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HEAVY METAL ACCUMULATION ABILITY & CHEMICAL FINGERPRINTING OF *CANNABIS*-A REVIEW

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Abstract: Heavy metals are non-biodegradable. Once they enter in food chain through plants, they get accumulated in human body by bioaccumulation and cause serious health problems due to their toxic nature. Increasing concentration of heavy metals in soil is therefore becomes a global issue. Phytoremediation is an eco-friendly and economic mitigation measure to remove metal contamination from the soil. *Cannabis*, the illicit drug bearing plants and the most widely trafficked drugs worldwide, has a unique property of accumulation of heavy metals from the soil. Therefore this crop can be used for phytoremediation. This review will discuss the chemical composition and use of *Cannabis*, its ability of accumulation of heavy metals, factors affecting its metal accumulation abilities, effect of metals on human health and chemical fingerprinting of *Cannabis* for identification of its origin.

***Index Terms* -** Heavy metals, bioaccumulation, *Cannabis*, chemical fingerprinting.

I. INTRODUCTION

The *Cannabis* plant comprises about five forty chemical substances. Cannabinoids are the group of substances present in the *Cannabis*. Among these cannabinoids, cannabidiol (CBD) and tetrahydrocannabinol (THC) are known for their healing properties and medicinal use (Russo, 2007). THC and CBD are the not only cannabinoids available, inspite of them there are hundreds of other cannabinoids are also available. *Cannabis* is an important crop as it is used in the treatment of pain, asthma, insomnia, spasms, loss of appetite, depression, nausea and vomiting associated with cancer chemotherapy, spasticity in multiple sclerosis, anorexia in HIV/AIDS, movement disorders and neuropathic pain (Klumpers, and Thacker, 2019). These medicines are obtained from leaves, flowers, seeds and stem of the *Cannabis* plant. Along with these medicinal properties, *Cannabis sativa L*. is also used to produces numerous edible, cosmetics and other products such as cooking/seasonal oil, dietary supplements, plant-based super foods, beverages, body care products, fuel, paint and *Cannabis* fiber (stalk part) for the manufacture of textiles, insulators, ropes, paper, and biomaterials. Raw leaves and buds of *Cannabis* are also rich in nutrients (Zafeiraki et al., 2021).

Because of variety of use, *Cannabis* is cultivated in many parts of world. As per UNODC reports United States, Morocco, Afghanistan, Mexico, Colombia, Paraguay, Jamaica, Canada, Nigeria and India are the ten largest *Cannabis* producing countries (Thomas and ElSohly, 2016). In some countries like Uruguay and Canada, consumption and sale of recreational Cannabis has been made legalized. Central Asia or western China is the geographic location which initiates the use of cannabis. The first documented case of use of Cannabis was reported by Emperor Shen Nung's (known as the father of Chinese medicine) pharmacopoeia. As per UNODC reports, use of *Cannabis* increases between 1990 to 2000. According to World Health

Organisation, about 147 million people that are 2.5% of the world population consume *Cannabis* and the major consumers are in North America, Western Europe and Australia.

Cannabis is possibly one of the earliest plants to be cultivated (Russo, 2007). Cannabis has been cultivated in Japan since the pre-Neolithic period for its fibres, as a food source and as a psychoactive material (Clarke and Merlin, 2013). It was found in Neolithic Age in China (Stafford and Bigwood, 1992; Barber, 1992), and later the Chinese used it to make clothes, shoes, ropes, and an early form of paper (Stafford and Bigwood, 1992). Cannabis was an important crop in ancient Korea, with samples of hempen fabric discovered dating back as early as 3000 BC (Duvall, 2014).

Indians are also known as the earliest consumer of *Cannabis* in the history. As per WHO data, India has 31 million users of Cannabis. According to National Drug Dependence Treatment Center, AIIMS, New Delhi, the prevalence of current *Cannabis* use among total population (10-75 years), all males, all females, children's (10-17 years), adults (>18 years) is 2.8%, 5.0%, 0.6%, 0.9% and 3.3% respectively (Dube & Dhingra, 2020). Overall, 0.25% Indians uses *Cannabis* in a dependent pattern (World Health Organisation). Indians consumed it as Charas (resin) Ganja (flower) and Bhang (seeds and leaves), out of which Bhang is very popular. Bhang is used by Indians on many occasions like Shivratri, Holi etc. as Prasad (Acharya et al., 2014) and also recommended as medicine for the treatment of catarrh, phlegm and diarrhea in Indian scriptures Sushruta Samhita (Mechoulam and Hanus, 2000). Therefore millions of people in India consume *Cannabis* for pleasure as well as for pain alleviation. Regardless of its advantages *Cannabis* remains a substance of misuse issue around the world (Dube & Dhingra, 2020).

