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Chapter 22
Contaminants of Concern in Cannabis: Microbes, Heavy Metals and Pesticides

John M. McPartland and Kevin J. McKernan

Abstract Microbiological contaminants pose a potential threat to cannabis consumers. Bacteria and fungi may cause opportunistic infections in immunocompromized individuals. Even dead organisms may trigger allergies and asthma. Toxins from microbial overloads, such as Shigla toxin and aflatoxins, may pose a problem—unlikely, but possible. The *Cannabis* plant hosts a robust microbiome; the identification of these organisms is underway. *Cannabis* bioaccumulates heavy metals in its tissues, so avidly that hemp crops have been used for bioremediation. Heavy metals cause myriad human diseases, so their presence in crops destined for human consumption must be minimized. Pesticide residues in cannabis pose a unique situation among crop plants—the Environmental Protection Agency (EPA) will not propose pesticides guidelines, because *Cannabis* is illegal on the federal level. The use of illegal pesticides is a rising crisis, and a breakdown in ethics. Testing for pesticide residues and maximal limits are proposed.

22.1 Introduction

Cannabis (the plant) and cannabis (the plant product) may be contaminated by microbes, heavy metals, or pesticide residues. The first two contaminants, microbes and heavy metals, present a Janus-face or "flip-side of the coin" in relation to *Cannabis*. Some bacteria and fungi are part of the plant's microbiome. They provide benefits to *Cannabis*. See the book chapter by Parijat Kusari and Oliver Kayser for more about the *Cannabis* microbiome. On the "flip-side of the coin," other bacteria and fungi cause disease, and these must be controlled.

J.M. McPartland (✉)
GW Pharmaceuticals place, 1 Cavendish Place, London W1G 0QF, UK
e-mail: mcpruitt@myfairpoint.net

K.J. McKernan
Courtagen Life Sciences, 12 Gill Street Suite 3700, Woburn, MA 01801, USA

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Heavy metals are harmful to humans, and these contaminants must be minimalized in cannabis destined for human consumption. *Cannabis* pulls heavy metals from soil with great efficiency. Therein lies a second Janus face: the plant has great potential as a tool for bioremediation. Bioremedial plants extract pollutants from soil and accumulate the pollutants in their tissues, for harvesting and removal.

Pesticide residues have no "flip-side of the coin," they are just bad. Growth in the cannabis industry, from outdoor hippie gardens to indoor commercial warehouses, has multiplied pesticide usage. Pesticide regulation in the USA is primarily a responsibility of the Environmental Protection Agency (EPA). The EPA will not register pesticides for use on *Cannabis* or set tolerance levels because the crop is illegal on the federal level (Stone 2014). For that same reason, no cannabis can be labeled as "Organic" by the USA Department of Agriculture.

This chapter focuses on microbes, heavy metals, and pesticide residues in cannabis inflorescences and seed oil. Other contaminants exist, such as butane residues in cannabis extracts. For these, the reader is directed elsewhere (Upton et al. 2013; Farrer 2015). Adulterants—deliberately added contaminants—are a separate issue, particularly hashish diluents and psychoactive adulterants (Bell 1857; Dragendorff and Marquis 1878; Indian Hemp Drugs Commission 1894; Perry 1977; Wilson et al. 1989; McPartland and Pruitt 1997; McPartland 2002; Caligiani et al. 2006; McPartland et al. 2008; Busse et al. 2008; Venhuis and de Kaste 2008; Scheel et al. 2012).

22.2 Microbial Contaminants

Cannabis is often characterized as a "disease-free" crop. In fact, a plethora of plant pathogens attack the plant. At least 88 fungal species cause diseases in *Cannabis* (McPartland 1992), as do eight pathovarieties of plant pathogenic bacteria (McPartland et al. 2000). Some phytopathogens are unique to *Cannabis* (McPartland 1984), and some organisms are ubiquitous. The most threatening diseases of flowering tops are caused by three ubiquitous fungi—*Botrytis cinerea* (the cause of gray mold), *Trichothecium roseum* (white mildew or pink rot), and *Alternaria alternata* (brown blight).

Phytopathogens cannot infect humans, except perhaps immunocompromized individuals. Opportunistic infections by *A. alternata* have been reported in patients receiving chemotherapy, recent organ transplant patients, and people with AIDS. Airborne conidia (spores) of *B. cinerea* and *A. alternata* cause mold allergies and asthma, particularly in greenhouse workers (Jurgensen and Madsen 2009).

From a consumer perspective, a separate population of bacteria and fungi is of greater concern than phytopathogens: post-harvest storage microbes (McPartland 1994a). Storage organisms are saprophytes, rather than pathogens. They can only invade dead plants after harvest. Fungi are the primary cause of storage contamination. They thrive under low oxygen levels, limited moisture, and intense competition for substrate.

