to Cs-137 (40 µGy/hr) over two generations but only in the exposed generations. Ou *et al*. examined the effects of spaceflight on methylation in *Oryza sativa* (rice).[151] Although these studies are potentially confounded by other factors, such as microgravity or magnetic fields on plant stress levels, it was observed that DNA methylation at the small number of sites examined (< 20) was generally increased. These results are in agreement with the observations of Kovalchuk *et al*., who also reported generally increased levels of DNA methylation in native *Arabidopsis* collected from the Chernobyl exclusion zone that had estimated absorbed doses in the range of 0.2-2 Gy.[137] While these studies suggest an overall trend towards hypomethylation of DNA at cytosine residues in response to IR exposure, it is important to recognize that the effects of DNA methylation are site-specific. Overall increases or decreases in methylation may mask critical shifts in methylation with regulatory consequences for specific genes. This suggests that targeted assays for methylation changes at specific sites, validated by transcriptomic or proteomic studies of the associated gene(s) and their product(s), may be more efficient and sensitive as a tool for detecting exposure to IR.

Section Summary. As detailed in the above sections, plants have great potential to be exploited as natural sentinels for nuclear activities. However, several overarching challenges have thus far prevented the use of plant sentinels in an operational context. Biological and environmental variables often have confounding effects. For example, a particular plant species may respond differently at various stages in its growth cycle or under seasonal environmental conditions. Plant responses may also differ depending on the dose or biological availability of the chemical species, which is affected by meteorological conditions and soil composition. Plants can also develop non-photochemical quenching responses to some external exposures which offers additional challenges, particularly for remote sensing. Lastly, some responses are generalizable to multiple stressors, thus producing non-specific signatures. Limited understanding of plant responses constrains, at present, utility for some applications. Only a small subset of plant species has been studied as potential sentinels, with many of these studies focusing on natural species found near nuclear accidents. Moreover, some biotechnologies, such as sequencing technologies for detecting epigenetic modifications, are recent developments. Finally, operational constraints may be a limitation if physical access to the site is required for collection of plant material; however, optical spectroscopy and remote sensing has shown promise that may increase their utility once proof-of-concept is more firmly established.

Native and Domesticated Animals

Animals are sensitive to environmental perturbation and have been extensively studied as sentinels of ecosystem change, particularly that related to anthropogenic disturbance.153 Analyses can include both qualitative and quantitative approaches to assess morphological and pathological changes; to interrogate excreta, bodily fluids, tissues, and biomaterials for contaminant residues; to evaluate genetic damage, metabolic changes, enzymatic markers, and other molecular endpoints related to exposure; and to analyze community- and population-level changes like species composition, density, and diversity.154,155,156 These methods provide an additional source of intelligence that serves as a "tipping and cueing" function or as an orthogonal means of verification.

Morphological and pathological changes. Environmental radiation exposure can, in some cases, produce observable changes to physical traits that do not require sophisticated analytical tools for interpretation. So-called "epigenetic" factors modulate gene expression based on both endogenous and exogenous cues, including to, *e.g.*, exposure to IR and chemicals, that can manifest as distinctive phenotypic modifications.[157] Proof-of-concept is provided in several epigenetic studies that examine the influences of *in utero* exposure to gamma radiation. For example, the Viable Yellow Agouti (A^{vy}) mouse model was used as a bioindicator for low-dose IR exposure (<0.1 Gy).[158] Exposure to IR resulted in sex-specific changes to gene methylation patterns that concomitantly altered coat color and reduced body mass. Moreover, exposure produced changes that were dose-specific.

Other studies in both Fukushima and Chernobyl underscore the importance of phenotypic changes as indicators of environmental radiation exposure. Butterfly larvae developed obvious physical malformations upon metamorphosis after ingesting leaves from sites contaminated with IR from the Fukushima accident.[159] Dose levels as low as 0.2 Bq/kg produced notable changes as compared to control groups. Chernobyl researchers made similar observations, identifying morphological abnormalities mediated by a phenomenon known as "fluctuating asymmetry" in stag beetles160 and barn swallows161 from sites contaminated with Sr-90 and Cs-134,-137. More recent studies on animals from the Chernobyl Exclusion Zone indicate that exposure to low, chronic doses of IR resulted in notable changes to heart, kidney, and brain mass.[162]

Multiple lines of research thus demonstrate that environmental exposure to IR is sufficient to elicit perceptible changes, although the nature and extent of such changes may vary according to the particular organism under study. Special consideration should be given to selection of species whose feeding ecologies and other lifestyle factors contribute to enhancement of absorbed dose and/or who are more inherently susceptible to IR effects.