During its growth, *Cannabis* accumulates natural contaminants as trace and macro elements through soil and water and anthropogenic contaminants through fertilizers, pesticides, and fungicides that are usually applied to crops (Galic et al., 2019). *Cannabis* is a versatile plant which has been an important source of food, fiber, oil and medicine from many years. *Cannabis* plant grown on metal contaminated soil accumulate metals in its edible parts such as stem, seeds and leaves, through which metals enters in human food chain (Korkmaz et al., 2010). Essential micronutrients (such as Fe, Zn, Cu etc.) are good for health in a specific amount in food. Presence of metals beyond their permissible limit can disturb metabolic activities (Khan et al., 2008). Accumulation of toxic metals (such as Pb, Cd, Ni, Cr etc.) in human organs has serious health issues. Due to accumulation of heavy metals, *Cannabis* is considered a potential source of risk for human health (Craven et al., 2019).

But on the other hand, ability of accumulation of metals from soil by *Cannabis* plant makes it an important source for removal of metals from soil. Several studies report an accumulation of Ni, Pb, Cd, Zn and Cr in this plant which makes the plant to be considered in soil phytoremediation processes (Mihoc et al., 2012). Therefore, the aim of the current study was to investigate the potential of *Cannabis Sativa L*. plant to accumulate the heavy metals from soil for phytoremediation, factors affecting its metal accumulation abilities, effect of metals on human health and to check the possibility of chemical finger printing of the plant origin.

II Chemical composition of *Cannabis*

Cannabis is a natural product. Its main psychoactive constituent is tetrahydrocannabinol (Δ^9-THC). As per the dibenzopyran ring system, the unsaturated bond is present in between 9 th and 10th carbon atom in the cyclohexane ring. Other substances occur in Cannabis are cannabidiol (CBD) and cannabinol (CBN). Other compounds include the cannabivarins and cannabichromenes; which are all collectively known as cannabinoids (ElSohly and Slade, 2005, Lewis et al., 2017).

Out of the two varieties of Cannabis - *Cannabis indica* and *Cannabis sativa*, due higher THC content, *Cannabis sativa* is the preferred choice by users. *Cannabis sativa* is a complex plant and content near about 426 chemical entities and more than 60 of them are cannabinoid compounds (Dewey, 1986). The four major compounds are d-9-THC, CBD, d-8-THC and cannabinol (Pertwee, 1997, 2008; Pamplona and Takahashi, 2012). These four compounds are similar in structure but differ in their pharmacological effects. THC is the main psychoactive ingredient, however, CBD, the second major component of the plant, does not affect motion, body temperature or memory on its own. However, higher doses of CBD can aggravate the lower doses of d-9-THC (Atakan, 2012). Structure of tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) is shown in Figure 1.

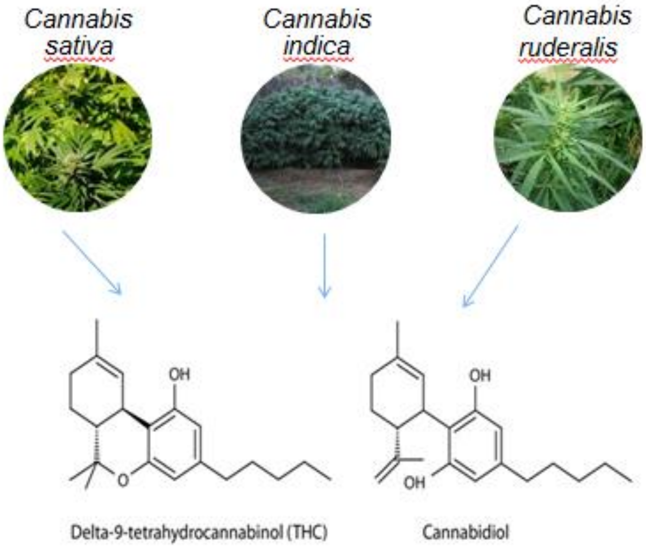

Figure 1: Structure of tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD)

The total concentration of delta-8 THC in *Cannabis* is lower than the concentration of delta-9 THC, but there is no difference in psychoactive and intoxicating effects of both type of THC. Cannabinol is the first cannabinoid isolated in pure form from the plant (Wood, 1899) and it has psychoactive effects (Mechoulam and Hanus, 2000).