The spectrum of post-harvest storage fungi has changed in the past 40 years. Most black-market cannabis available in the 1980s came from Latin America. It was "sweat cured" by drying herb in a pile, covered by cloth. Heat arising from fermentation quickly cured the product, but allowed storage organisms to gain a foothold. Then the cannabis was compressed into bricks for smuggling, and stored under ambient humidity and warm temperatures. Under these conditions, fungi from four genera commonly contaminated the product: *Aspergillus, Penicillium, Rhizopus,* and *Mucor* (Fig. 22.1).

Kagen et al. (1983) isolated three worrisome *Aspergillus* species from marijuana: *A. niger, A. fumigatus*, and *A. flavus*. Schwartz (1985) scraped an aspergilloma ("fungus ball") caused by *A. niger* from the sinuses of a marijuana smoker suffering severe headaches. Llamas et al. (1978) implicated *A. fumigatus*-contaminated marijuana in a case of bronchopulmonary aspergillosis. Aspergillosis is an invasive disease, unlike an aspergilloma. It usually stays localized (e.g., a pneumomycosis) but sometimes becomes systemically disseminated. Chusid et al. (1975) reported *A. fumigatus* causing near-fatal pneumonitis in a 17-year old. They noted that the patient buried his marijuana in the ground for "aging." *Penicillium, Rhizopus,* and *Mucor* have also been cultured from moldy cannabis (Kagen et al. 1983; Kurup et al. 1983; Bush Doctor 1993).

Mycotoxins produced by fungi are hepatotoxic, nephrotoxic, and carcinogenic. Ochratoxins, citrinin, and patulin are produced by *Aspergillus* and *Penicillium* species. Paxilline is produced by *Penicillium paxilli.* Trichothecenes gained notoriety for their reputed use in biological warfare ("yellow rain"). Trichothecenes are

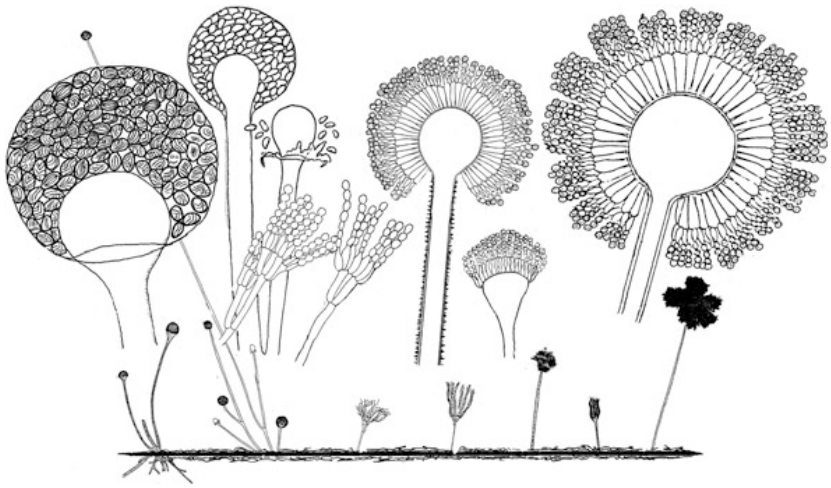


Fig. 22.1 Common storage fungi in the 1980s. From *left* to *right*: *Rhizopus stolonifer, Mucor hiemalis, Penicillum chrysogenum, P. italicum, Aspergillus flavus, A. fumigatus,* and *A. niger*. *Top row* sporophores cross-sectioned to reveal internal structures (400x). *Bottom row* natural habitat (25x). From McPartland (1989), reprinted with permission

produced by *Fusarium oxysporum*, a biological control fungus deployed against illegal *Cannabis* cultivation (McPartland and West 1999). Aflatoxins are the most common mycotoxins.

Aspergillus species (*A. flavus, A. parasiticus*) produce aflatoxins in warm and humid conditions—optimally 33 °C (91.4 °F), and 0.99 water activity. Aflatoxins are acutely poisonous as well as carcinogenic. Llewellyn and O'Rear (1977) identified aflatoxins in cannabis, but under artificial conditions. They added 15 ml water to 5 g pulverized flowering tops, autoclaved the material, and inoculated it with *A. flavus* or *A. parasiticus.* After 14 days at 25 °C (77 °F), the fungi sporulated and produced "moderate" amounts of aflatoxins. Importantly, no studies have reported aflatoxins in cannabis under normal storage conditions (McPartland and Pruitt 1997).

Kurup et al. (1983) isolated three thermophilic actinomycetes from questionably sourced material, *Thermoactinomyces candidus, T. vulgaris,* and *Micropolyspora faeni*. These endospore-forming microbes cause "farmer's lung," which is a hypersensitivity reaction rather than an infection.

Turning to bacteria, Ungerleider et al. (1982) cultured several members of the *Enterobacteriaceae* from NIDA-sourced cannabis—species of *Klebsiella, Enterobacter,* and *Enterococcus* (group D *Streptococcus*). It should be noted that NIDA marijuana at that time was sweat cured by placing harvested material on concrete floors (B. Thomas, pers. commun. 1999)—an unacceptable method today. A disease outbreak caused by another member of the *Enterobacteriaceae—Salmonella muenchen*—was associated with cannabis (Taylor et al. 1982). The investigators concluded that the plant material, sourced from Mexico, was contaminated or adulterated by untreated manure—another unacceptable method today.