Excreta, bodily fluids, tissues, and food products. Systems for consideration may include both terrestrial and aquatic/marine organisms depending on access and the particular iso-

topes of interest. Although it is not within the scope of the present paper to provide a comprehensive description of the various environmental and metabolic pathways that may influence signature uptake, it is worth noting that environmental fates and chemistries will significantly impact the chemical species, concentrations, and biological availability of chemical as well as radiological and nuclear signatures of interest. Substantial transfer of radionuclides and other contaminants into animal matrices (*e.g.*, food products) may occur based on the particular features of the release or the contaminated system, but proposed sentinel species and sampling matrices should be carefully deliberated in light of the above.

Marine and aquatic animals. Numerous marine animals are known to accumulate radionuclides, and many of these, including bivalves (clams, scallops, and mussels), crabs, shrimp, and other coastal inhabitants, have been used for environmental monitoring. Aquatic animal accumulators include crayfish and bony fishes. Several long term studies have evaluated Cs-137 concentrations in Baltic Sea fauna resulting from the Chernobyl accident.[163] Assessment of bioaccumulation and biomagnification in both fish and seals revealed considerable variability among species in terms of radioisotope retention, presumably because of differences in metabolism or trophic position. In addition, levels of Cs-137 in seal tissues were higher than would be predicted by the physical half-life of the radionuclide, indicating that recirculation from sediments and/or inputs from freshwater sources was likely occurring. Similarly, evaluations of zooplankton and mesopelagic fish in the Northwest Pacific Ocean following the Fukushima accident resulted in detection of Fukushima-derived Cs-134 and -137 (as well as Ag-110m in zooplankton) in the tissues of surveyed species, although offshore stirring and mixing of oceanic waters led to considerable heterogeneity in distribution of radionuclides and associated presence in biota.[164]

Terrestrial animals. This category includes vertebrates and invertebrates that inhabit both riparian (i.e., land associated with a water course) and terrestrial environments. Environmental or reclamation studies that evaluate ecosystem health based on animal systems use a typical suite of sentinel species which include snails, frogs, ducks, crabs, and bees, whereas studies more concerned with evaluating contamination of human food sources focus on species that represent entry points into agri-food chains. For the presently proposed application, the latter is likely to represent a more accessible source of sampling. Agricultural, free-ranging domesticated, and game animals can be exposed to IR resulting from nuclear and radiological incidents via multiple routes including inhalation and ingestion of contaminated plants, water, and soils. Three primary radionuclides, including radioiodine, radiocesium, and radiostrontium, are highly mobile in environmental matrices and readily transfer to animal tissues and products.[165] Larger species like ungulates consume up to 25% of their body weight daily in primary biomass such as grains, stems, and leaves that can retain radiostrontium and, to some extent, radioiodine for extended periods, thus body burdens can be appreciable depending on factors like, *e.g.*, general nutrient availability and ingestion of clean versus contaminated feed. Exposure to radiocesium also occurs through grazing activity that results in ingestion of contaminated soils. Numerous studies validate collection of milk, muscle tissue, and excreta as a means to evaluate exposure to specific radionuclides.[166]

Genetic damage, metabolic changes, enzymatic markers, and other molecular endpoints. A number of biomarkers that can serve as tools for identifying exposure events have been identified. Assays have been developed and validated for biomarkers including, among others, cytogenic changes, DNA damage, transcriptional changes, and oxidative stress repair pathway activation. Assessments of biota inhabiting the radiation-contaminated areas of Chernobyl and Fukushima have yielded equivocal evidence of genetic damage and increased mutational rates[155]; however, the scientific community continues to lack consensus regarding the long-term effects of chronic exposure on wildlife that inhabits those areas.[167] It is not clear that levels of radiation in the aforementioned sites are sufficient to induce DNA damage which will exceed biological repair capacity, nor that the standard linear dose-response paradigm, which maintains that DNA lesions will increase linearly with energy deposition, applies to doses below a certain threshold. Other markers may have greater utility for the applications proposed in the present thesis.