III Plant types of *Cannabis*

Hemp plant has three species *Cannabis sativa L.*, *Cannabis indica* and *Cannabis ruderalis* which differ from each other on the basis of presence of Delta-9-tetrahydrocannabinol (THC) content in them (Atakan, 2012). ***Cannabis sativa* L.,** which is also known as hemp, is considered as a non-psychoactive form of *Cannabis*. It contains smaller amount of THC (less than 0.3%). It is tall and randomly branched plant. This plant is high in fiber and grain and important for industrial use. Whereas *Cannabis indica* have a higher Δ9-THC and a lower Cannabidiol (CBD) content than *Cannabis sativa* L (Atakan, 2012). It is comparatively short in length, conical in shape and densely branched and the fiber quality of it is not so good. It is used to make drugs for recreation and medicine.

Cannabis ruderalis have "thin, slightly fibrous stems" with little branching that contains low THC. It is used in medicine, especially for the treatment of depression. This variety is different from *Cannabis sativa L.*, *Cannabis indica* . The flowers are produced by *Cannabis ruderalis* with aging and it is not dependent on the light cycle (photoperiod). This kind of flowering is also known as auto flowering (produce flowers based on its age rather than the light cycle) whereas *sativa* L. and *C. indica* varieties are photoperiod sensitive for flowering. Different variety of *Cannabis* plants are mentioned in Table1.

Table 1: Variety of *Cannabis* plant

Variety of *Cannabis* plant	**Δ9-THC content (Psychoactive compound)**	**Characteristics of plant**	**Use**	**Reference**
Cannabis sativa L.	less than 0.3%	• Tall and randomly branched • High in fiber and grain • Photoperiod sensitive	In production of fiber and grain products and industrial uses	Langa et al., 2024; Thomas and ElSohly, 2016; Klumpers and Thacker, 2019; Atakan, 2012
Cannabis indica	18% to 38%	• Plant is relatively short conical and densely branched • Poor fiber quality • Higher Δ9-THC and a lower CBD • Photoperiod sensitive	Used to make drugs for recreation and medicine	
Cannabis ruderalis	3%	• Very short in height with a few branches and has wide, fat-bladed leaves • Higher in CBD • Auto flowering	Valuable for treatment of anxiety, cancer, sclerosis and loss of appetite.	

Different seed varieties of Cannabis plant are also available which differ in their THC and CBD contents and some of them are listed in Table 1. These varieties of *Cannabis* have different ability of accumulation of metal. Wielgusz et al., 2022 used two varieties Henola' and 'Białobrzeskie to study the accumulation of Cd by them and found that the highest cadmium concentration in seeds and biomass, were revealed for Henola variety. Galic et al., 2019 used *Fibrol,Fedora 17, Futura 75,* and *Santhica 27* for accumulation of Cd, Cu, Ni, Pb, Zn, Cr, Hg, Co, Mo and As and found that in alkaline soil *Fibrol* had the highest accumulation ability of Mo, *Fedora 17*, *Futura 75*, and *Santhica 27* had the hyper accumulator of Zn, *Fibrol* and *Santhica 27* of Hg, whereas in acidic soil *Fibrol* and *Santhica 27* are hyper-accumulators of Cd; Fibrol and *Santhica 27* accumulates Zn and *Fibrol* accumulates Hg. Zafeiraki et al., 2021 used nine varieties - Finola, Futura 75, Fedora 17, Gamagnola, Fellina 32, Dora, CS, Fibror 79, and Compolti for the study of accumulation of micro and trace elements by them. They found that Fedora 17 sample accumulate highest concentration of Ca, Fibror 79 accumulates Fe and CS accumulates Zn the most. Varieties of *Cannabis* seeds are listed in Table 2.

Table 2: Varieties of *Cannabis* seed

***Cannabis* seed variety**	**Characteristics**	**Reference**
Finola	• Growth cycle : 100 to 120 days • Low THC and high CBD content • Used for CBD production	Wielgusz et al., 2022; Galic et al., 2019; Zafeiraki et al., 2021; Pavlovic et al.,2019; Galasso et al., 2016; Arango et al., 2024; Burgel et al., 2020
Futura 75	• Growth cycle : 97 to 102 days • Can be used for several applications: Fibre markets, Shives markets • Produces above-average levels of CBD, approximately 3%.	
Fedora 17	• Growth cycle : 129 to 134 days • Used for agro-food and cosmetics applications • Seed contains 23.9% protein, 60.5% Omega 6, 14.5% Omega 3 and 31.9% oil content	
Fellina 32	• Growth cycle : 133 to 138 days • Can be used for Seed markets, Fibre markets, Shives markets	
Dora	• Vegetative cycle: 145 days	