Some of these organisms, particularly *Rhizopus, Mucor,* and thermophilic actinomycetes, reduce cannabis to a deteriorated state that is no longer acceptable by today's consumers. The product is dark brown, crumbly, smells musty or moldy, and produces a brown or sooty smoke (McPartland et al. 2000). Although methods of sweat curing are still promoted on websites, today's product is carefully air dried, often vacuum-sealed (sometimes under nitrogen), and stored in cold, dry conditions. This process maintains potency and also prevents the growth of storage organisms.

Here in the 21st century, *Aspergillus*- and *Penicillium*-contaminated cannabis still poses a problem (Rechlemer et al. 2015; Cescon et al. 2008; Szyper-Kravitz et al. 2001; Verweij et al. 2000). Martyny et al. (2013) sampled grow operations in Colorado for airborne fungal spores. *Aspergillus* and *Penicillium* spp. predominated indoors, and *Cladosporium* spp. predominated outdoors. *Cladosporium* may be an emerging problem; this fungus also infests hemp mills (McPartland 2003). About 1% of cannabis supplies received by Harborside Medical Cannabis Dispensary in Oakland, California were returned to vendors because of unacceptable levels of *Aspergillus* contamination (DeAngelo 2010).

22.3 Microbial Testing

The Office of Medicinal Cannabis in the Netherlands initiated microbial testing (Hazekamp 2006, 2016). Bedrocan BV, the primary supplier of medical cannabis in the Netherlands, tests harvested plants as well as final packaged products. They use two petri plate-based screening tests recommended by the European Pharmaopoeia—one for total aerobic microbial count (TAMC), the other for total yeast and mold count (TYMC). Degree of contamination is quantified by counting the number of colony-forming units arising from one gram of plated cannabis (CFU/g). They placed upper limits of <100 CFU/g for TAMC, and <10 CFU/g for TYMC—which is close to sterility. Certain specific pathogens must be completely absent—*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and bile-tolerant Gram-negative bacteria such as *Escherichia coli.* Furthermore, the absence of fungal mycotoxins must be confirmed by additional quality control testing (Hazekamp 2016).

Health Canada (2008) mandated similar tests, with different upper limits: <100 CFU/g for TAMC, and <100 CFU/g for TYMC, as well as specific tests for Coliform bacteria (<3 MPN/g), and *E. coli* (absent). Their upper limit for aflatoxins B1, B2, G1, G2, and ochatoxin A is <20 μm/kg cannabis.

In the USA, medical cannabis was first legalized by California in 1996. Microbial testing was not mandated until 2011, when New Jersey instituted sample testing for pests, mold, mildew, heavy metals and pesticides, and the certification of "organic" medical cannabis (NJMMP 2011).

The American Herbal Pharmacopoeia (AHP) issued specific protocols for microbial testing (Upton et al. 2013). The AHP's protocols were based on tests for commodity food products issued by the EPA and the Food and Drug Administration, as well as assays for cannabis used in Holland (Hazekamp 2006). The tests consist of a series of petri plate- or film-based assays for bacterial, yeast, and mold.

For orally consumed cannabis, the AHP recommended four tests: (1) total yeast and mold count, (2) total coliforms, (3) *Escherichia coli*, (4) *Salmonella* spp. In addition, they recommended immunochemical methods to screen for aflatoxins. For products to be inhaled, more stringent tests were recommended: (1) total yeast and mold count, (2) total aerobic count, (3) bile-tolerant gram-negative bacteria, (4) *E. coli* and *Salmonella* spp., and aflatoxin assays. The AHP proposed specific limits in CFU/g counts, but emphasized that these values did not represent pass-fail criteria. Rather they were recommended levels when plants are cultivated and harvested under normal circumstances.

The states of Colorado and Washington issued specific testing protocols, reviewed by Holmes et al. (2015). Colorado's list of fungi required for testing was based on publications from the 1980s, including some species that may not be relevant to current, domestically-produced cannabis. Washington's protocols were adopted from the AHP. Holmes et al. (2015) criticized the use of screening tests, noting they are based on guidelines for food product facilities (and not necessarily the testing of end products). Some of the tests are quite outdated (e.g., bile-tolerant

gram negative bacteria). Furthermore, anonymous CFU/g counts do not identify relevant pathogens, or the threat of fecal contamination. Instead Holmes recommended testing herbal cannabis for specific pathogens: *Escherichia coli, Salmonella* spp., and four species of *Aspergillus*: *A. flavus, A. fumigatus, A. niger,* and *A. terreus.*

In 2015 Colorado changed its testing regimen: (1) total yeast and mold count (limit $<10^4$ CFU/g), (2) *Salmonella* (limit <1 CFU/g), (3) Shiga-toxin producing *E. coli* (STEC, limit <1 CFU/g). Colorado recommended testing for three species of *Aspergillus*: *A. flavus, A. fumigatus,* and *A. niger,* although this was never implemented.