For example, studies of inhaled uranium exposure demonstrate that urine concentrations of β2-microglobulin serve as a good proxy for yellow-cake exposure.[168] Sensitivity of the assay and the time scale post-exposure that the organism would be able to detect the signal was reported to range from 0.001 to 5 Gy and minutes to years, respectively. In general, longer or higher exposures provide more detectable signatures.[169] Epigenetic markers have also been associated with exposure to radiation. Both laboratory and field studies demonstrate characteristic modifications, putatively to induce DNA stability, under conditions of low, chronic exposure to gamma radiation.[170] Epigenetic changes like histone modifications, DNA methylation, and non-coding RNAs can influence end-state gene products (e.g., proteins) without altering underlying DNA sequences. Moreover, said changes can persist across multiple generations, thus may be "conduits for environmental influence" on the genome as long as a given environmental change persists.[171] Transcriptomic changes are likewise sensitive indicators of low dose radiation exposure. Several studies have documented modifications in gene expression patterns, particularly related to immune responses and inflammatory pathways, following exposure to low doses of IR and provide evidence that transcriptomics analysis are useful in evaluating the effects of prolonged external exposures.[172,173] Other "-omics" approaches for evaluating the effects of exposure include proteomics and metabolomics. Recent studies have investigated, *e.g*., post-translational modifications to proteins critical to modulating a number of important biochemical pathways and determined that gamma exposures as low as 0.1 Gy were sufficient to induce significant changes.[174]

Whereas interrogation of molecular endpoints represents a promising direction for evaluating exposure to radiation, research remains largely in the fundamental stages. Challenges associated with development of methods and refinement of analytical capabilities to identify points of coalescence across highly variable genetic backgrounds have hindered practical application of such approaches. More work is required to identify the specific endpoints that may be useful.

Population- and Community-Level Changes. Population- and community-level effects are also evident in areas contaminated by radiation. The relative abundance of different species in and around Chernobyl (where doses range from an estimated 0.01 to136 μSv/hr) appears to be influenced by variation in background radiation. Researchers found that mammals and birds showed the strongest negative relationship between abundance and background radiation levels as compared to dragonflies, butterflies, amphibians, and reptiles.[155] Similarly, numerous studies demonstrate the impacts, whether beneficial or deleterious, of modern war and military activities that result in exposure to radiation.[175] Such results suggest that relative abundance of select species is indicative of radiological and other types of contamination associated with nuclear activity. A non-trivial caveat is that studies like those cited above required (1) continual access to sites of interest and (2) longitudinal sampling to draw statistically robust conclusions. Certain endpoints (*e.g*., genetic endpoints) could assist in drawing inferences from more limited collection efforts, but sample sizes resulting from a given effort would still need to be large enough to promote confidence in conclusions.

Humans

A number of potential endpoints for evaluating occupational exposure are provided in the associated overview article "Harnessing the Environment to Identify Nuclear Processes: Biologically-Mediated Approaches" and include direct measurements of radionuclides in biological samples, inference of exposure through analysis of serum enzyme levels, genomic and proteomic changes, and community restructuring of the skin and oral microbiomes. Detail regarding signatures for which such endpoints are known to be relevant is provided therein. Additionally, many of the approaches delineated in the "Native and Domesticated Animals" section, above, and select approaches from the "Biological Materials" section, below, will apply.

Biological Materials

Certain species act as unique collection systems by incorporating environmental contaminants into anatomical structures, such as shells or exoskeletons. Marine and freshwater invertebrates, including benthic (sediment dwelling) invertebrates, are often used as biomonitors to assess population-level effects of anthropogenic pollution, thus have been the subject of considerable study evaluating accumulation and incorporation of contaminants into different

body tissues. For example, the mussel *Mytilus edulis* has been widely used as a sentinel organism for monitoring pollution in aquatic habitats, and laboratory studies have demonstrated its ability to concentration radionuclides such as Pu, Am, and Sr into shell material.[176] Studies of terrestrial invertebrates like the gastropod *Helix aspersa* have also demonstrated that the shell is a primary depot for certain contaminants like heavy metals.[177,178] Although information on trophic transfer (i.e., up the food chain) of radionuclides is relatively limited, some studies indicate that biomagnification of radionuclides and heavy metals occurs during transfer to predatory species (*e.g., Babylonia formosae habei*), likely due to high assimilation efficiencies of foodstuffs.[179] Given their demonstrated efficiency as bioconcentrators, macroinvertebrate exoskeletons could offer viable alternatives to traditional collection systems. Moreover, because individual shell layers are developed during specific stages of life, it may be possible to infer the time range during which exposure occurred. New techniques in mass spectrometry (*e.g*., multi-isotope imaging mass spectrometry) show promise in terms of characterizing isotopic ratios in biological materials at the subcellular level, thus can putatively support analysis of the materials described above.[180]