	• THC: <0.2%, CBD : 2 - 3%	
Carmagnola Selected (CS)	• Vegetative cycle: 160/180 days • THC: <0.2%, CBD: up to 25%	
Fibror 79	• Vegetative cycle: 130-150 days • **THC**: <0.2%, **CBD** : 2-3%	
Compolti.	• Vegetative cycle: 140/160 days • THC: <0.2%, CBD: 2-10%	
Santhica 27	• Vegetative cycle: 120/140 days • **THC:** <0.2%, **CBD :** up to 3%	
FIBROL	• Vegetative cycle: 135 days • **THC:** <0.2%, **CBD :** up to 25%	

IV Uses of *Cannabis Sativa L.*

Cannabis sativa is a species of the *Cannabinaceae* family of plants. The dried leaves and flowers (buds) of the Cannabis plant are known as marijuana, which can be smoked or consumed in an edible. The resinous secretions of the plant are known as hashish, which can be smoked or eaten and the fiber of the Cannabis plant is cultivated as industrial hemp which can be used in textile manufacturing.

Medical marijuana comes in different forms such as oil, tablet or capsule. Medical Cannabis is commonly used for severe or long-term pain, nausea and vomiting due to chemotherapy and painful muscle spasms. It is also used for Amyotrophic lateral sclerosis (ALS), Cancer-related pain, Glaucoma, HIV/AIDS-related weight loss or nausea/vomiting, Huntington's disease, inflammatory bowel disease, Multiple sclerosis, Muscle spasms, Neuropathy, Parkinson's disease and Panic disorders.

The main Cannabis active compounds tetrahydrocannabinol (THC) and cannabidiol (CBD) are used for food items (Charleboisa et al., 2018). The food items such as pizza, chocolates, breakfast cereals, gummy consistent products, cookies/brownies contain Cannabis. In addition to this, beverages such as coffee, and tea, hemp-infused kinds of milk, and barley-based sodas have also been produced and commercialized (Rasera et al., 2021). Cannabis compounds incorporated into food products through edible oil, which is extracted from Cannabis.

The cannabinoids present in Cannabis sativa L. are very useful for the treatment of inflammatory skin conditions and skin cancer. Therefore they are important ingradient for cosmetics such as sunscreens. Hemp Seed Oil contains flavonoids, terpenes, carotenoids and phytosterols which ensure its anti-inflammatory and anti-aging action. Along with this,, its ω-6/ω-3 content is perfect for the skin. It is quickly absorbed and non-comedogenic oil (Mnekin and Ripoll, 2021). Some of the use of Cannabis in different products is listed in Table 3.

Table 3: Use of *Cannabis* in different products

Category	Products containing Cannabis	Reference
Medicine	Sativex for neuropathic pain and spasticity, Dronabinol / Marinol and Nabilone / Cesamet for nausea and vomiting, Endocannabinoids for Alzheimer's disease, Cannabinoids for sleep disorder	El Oihabi et al., 2024
Food product	Cookies, brownies, gummies, beverages, flavored yogurt, hemp flour, baked products, hemp milk, protein seed powder, and flavoring sauce	Iftikhar et al., 2021
Cosmetics	Sunscreen, anti-inflammatory and anti-aging cream, cream for treatment of skin conditions such as acne	Mnekin and Ripoll, 2021; Oláh et al., 2014; Baswan et al., 2020

V Heavy metals accumulated by *Cannabis Sativa L.*

Heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), lead (Pb), copper (Cu), zinc (Zn), nickel (Ni) are common pollutants for the soil. These pollutants are biologically toxic, widely distributed, and remains in the soil for long period. When plants grow in this type of contaminated soil, they accumulate these pollutants and consequently this contamination is transmitted in animal and human bodies (Buszewski et al. 2000). The uptake mechanism of heavy metals by the plant is selective i.e. different plants acquire different metals preferentially over others (Angelova et al. 2004).

Selected metal concentration in leaves of *Cannabis sativa L.* of four different samples from Ethopia was studied by flame atomic absorption spectrometry and level of metal concentrations are found to Ca (657–1,511), Zn (321–380), Ni (124–172), Cu (122–176), Cd (3–10), Pb (8–10), and Cr (4–8) in μg/g . The concentration of Zn was the highest among trace metals in this study (Zerihun et al., 2015).