Aspergillus is a large genus with 250 species, and separating three specific species from the others is not easy. Traditionally, identification required culturing on *Aspergillus*-selective plating media, and morphological measurements by a specialist (Samson et al. 2004). Due to the challenges associated with species-specific detection, Colorado changed their testing requirements again in 2016, to a 10,000 CFU/g total yeast and mold test, but left in place single CFU/g testing for *E.coli* and *Salmonella* spp.

Microbial tests that require CFU/g detection are prone to sampling bias, since the cannabis sample (usually 250 mg to 1 g) is usually wetted with 3–4 ml of Tryptic Soy Broth (TSB), a general purpose culture medium. This large volume cannot be placed into a given petri dish, PCR reaction, or culture based detection device. Thus a subsample of the large volume is taken after a defined growth time (termed enrichment) to accommodate for the subsampling.

Because of these difficulties, and to accelerate testing turn-around time, some laboratories now use quantitative polymerase chain reaction (qPCR) assays. This method detects DNA sequences in cannabis samples. Primers for 18S rDNA ITS (Internal Transcribed Spacer) are particularly useful for identifying specific *Aspergillus* species.

The drawback to qPCR is the method's indifference to living or non-living DNA. To accommodate this, an enrichment step is performed, where the cannabis samples are incubated overnight in TSB broth prior to qPCR detection. Overnight growth in TSB ensures only live organisms are measured, but raises questions over preferential culture conditions for broader total yeast and mold tests. To address this conundrum, some labs perform a qPCR on total yeast and molds, and positive results are confirmed with an additional test extracted 24 h later to ensure the signal from the pre-incubation test was from live organisms.

McKernan et al. (2015) compared results between qPCR and three petri plate- or film-based detection systems: 3 M Petrifilm™, Simplate-Biocontrol Systems™, and BioLumix™. They tested 17 dispensary-obtained cannabis samples. Six samples tested positive with the qPCR assay, five samples tested positive with the Biocontrol Systems™ assay (>10,000 CFU/g), four samples test positive with the 3 M Petrifilm™ assay (>10,000 CFU/g), and only one sample tested positive with the BioLumix™ assay, which is a simple pass-fail test.

McKernan and colleagues then subjected ITS amplicons to DNA sequencing, to identify specific fungi. All three *Aspergillus* species on the bad list turned up:

A. flavus (one sample), *A. fumigatus* (one sample), and *A. niger* (three samples). Twelve other *Aspergillus/Emericella* species were detected: A. *candidus, A. ostianus, A. sepultus, A. sydowii, A. tamari, A. terreus, A. versicolor, E. rugulosa, E. nidulans, E. filifera, E. repens, E. bicolor*. Two of these produce toxins, *A. versicolor* and *A. terreus*.

ITS amplicons identified 17 *Penicillium* species. The most common fungus was *P. paxilli,* surpassing all *Aspergillus* species. This species has not previously been reported in association with *Cannabis* or cannabis. *P. paxilli* produces paxilline toxin, so McKernan and colleagues confirmed its presence with PaxPss1 and PaxPss2 DNA primers. Paxilline has been shown to decrease the antiseizure benefits of cannabidiol in a mouse epilepsy model (Shirazi-Zand et al. 2013).

Although Holmes et al. (2015) questioned the need to test cannabis for *E. coli, Listeria* spp., and *Pseudomonas aeruginosa*, McKernan (unpublished study 2016) has identified several *Pseudomonas* species in cannabis with DNA testing. The most dangerous pathogen, *P. aeruginosa,* was not seen. The array of organisms that need to be screened is not yet formalized.

Screening herbal cannabis for moisture content (MC) is another approach. Bush Doctor (1993) and McPartland et al. (2000) recommended drying herbal cannabis to 10–12% MC. Fungi and bacteria cannot grow below 15% MC. Herb dried below 10% MC becomes brittle and disintegrates easily. Hazekamp (2006) recommended 5–10% MC. The AHP monograph recommended not more than 15% MC (Upton et al. 2013). Holmes et al. (2015) used water activity (a_w) as a metric; a_w measures the partial vapor pressure of water in a substance. The a_w of pure distilled water equals 1.0. Bacteria usually require a minimum of 0.9 to grow, and fungi require a minimum of 0.7. Holmes and colleagues recommended a maximum a_w of 0.65 for herbal cannabis, approximately 13% MC.

22.4 Microbial Harm Reduction

Prevention is the best strategy to avoid microbial contamination. Growers must harvest disease-free *Cannabis*. This book's chapter by David Potter discusses GW Pharmaceutical's methods of growing healthy *Cannabis*—by controlling humidity, using biological controls and natural predators, and without resorting to pesticides. The use of pesticides is addressed below.