Calcified tissues such as exfoliated deciduous teeth181 and walrus tusks[182] are also known to be exceptional lifetime integrating dosimeters through the application of electron paramagnetic resonance (EPR). With regard to the former, samples retained from root canals, denture fittings, wisdom teeth extraction, and so on, can be obtained with the assistance of dental surgeons and then submitted to an EPR lab for dose reconstruction. High precision and accuracy can be obtained using detailed protocols and techniques[183] although much of the process is also amenable to automation184. When EPR is used in combination with alanine dosimetry, detection limits can be as low as 10s of millisievert.[185] Based on differential attenuation of medical x-rays and external gamma as they penetrate teeth, the contribution from diagnostic exposures is easily distinguishable by measuring the dose-depth profile.[186]

Thermoluminescence (TL) and optically stimulated luminescence (OSL) also have been used to characterize the above and comparable materials. Although TL and OSL are more commonly associated with personnel dosimetry (e.g., radiation worker badges), use of OSL via distributed dosimeter arrays has demonstrated application for reconstruction of historical weapons grade plutonium locations and distributions.187 Similarly, TL and OSL have demonstrated ability to establish dose-depth profiles sufficient for reconstruction of historical radiation fields for Am-241 in bricks[188] and retrospective assay of historical uranium enrichment levels in bricks and other ubiquitous building materials, even when the nuclear material no longer exists.[189] Detection levels approaching natural background have been demonstrated for personal items commonly found in the public,[190] although further research is needed to validate findings. It is reasonable to suppose that the same approaches could be extended to biologically-derived materials.

Ecological Networks

Mycorrhizosphere. Mycorrhizae ("myco" = fungus + "rhizo" = root) are widespread networks of symbiotic water, nutrient, and information exchange between plants and soil fungi, and approximately 80% of terrestrial plants form such mycorrhizal associations. The filaments or "mycelium" of fungi thread through the root systems of plants, receiving carbohydrates and returning water and minerals. In addition to the exchanges between plant and fungal partners, plants can distribute resources to other plants of the same or different species in a community through shared, interconnecting fungi.[191] Furthermore, plants can direct chemical signals of distress to neighboring plants through shared fungal networks, communicating specific threats such as disease or grazing.[192]

A single plant can link with multiple species of fungi and vice versa. The aggregation of unit connections between plant nodes and fungal relays form vast underground systems that have been dubbed "myconets" or "the wood-wide web".[193] Myconets both physically and functionally resemble human information networks, and so efficient is their organization and distribution that network engineers examine myconets to inform their own designs.[194] Due to the interconnectivity of fungi, disturbances to any part of an ecosystem are translated into intelligible signals distributed across the local myconet.[192-194,195]

The influx of the unique radiochemistries and energy produced by fissile materials or nuclear processes into an ecosystem can be relayed across a myconet. Fungi are also known to bioaccumulate heavy metals and radionuclides, which may lead to establishing "mycological fingerprints" as biorecognition elements for isotopes of interest. Myconet-based detection offers the potential to leverage sensing capacities of multiple plant and fungal species across an ecosystem for use in broad surveillance scenarios. Distress signals can be transmitted underground through chemical communications or through the air over significant distance by volatile chemicals, pollen, or spores. Myconets can be monitored through chemical analysis of root samples or downwind air sampling of volatile chemicals. While myconets possesses great potential to capture and transmit information from multiple biological sources, the science to receive and process biochemical signals associated with myconets is still nascent. Further investigation of these ecological networks is necessary to engage this resource as a viable recognition element in sensing technology.

Conclusion

The biological world offers a wealth of possibilities for development of environmental monitoring systems that are uniquely capable for the identification and characterization of nuclear activity. The previously described systems can provide, alone or in concert, clear indication of the nature and extent of contaminating events through careful and systematic interrogation. Techniques for leveraging the information they provide have become increasingly refined as have computational approaches that help to make sense of the manifold layers of information likely to result, especially where multiple environmental stressors may be present. Although several of the concepts delineated here can be adapted for immediate use, others will require additional research to develop fully mature capabilities for incorporation into the nuclear monitoring toolkit.

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