Heavy metal concentration in root, leaves and stems of *Cannabis* plant grown from variety of hemp seeds Fibrol, Fedora 17, Futura 75, and Santhica 27 was investigated by Galic et al., 2019 in Croatia. Metals Cd, Cu, Ni, Pb, Zn, Cr, Hg, Co, Mo and As were analysed in samples and it was found that majority of varieties accumulated heavy metals in roots then that of leaves and stems. This study also found that pH of the soil affects the accumulation of heavy metals by the plant. It was observed that removal of metals such as Cd, Ni, Pb, Hg, Co, Mo and As was higher in acidic soil. Potential ability for accumulation of metals by plant was observed in alkaline soil in order Cu>Cr> Cd>Mo>Hg>Zn>Ni>Co>As>Pb, while for acid soil in order Zn>Cd>Cr>Ni>Hg>Cu> Mo>As>Co>Pb. This study also revealed that out of the different variety of hemp seeds, *Fibrol* had the highest accumulation capacity in alkaline soil, whereas it was *Fedora 17* in acid soil. So this study showed that pH of the soil and variety of hemp seed affects the accumulation capacity of heavy metals by *Cannabis* plant.

Zafeiraki et al., 2021 analysed 29 macro and trace elements, including both beneficial and toxic elements (heavy metals and metalloids) in leaves and flowers of 90 samples of 9 varieties of *Cannabis* by using coupled plasma mass spectrometry (ICP-MS) in Greece. These varieties include Finola, Futura 75, Fedora 17, Gamagnola, Fellina 32, Dora, CS, Fibror 79, and Compolti. Micro elements sodium (Na), magnesium (Mg), phosphorus (P), potassium (K) and calcium (Ca), trace elements aluminium (Al), titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium(Se), strontium (Sr), molybdenum (Mo), silver (Ag), tin (Sn), antimony (Sb), barium (Ba), thallium (Tl) and uranium (U), toxic elements arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb). It was found that micro elements ranged between 28 and 138.378 ppm, and of trace elements between 0.002 and 1352.904 ppm and the two toxic elements were below the prescribed limits established by the WHO. It was also noticed that *Cannabis* leaves and flowers were more contaminated with trace and macro elements than compare to seeds.

A study of heavy metal analysis of leaves and seeds of *Cannabis* in Nigeria found that the *Cannabis* leave seems to be more dangerous to health than the seeds. Because when the leaves and seed samples were analysed for As, Cd, Cr, Fe, Ni, Pb Hg and Mn by atomic absorption spectrometers model Perkin Elmer 3110, it was found that metal levels in Cannabis leaf exceeded those of Cannabis seed except in Mn (Eboh and Thomas, 2005).

Mihoc et al., 2013 studied the distribution of microelements in whole hempseeds (Cannabis Sativa L.) and in their fractions. In this study, Fe, Mn and Zn were analysed by air-acetylene flame AAS and Cu, Ni, Cr, Pb and Mo were analysed by graphite furnace and the results were obtained as Fe (130-164 mg/kg), Mn (89-108 mg/kg), Zn (42-57 mg/kg), Cu (10-12 mg/kg), Ni (1.6-6.1 mg/kg), Cr (598-877μg/kg) and Mo (265-652μg/kg). Pb concentration in hemp seeds belongs to the range of 217-626 μg/kg excepting the Armanca variety. Iron is concentrated in shell, zinc and nickel in hemp heart, while manganese and copper are equally balanced in both the core and the shell.

In a study by Khan et al., 2008, samples of leaves, stem and roots of *Cannabis sativa L.* plant were collected from five different regions of Pakistan (Charsada, Pabbi, Nowshera, Peshawar and Bara) and were analysed for Cu, Ni, Fe, Mn, Zn, cr, Pb and Co by atomic absorption spectrometer. Concentration of Cu ranged between 20 -100 mg/g, Mn in between 300 – 350 mg/g, Zn in between 019 – 0.45 mg/g and Pb in between 0.01 – 0.07 mg/g in plant samples. But the concentration of Mn in plant sample was well below than the critical level and no Cd was detected in all the plant samples. In this study, it was found that the Cannabis plant grown in the contaminated soil has high risk of having heavy metals concentration beyond their permissible limit.

The twenty one samples of hempseed, collected from North - western Turkey, were analysed for metal content in them. The metals analysed were Cd, B, Al, Co, Cu, Mo, Ni, Zn, Fe, Mn, Pb and Cr. The result of analysis showed that Cadmium content of the hempseeds was found within the range of 5 – 23 mgkg-1 and among the trace elements, the highest concentration found was of iron (98 -121 mgkg-1) trailed by concentration of manganese (70 - 102 mgkg-1) and zinc (46 - 72 mgkg-1). On the other hand, the lowest concentration found was of cobalt (0.06 - 0.17 mgkg-1) followed by molybdenum (0.25 - 0.62 mgkg-1) and nickel (0.55 - 1.66 mgkg-1) in the samples. Hence the study clearly indicates that the hempseeds from North-western Turkey are quite low in concentration of heavy metals when compared with the standards set for human well-being and this in turn shows that they can be used as valuable food source for human nutrition (Korkmaz et al., 2010).