To kill microbial contaminants in medical cannabis, Ungerleider et al. (1982) used radioactive ^{60}Co gamma rays, a dose of 15,000–20,000 grays. Dutch and Canadian medical cannabis is treated with 10,000 grays (Hazekamp 2006; Health Canada 2008). Microbial counts in Dutch cannabis are tested before and after irradiation, because "bad" quality cannabis should not be rescued by irradiation (Hazekamp 2016). In comparison, packaged meat and poultry may be irradiated with up to 70,000 grays. Gamma radiation remains controversial—it may destroy terpenoids, and it does not destroy mycotoxins (Lucas 2008).

Hazekamp (2016) evaluated the effects of 10,000 grays in four cultivars of THC- or CBD-dominant *Cannabis*. Quantification with ultra performance liquid chromatography (UPLC) and gas chromatography-flame ionization detector (GC-FID) showed that levels of total THC and/or CBD were not altered by irradiation treatment in any of the cultivars tested, compared to controls. Irradiation decreased four monoterpenoids—α-guaiene (10%), cis-ocimene (7–23%), β-myrcene (8–18%), terpinolene (16–38%), and seven sesquiterpenoids—guaiol (6%), nerolidol (7%), trans-β-farnesene (7–10%), β-caryophyllene (6–10%), γ-selinene (13–17%), eudesma-3,7(11)-diene (14%), and γ-emelene (8–19%). Hazekamp compared these reductions to similar decreases arising from short term storage in a paper bag (Ross and Elsohly 1996).

Hazekamp (2006) compared the inoculum load of irradiated medical-grade herbal cannabis (MC) to that of untreated recreational coffeehouse cannabis (CC). An *Enterobacteriaceae* assay revealed <10 CFU/g in MC samples (n = 2), and a mean of 1.4×10^4 CFU/g in CC samples (n = 11). An assay for molds and aerobic bacteria revealed <100 CFU/g in MC samples, and a mean of 5.4×10^4 CFU/g in CC samples. Because screening tests do not identify species, one CC sample was sent out for further testing, which identified *E. coli* and *Aspergillus, Penicillium*, and *Cladosporum* spp.

Ruchlemer et al. (2015) tested three other ways to sterilize cannabis: gas plasma, autoclaving, and ethylene oxide. These methods decreased THC content 12.6, 22.6, and 26.6%, respectively. Levitz and Diamond (1991) killed condia (spores) of *A. fumigatus, A. flavus*, and *A. niger* in marijuana by baking herb at 150 °F (300 °C) for 15 min. Water pipes do not prevent the transmission of fungal spores from contaminated cannabis (Moody et al. 1982), not even water pipes with filters (Sullivan et al. 2013). Fungi and bacteria are capable of passing through vaporizers (Ruchlemer et al. 2015). Some toxins produced by fungi and bacteria, such as Shiga toxin, are resistant to heat treatment (pasteurization).

22.5 Janis Face—Endophytes

A microbiome is the ecological community of commensal, symbiotic, and generally non-pathogenic microorganisms that inhabit plants, animals, and us. The plant microbiome is a key determinant of plant health and productivity, and has gained attention recently (Turner et al. 2013). Over a century ago, however, botanists first recognized mutualistic associations between plants and fungi, termed mycorrhizae.

Emil Arzberger, a USDA scientist, discovered fungi living in the roots of healthy *Cannabis* plants back in 1925. He died shortly thereafter, without reporting his results; they were rediscovered in USDA archives (McPartland et al. 2000). The endorhizal (root-inhabiting) microorganisms that colonize *Cannabis* improve plant nutrition and disease resistance (McPartland and Cubeta 1997; Citterio et al. 2005; Winston et al. 2014).

Researchers have turned their attention to phylloplane organisms, which live in nooks and crannies above the leaf epidermis (epiphytes) or in spaces below the epidermis (endophytes). Phylloplane organisms protect their plant hosts by repelling pathogenic organisms. The yeast-like fungus *Aureobasidium pullulans* is a ubiquitous epiphyte, and it has been isolated from *Cannabis* (Ondrej 1991). It oozes chitinases and other enzymes that attack other fungi, including the dreaded gray mold fungus, *Botrytis cinerea.*

Gautam et al. (2013) identified a number of *Cannabis* endophytic fungi. They eliminated epiphytes from their study by surface-sterilizing plant material with sodium hypochlorite (bleach) for 40s. They rinsed material with sterile distilled water, and plated it on agar with antibacterial antibiotics. Fungi were identified by their morphological and cultural characteristics. Gautam and colleagues identified three *Aspergillus* species (*A. niger, A. flavus, A. nidulans*), two *Penicillium* species (*P. citrinum, P. chrysogenum*), and *Rhizopus stolonifer*. They also identified five other species known to be foliar pathogens of *Cannabis: Curvularia lunata, Alternaria alternata, Cladosporium* sp., *Colletotricum* sp., *Phoma* sp. "One plant's protective phylloplane fungus is another plant's latent pathogen" (McPartland et al. 2000).