Ghani et al. 2012 studied the levels of selected heavy metals Fe, Mn, Cu, Zn and Pb in Cannabis sativa L. in Soon Valley, Khushab District in Pakistan. The plants were collected from pollution free site. The heavy metal concentration in plant was determined by AAS. The study found the concentration of heavy metals as Zn 2.25 mg/L, Cu 26.6 mg/L, Fe 135.2 mg/L, Mn 40.30 mg/L and Pb 0.30 mg/L. The levels of heavy metals determined in the analyzed samples were found below the maximum tolerance limit. Therefore analyzed samples can be used as good source of important elements. Heavy metal accumulation in various parts of Cannabis plant is mentioned in Table 4.

Table 4. Heavy metal accumulation by *Cannabis* plant

S.No	Study area	Part of plant used for study	Metals analysed	Reference
1	Ethiopia	Leaves	Ca, Zn, Ni, Cu , Cd, Pb, and Cr	Zerihun et al., 2015
2	Croatia	Plant, Root, Leaves, stem	Cd, Cu, Ni, Pb, Zn, Cr, Hg, Co, Mo and As	Galic et al., 2019
3	Greece	leaves/flowers	B, Na, Mg, Al, P, K, Ca, Ti, V, Cr, Mn, Fe,Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Hg, Tl, Pb, and U.	Zafeiraki et al., 2021
4	Nigeria	leaves and seeds	As, Cd, Cr, Fe, Ni, Pb Hg and Mn	Eboh and Thomas, 2005
5	Romania	Seeds	Fe, Mn, Zn, Cu and Ni	Mihoc et al., 2013
5	Pakistan	Plant Root, Leaves, stem	Cr, Cu, Pb, Cd, Fe, Ni, Mn	Khan et al., 2008
6	Turkey	hempseed	Cd, B, Al, Co, Cu, Mo, Ni, Zn, Fe, Mn, Pb and Cr	Korkmaz et al., 2010
7	Pakistan	Whole plant	Fe, Mn, Cu, Zn and Pb	Ghani et al. 2012

VI Factors affecting accumulation of heavy metals by *Cannabis Sativa L.*

6.1 Effect of soil

Musio et al., 2022 Studied Cannabis plant cultivated in soil contaminated by Cd, Cr, Cu, Pb, Ni and Zn which had pH 6-7 and the trend of accumulation of metals was Cu > Zn > Cr > Ni > Cd >> Pb.

In a study made by Citterio et al., 2003 used two types of soil with different concentration of heavy metals for *Cannabis* cultivation and found that entire plant uptake capacity of Cd was 684 and 7500 g ha−1year−1 and of Ni 285 and 2026 g ha−1year−1 for the first and second type of soil variety. So they concluded that the *Cannabis* plant can be used for remediation of polluted soil.

Cannabis plant grown in soil contaminated with heavy metals accumulates heavy metals in good amount. Therefore before using *Cannabis* leaves stem, and roots for production of food items and medicines, it is important to estimate heavy metal concentration in plant as well as in soil where plant is grown, otherwise these heavy metals will enter in food chain and affect the health of the consumers.

6.2 Effect of pH

In their study, Galic et al., 2019 found that accumulation of heavy metals by Cannabis plant depends on the pH of the soil. They used *hemp* seeds -*Fibrol*,*Fedora 17*, *Futura 75*, and *Santhica 27* for the study and accumulation of Cd, Cu, Ni, Pb, Zn, Cr, Hg, Co, Mo and As were analysed. It was observed that removal of Cd, Ni, Pb, Hg, Co, Mo and As was higher in acid soil. The ability of *Cannabis* plant for phytostabilization was observed in alkaline soil in order Cu>Cr>Cd>Mo>Hg>Zn>Ni>Co>As>Pb, while for acid soil in order Zn>Cd>Cr>Ni>Hg>Cu> Mo>As>Co>Pb.

Wielgusz et al., 2022 studied effect of pH of soil on accumulation of Cd by two varieties Henola' and 'Białobrzeskie and found that at higher soil pH Białobrzeskie variety accumulates the highest concentration of Cd, but this type of relationship has not been observed for Henola variety. So they concluded that pH of the soil affects the cadmium uptake by some hemp varieties but not by the all varieties.

6.3 Effect of plant type and growing medium

Cannabis plants grown from different types of seed also have different capacity of accumulation of heavy metals. Galic et al., 2019 used *hemp* seeds -*Fibrol*, *Fedora 17*, *Futura 75*, and *Santhica 27* for accumulation of Cd, Cu, Ni, Pb, Zn, Cr, Hg, Co, Mo and As. They found that the seed variety *Fibrol* can be used for the removal of heavy metals because it had the highest accumulation ability of heavy metals in alkaline soil (2.65 mg/g - Mo), while in acid soil it was *Fedora 17* (16.1 mg/g - Cd). It was also noticed that in alkaline soil, *Fedora 17*, *Futura 75*, and *Santhica 27* were hyper-accumulators of Zn; *Fibrol* and *Santhica 27* of Hg and all varieties were hyper-accumulators of Mo. On the other hans, in acid soil, *Fibrol* and *Santhica 27* were the hyper-accumulators of Cd; Fibrol and *Santhica 27* of Zn; *Fibrol* of Hg and all varieties were hyper-accumulated of Mo. Therefore, these results recommend *Cannabis* as a suitable adsorbent for phytoaccumulation of heavy metals.

Zafeiraki et al., 2021 investigated 90 samples of 9 varieties of *Cannabis* for metal accumulation ability and found Fedora 17 had the highest capacity of accumulation of Ca (138378 mg/g) whereas Fibror 79 accumulates Fe the most.

Mihoc et al., 2013 used Zenit, Diana, Denise, Armanca and Silvana seeds for heavy metal accumulation and found that distribution of heavy metals are not only different in different varieties but also different in the different parts of the seeds. This study found that Iron is concentrated in shell, zinc and nickel in hemp heart, while manganese and copper are equally balanced in both the core and the shell.

VII Health issues due to consumption of heavy metals

Heavy metals are found around us in environment and food items are the main source of consumption of heavy metals by us. Heavy metals are required for maintaining good health in small quantities, but when their concentration cross their permissible limit in human body, they becomes toxic and causes many kinds of diseases. Higher concentration and long-time exposure to heavy metals can affect brain, lungs, liver, kidney, blood composition and other important organs and can lead to disease like multiple sclerosis, Parkinson's disease, Alzheimer's disease, muscular dystrophy and cancer (Jarup, 2003). Therefore estimation of heavy metals and their source is essential to take proper mitigation measures to prevent form their adverse health effects (Ferner, 2001). Effects of higher concentration of some heavy metals are mentioned in Table 5.

Table 5. Health effects due to consumption of heavy metals

Metal	Source	Health Effect	Permissible limit in food items	Reference
Cu	Food and water	Diarrhea, headaches, and in severe cases, kidney failure.	73.3 mg/g	Mensah et al. 2009
Cd	Food	Can cause kidney disease, fragile bones and lung damage	0.2 mg/g	Bernard, 2008
Cr	Water, soil	damage to DNA and proteins, carcinogenic	35 mcg	Stohs & Bagchi, 1995, Food, F. D. A 2016
Ni	Air, water, and soil	Allergy, cardiovascular and kidney diseases, lung fibrosis, lung and nasal cancer.	67.9 mg/g	Genchi et al., 2020
Pb	Air, water and soil	Affect the central nervous system and the gastrointestinal tract and cause mental retardation, birth defects, psychosis, autism, allergies, dyslexia, weight loss, hyperactivity, paralysis, muscular weakness, brain damage, kidney damage and may even cause death	0.3 mg/g	Martin & Griswold, 2009
Zn	Water and food	Gastrointestinal effects, abdominal pain, nausea, and vomiting, lethargy, anemia, and dizziness	99.4 mg/g	Porea et al., 2000
Fe	Water and soil	Gastro intestinal bleeding, vomiting and diarrhea, Cancer	425.5 mg/g	Osweiler *et al.*, 1985, Nelson, 1992
As	Air, food and water.	carcinogenic and can cause cancer of lungs, liver, bladder and skin	0.2 mg/kg	Chowdhury *et al.*, 2000; Shibata et al., 2016; Cubadda et al., 2017
Hg	Foods (marine foods) and beverages	can damage the brain, kidneys and the developing fetus	0.7 µg/kg	Alina *et al.*, 2012; Ye et al., 2016

VIII Phytoremediation ability of *Cannabis Sativa L.*

Studies carried out on *Cannabis sativa L*. by different researchers established the fact that the plant had the ability to accumulate heavy metals from the soil. Therefore this plant can be used as hyper accumulator for toxic metals such as lead, cadmium, copper, chromium and cobalt (Table 4) which are threat to the ecological system. Higher concentration of these toxic metals in environment is a result of various anthropogenic activities such as smelting, sewage sludge distribution and automobile emissions (Foy et al. 1978; Chronopoulos et al. 1997; Prasad et al., 1999; Dahmani-Muller et al. 2000). This plant is helpful in removal of these toxic metals from the soil which is contaminated by such anthropogenic activities. *Cannabis sativa L*. has the capacity to phytoaccumulation through roots of plant to absorb, translocate and concentrate heavy metals from contaminated soil. Therefore this plant can be used to convert the wasteland into cultivated land, especially the land contaminated with heavy metals like lead, copper, zinc, and cadmium. (Angelova et al. 2004).