Kusari et al. (2013) tested plants obtained from Bedrocan BV. Samples were surface sterilized with ethanol and bleach, and cultured on agar with antibiotics. Kusari and colleagues used molecular methods for species identification: DNA extraction and PCR amplification using primers for ITS1, 5.8S, and ITS2 regions of ribosomal DNA. Amplicons were sequenced, and the sequences were BLASTed for matches in the EMBL nucleotide database. The predominant endophyte was *Penicillium copticola*. Other species included *P. meleagrinum, P. sumatrense, Eupenicillium rubidurum, Chaetomium globosum, Paecilomyces lilacinus*, and *Aspergillus versicolor.* None of these fungi have previously been associated with *Cannabis* except for *C. globosum* (McPartland et al. 2000). Kusari and colleagues demonstrated that these endophytes antagonized in vitro growth of two common *Cannabis* pathogens, *Botrytis cinerea* and *Trichothecium roseum.*

The aforementioned study by McKernan et al. (2015) highlighted the predominance of *Penicillium* species in a majority of samples tested. They proposed that a number of these were endophytes. They likely isolated epiphytes as well as endophytes, because they dispensed with surface sterilization and plating, and went straight to molecular identification. Five organisms they isolated were phytopathogens previously reported causing *Cannabis* diseases: *Diplodia* spp. (McPartland 1994b), *Pestalotiopsis* spp. (McPartland and Cubeta 1997), *Botryosphaeria dothidea* (McPartland 1994c), *Fusarium oxysporum* (McPartland and Hillig 2004a), and *Pseudomonas syringae* (McPartland and Hillig 2004b).

These studies reveal a surprisingly depauperate *Cannabis* foliar microbiome. A recent study of *Genlisea* species, using similar methods, identified 92 genera of organisms (Cao et al. 2015). See Delmotte et al. (2009) for rich microbiomes in other plant species. Many of the 97 species of fungi that Gzebenyuk (1984) isolated from hemp stems in Russia may be phylloplane organisms.

Phylloplane research should be extended to a comparison of indoor crops and outdoor crops. Outdoor crops may show a seasonal community succession. Comparing the microbiome in *Cannabis* from different climates and continents would be informative. Winston et al. (2014) demonstrated *Cannabis* cultivar-specific differences in endorhizae (root-inhabiting bacteria). Their study was limited to drug-type hybrids; this work should be extended to fiber-type cultivars and wild-type plants.

22.6 Heavy Metals and Radionucleotides

Contamination by heavy metals is a health concern because these elements accumulate in the body. They are toxic, carcinogenic, and cause a variety of diseases. Particularly dangerous elements include cadmium, mercury, lead, arsenic, and nickel. Radionucleotides present in the environment may also contaminate plants, and contribute to the risk of lung cancer.

Siegel et al. (1988) measured 440 ng mercury per gram of cannabis in Hawai'i, where the volcanic soil contains naturally high levels of mercury. Siegel notes that mercury is absorbed 10 times more efficiently by the lungs than by the gut. He calculated that smoking 100 g of volcanic cannabis per week could lead to mercury poisoning.

Volcanic soil also contains significant levels of cadmium. Grant et al. (2004) attribute this to elevated levels of cadmium in Jamaican-grown tobacco and cannabis. However, anthropogenic emissions, from fossil fuel combustion and mining/smelting activities, are the primary source of cadmium.

Tainted fertilizer is another source of heavy metal contamination. Safari Singani and Ahmadi (2012) showed that *C. sativa* readily takes up lead and cadmium from soils amended with contaminated cow and poultry manures. Even reportedly "clean" fertilizer seems to increase the uptake of cadmium by *C. sativa* (Ahmad et al. 2015). Phosphate ions are the main carriers of heavy metal contamination, and hydroponic fertilizers are particularly vulnerable to contamination (Karadjov 2014). Phosphate fertilizers targeted at *Cannabis* growers ("bud blooms") have particular problems with arsenic, in some cases 10–50 ppm (N. Palmer, pers. commun. 2016). Rockwool, a.*k.a.* mineral wool fiber, used as hydroponic growth medium, may also be contaminated.

In a study on hemp seeds, Mihoc et al. (2012) report a problem with cadmium contamination; they measured levels of 1.3–4.0 mg/kg. Eboh and Thomas (2005) showed that concentrations of arsenic, cadmium, chromium, iron, nickel, lead and mercury were greater in leaf material than in seeds. Moir et al. (2008) measured heavy metals in marijuana smoke, including mercury, cadmium, lead, chromium, nichel, arsenic, and selenium. Deep inhalation, typical of marijuana smokers, doubled the exposure to heavy metals.

Health Canada (2008) mandated upper limits for arsenic (0.14 μm/kg body weight per day), cadmium (<0.09 μm/kg), lead (<0.29 μm/kg), and mercury

(<0.29 µm/kg). The AHP proposed maximal limits for orally consumed cannabis products: mercury 2.0 µm/day, arsenic 10.0 µm/day, and cadmium 4.1 µm/day (Upton et al. 2013).