IX Chemical fingerprinting for origin of *Cannabis Sativa L*

Researchers had used laser ablation ICP-MS techniques to determine the trace element patterns 'fingerprint' associated with Cannabis crops in order to identify their specific geological environments. Shibuya et al., 2007 used inductively coupled plasma mass spectrometry technique (HR-ICP-MS) to verify the existing differences in the elemental pattern of samples seized in the main Brazilian regions of *Cannabis* production. They analysed carbon and nitrogen stable isotopes, copper, cobalt, barium, lanthanum, zinc, iron, yttrium and manganese in the 153 seized *Cannabis* samples and found that samples seized from three different regions (30 samples from region1, 98 samples from region 2 and 25 samples from region 3) have different chemical profiles which is in accordance with the geological characteristics of each region.

Shibuya et al., 2007 found that nutrient contents in samples from Region 1 (Mato Grosso do Sul) were high, mainly for Cu and Fe followed by Aluminium, Ga and Ba levels. Maximum soils of this region are of volcanic origin (magmatic rocks, most of them pholeiitic basalt and, acidic in nature with excess Al and higher concentration of Fe and Mn. Samples seized in Region 2 (Marijuana Polygon) are containing high levels of U, Th, Pb, Mn and rare earth elements, particularly lanthanum and cerium. The soils of this area are mainly formed of granitic rocks and granulites, which are naturally enriched by lanthanides. The concentrations of Cu, Mo and Zn nutrients obtained for Region 3 (Amazon) samples were in similar pattern with lower concentration to the levels reported for Region 2. The samples seized in Region 3 also showed low concentrations of cobalt (below 100 ng g-1), than the other regions (600 ng g-1). Intense leaching of the soils was considered the cause for low levels of concentration of the elements in samples from the Amazon Region.

áJohn Watling 1998 analysed the *Cannabis* samples seized by police from different parts of Western Australia by using LA-ICP-MS technique. Large variety of sub-cropping rock types of this area had given it a widespread and diverse soil profile and mineralization. The study found that *Cannabis* grown in Kalgoorlie (major gold producing areas of Australia's) contains gold signature while Cannabis grown on mineral sands industry contains an rare earth element signature, Hydroponically grown Cannabis exhibit trace element association patterns with high molybdenum concentrations than field' crops.
These studies showed that chemical fingerprinting (study of elemental pattern) of *Cannabis* can provide important information about its growing sites which can help the police department to develop a national databank for monitoring the geographical origin of Cannabis consumed in the country and this gives police a better chance of finding the remaining source crop. This will help the police to track its distribution chain and to stop the illegal use of it.

X Conclusions

Heavy metal pollution has become a major concern for agriculture and food chain in all over the world because of their toxic effects. Phytoremediation is a one of the most effective method for removal of heavy metal contamination from soil. *Cannabis sativa L.* is a plant with potential ability to use as medicine and food product but it has a very good capacity of heavy metals accumulation, due to which it can be used for phytoremediation of the contaminated soil. In this review it was found that *Cannabis sativa L* can accumulate metals like Zn, Ni, Cu, Cd, Pb, Cr from the soil. Therefore it can be used for removal of these metals from the soil which may be contaminated by theses metals naturally (due to geological composition or volcanic eruption) or anthropogenic activities (industrial development or use of excessive fertilizers). It was also found that soil type, pH of the soil and variety of plant seed affect the ability of plant accumulation of metals. *Cannabis* is useful crop because it is widely used in medicine, food and cosmetics, it is important for the manufacturer to have an idea about its geographical origin and heavy metal concentration in its leaves, seeds and stems. Otherwise these heavy metals enter in human body through these products and damage it by their toxic effects. Moreover the analysis of elementary pattern of *Cannabis* is also useful for identification of its origin and to prepare its chemical finger prints. The chemical fingerprinting of *Cannabis* can help to prepare a wide databank which can help the legal authorities of a country to tract the source of *Cannabis* samples and prevent the illegal use of tis crop.

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