22.7 Janis Face—Bioremediation

Cannabis is so efficient at absorbing and storing heavy metals that it has gained attention as a "bioremediation crop." Bioremediation uses plants or microorganisms to remove pollutants. Plants such as *Thlaspi caerulescens* (= *T. alpestre*) extract toxins from soil and accumulate the toxins in their tissues. The plants are harvested and the toxins removed. *Cannabis* is an excellent candidate for bioremediation (Shi and Cai 2009), although the amount of metal taken up by *Cannabis* pales in comparison to *T. caerulescens* (Giovanardi et al. 2002; Löser et al. 2002; Citterio et al. 2003; Meers et al. 2005).

Jurkowska et al. (1990) measured high levels of lithium in hemp (1.04 mg/kg), higher than the other crop plant tested, including barley, maize, mustard, oats, radish, rape, sorrel, spinach, sunflower, and wheat. *Cannabis* has been sown on toxic waste sites contaminated with cadmium and copper in Silesia. The metals are recovered by leaching the harvested seed with hydrochloric acid (Kozlowski 1995).

Other studies have shown that hemp accumulates heavy metals in its roots (Giovanardi et al. 2002; Citterio et al. 2003; Shi and Cai 2009), and in leaf material (Giovanardi et al. 2002; Arru et al. 2004). Plants with mycorrhizal fungi growing in their roots show greater translocation of heavy metals from roots to shoots (Citterio et al. 2005). Perhaps mycorrhizal-inoculated plants are healthier, and therefore can better tolerate heavy metal stress.

Ciurli et al. (2002) showed potential for bioremediation using 'Fibranova' fiber-type plants, which tolerated growth in zinc-contaminated soil. They also showed that experiments of this type need to be done in soil, and not in a hydroponic-based screening test. The plants tolerated zinc salts much better in soil than in hydroponic culture.

Cannabis bioaccumulates sodium chloride, which kills it—despite the fact that chloride is an essential nutrient, and sodium is beneficial in trace amounts. Salty breezes near the sea are sufficient to stunt hemp crops. Italian accessions are being tested for tolerance to salt water, 2.5% NaCl (G. Grassi, pers. commun. 2016).

Cannabis can extract toxic polycyclic aromatic hydrocarbons from soil, such as benzo[a]pyrene and chrysene (Campbell et al. 2002). *Cannabis* also extracts radioactive caesium-137 and strontium-90 from contaminated soil (Vandenhove and Van Hees 2005; Hoseini et al. 2012). Hemp crops were planted near the Chernobyl site for the purpose of removing radionucleotides (Anonymous 2000).

Löser et al. (2002) were not impressed with the ability of *C. sativa* to uptake heavy metal-polluted river sediment. Although the plants took up zinc, cadmium,

and nickel, about 95% of the plants died within a week. Apparently different cultivars vary in their ability and tolerance in taking up cadmium from contaminated soils (Shi et al. 2012).

22.8 Pesticide Residues

Pesticide residues pose a uniquely unpredictable risk to consumers, because cannabis is usually smoked and inhaled, unlike most agricultural products. Up to 69.5% of pesticide residues remain in smoked cannabis (Sullivan et al. 2013). The use of illegal pesticides is a rising crisis, and a breakdown in ethics. Voelker and Holmes (2015) estimated that pesticide residues are found on close to half of the cannabis sold in Oregon dispensaries.

Sloppy and unscrupulous *Cannabis* growers utilize "over the counter" pesticides available in garden supply stores. Some of these are only approved for landscape plants, not food plants. Hydroponic shops repackage pesticides for ornamental plants, such as bifenazate and abamectin, for sale to *Cannabis* cultivators (McLean 2010). A dubious corporation marketed Guardian, a "100% natural" miticide, which contained undisclosed abamectin—resulting in the recall of cannabis in several states (Associated Press 2016).

McPartland et al. (2000) published a list of pesticides used by growers, derived from anecdotal reports and the literature. This veritable witches brew included abamectin, acephate, benomyl, carbaryl, carboxin, chlorpyrifos, chlorothalonil, chlorpyrifos, diazinon, dichlorvos, dicofol, dimethoate, fenbutatin oxide, iprodione, malathion, maneb, parathion, vinclozolin, and a slew of synthetic pyrethroids. The Centre for Disease Control in British Columbia studied former marijuana grow operations in residential homes. Their list of pesticide residues found in former grows included chlorpyrifos, diazinon, and 11 synthetic pyrethroids (NCCEH 2009).

Medical cannabis products in southern California have been contaminated with diazinon, paclobutrazol, and synthetic pyrethroids (Sullivan et al. 2013). The AHP published a list of pesticides that are most likely to be used on *Cannabis*, including 12 insecticides/miticides (abamectin, acequinocyl, bifenazate, etoxazole, fenoxycarb, imidacloprid, spinosad, spiromesifen, spiromesifin, and several synthetic pyrethroids), four fungicides (imazalil, myclobutanil, trifloxystrobin, paclobutraxol), and three plant growth regulators (daminozide, paclobutraxol, chlormequat chloride).

Testing of medical cannabis products in central California identified 12 pesticides and growth regulators, in up to 49.3% cannabis samples (Wurzer 2016). Myclobutanil led the list (40%), followed by bifenazate (20%), spiromesifen (15%), imidacloprid (4.6%), and spinodad (1.3%), as well as abamectin, acequinocyl, bifenazate, daminozide, fenoxycarb, pyrethrum, and spirotetramat.

A survey of 389 cannabis samples obtained from Oregon dispensatories found residues of 24 pesticides and growth regulators: abamectin, azadirachtin, bifenazate,

bifenthrin, carbaryl, chlorfenapyr, chlordane, chlorpyrifos, coumaphos, cypermethrin, diazinon, dichlorvos, ethoprophos, imidacloprid, malathion, metalaxyl, mevinphos, myclobutanil, paclobutrazol, permethrin, piperonyl butoxide, propoxur, and 4-4'-DDE (Voelker and Holmes 2015). Two percent of the samples contained >100,000 ppm pesticides. Piperonyl butoxide was the most commonly seen contaminant, with up to 407,000 ppm in one sample. This is a synthetic compound linked with human disease.

Russo (2016) purchased 26 cannabis samples (24 concentrates, 2 cannabis inflorescences) from legal stores in Washington State, and passed the samples via a witnessed chain to a state certified legal licensed laboratory. Pesticides residues were detected in 22 samples (84.6%), including 24 distinct agents of every class: insecticides (organophosphates, organochlorides, carbamates, neonicotinoids), miticides, fungicides, an insecticidal synergist, and growth regulators. One sample was contaminated with nine agents, include the fungicide boscalid (112,033 ppb) and the extremely toxic insecticide carbaryl (25,483 ppb). Samples obtained from indoor grows had a higher risk of contamination than samples obtained from outdoor grows.

Fertilizers may also contaminate *Cannabis*. Spraying plants with liquid fertilizers may result in the formation of *N*-nitrosamines, which are potent carcinogens (Farnsworth and Cordell 1976). Ramírez (1990) reported four policemen contracting pulmonary histoplasmosis while pulling up marijuana plants. The plants were likely fertilized with bird guano contaminated with the fungus *Histoplasma capsulatum*. The use of human dung has been associated with outbreaks of hepatitis viral infections (Cates and Warren 1975; Alexander 1987).

The EPA claims its failure to act in the interests of the American public is simply because it "has yet to receive any applications for pesticide use on marijuana and, therefore, we have not evaluated the safety of any pesticide on marijuana" (EPA 2016). In the absence of federal regulations, individual stakeholders and states have formulated guidelines.

In the spirit of harm reduction, the Maine legislature allowed the application of 25(b) pesticides on *Cannabis* (State of Maine 2013). These are minimal-risk pesticides exempted by the EPA—mostly botanicals (e.g., rosemary oil, thyme oil, garlic oil, corn gluten meal, eugenol), and other substances such as 2-phenylethyl propionate and potassium sorbate (EPA 2015). The Colorado Department of Agriculture and the Washington Department of Agriculture released larger lists of allowable pesticides (CDA 2016, WSDA 2016). Most of these pesticides are permitted in *The National List* of materials designated by the Organic Foods Production Act of 1990. They include botanical poisons (e.g., neem oil, garlic oil, azadirachtin, pyrethrins), minerals (e.g., potassium salts, copper, sulfur), and biological control organisms (e.g., *Bacillus thuringiensis, Streptomyces griseoviridis*). Both states allowed piperonyl butoxide. All these materials are described at book-length elsewhere (McPartland et al. 2000).

Assaying for pesticide residues is more difficult than microbial testing. Each pesticide must be tested individually, and the secretive use of pesticides leaves regulators in the dark (Stone 2014). The Oregon Health Authority posted a list of 59

pesticides required for testing before cannabis can be release for sale (Farrer 2015). Voelker and Holmes (2015) suggested testing for 123 pesticides, with tolerance limits of 100 ppb. Feldman (2015) documented pesticide regulations in other states.

Detecting pesticides requires expensive analytical methods, such as GS-MS and HPLC (Upton et al. 2013). Adequate pesticide testing costs around $400; laboratories charging only $100 are substandard (T. Flaster, pers. commun., 2016). To wit, few independent laboratories have been accredited for pesticide testing in cannabis—zero in Colorado (N. Palmer, pers. commun., 2016).

There have been several high-profile cases of cannabis removed from sale due to pesticides. In 2011 California issued a cease-and-desist order against the sale of cannabis contaminated with daminoside and paclobutrazol (Upton et al. 2013). In 2012, a whistleblower at Maine's largest medical cannabis dispensary revealed that nine types of insecticides and fungicides were being applied to *Cannabis;* the dispensary was fined $18,000 (Shepard 2013). Colorado regulators quarantined thousands of plants grown by a dispensary chain that used myclobutanil, a turfgrass fungicide; consumers filed a lawsuit against the corporation (Wyatt 2015). This was only one of nine marijuana recalls in Denver that year (Baca and Migoya 2015).

Mikuriya et al. (2005) reported the first case of hospitalization due to concealed pesticide use. The case report involved a bud trimmer working with cannabis contaminated with avermectin (abamectin), which a grower used against spider mites.

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