ALMA MATER STUDIORUM - UNIVERSITY OF BOLOGNA

EARTH RESOURCES ENGINEERING

Department of Civil, Chemical, Environmental,

and Materials Engineering – DICAM

Master Thesis

"PHYTOREMEDIATION OF CONTAMINATED SOILS BY USING *Cannabis sativa L.* AND THE ASSOCIATED VALORIZATION OF THE RESULTING BIOMASS"

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Panis vita, Canabis protectio, Vinum laetitia

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DECLARATION OF AUTHORSHIP

This project is a Master Thesis developed in collaboration with Department of Civil, Chemical, Environmental, and Materials Engineering - DICAM - at Alma Mater Studiorum University of Bologna. It covers 18 CFU and has been written by Nunziottavio Parrilli, master student of Earth Resources Engineering.

The thesis is a combination of two researches:

- Research A (6 CFU): phytoremediation of contaminated soil (heavy metals and organic xenobiotic compounds) using hemp (*Cannabis sativa L.*);
- Research B (12 CFU): valorization of biomass produced during this process.

This work was done wholly while in candidature for a master degree at this University. Where published works of others have been consulted, this was always clearly attributed.

Alma Mater Studiorum - University of Bologna, DICAM

Date

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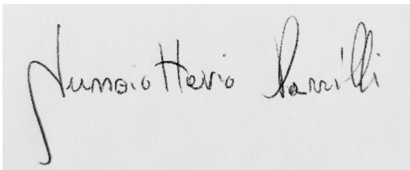

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ABSTRACT

Increasing urbanization, industrialization and over population cause fast and considerable degradation of soil and vegetation cover, which necessitate pursuing the methods of managing derelict industrial lands. On such chemically devastated lands vegetation plays increasingly important ecological and sanitary role. Proper management of plants from such areas may significantly contribute to restoring the natural environment. Numerous efforts have been undertaken recently to find methods of cleaning up soils contaminated by xenobiotic compounds, such as phytoremediation.

The aim of this thesis work is to analyse the influence of heavy metals and organic contaminants of soil on the growth yield and content of pollutants in *Cannabis sativa* L. This plant was selected because *a)* it grows rapidly, reaching full harvest in just 100-120 days (depending on local conditions) and produces a sphere of roots that extends from 1.5 to 2.5 meters into the soil, without requiring nutrients, herbicides and pesticides; *b)* it is a non-food crop, suitable for the production of a wide variety of renewable products more efficiently than what can be done with other non-food crops; and *c)* it produces components with relevant market opportunities through a stepwise breakdown. Hemp biomass can be used for the preparation of several commercial items, including paper, textiles, clothing, biodegradable plastics, paint, insulation, biofuel, food, and animal feed.

However, hemp presents a key disadvantage: it is also used as an illegal narcotics and often that costs discriminates between fiber types and drug type use of hemp crop. Consequently, only cultivars which set seed in a given member state are permitted to be grown in the EU.

Key words: phytoremediation, *Cannabis sativa*, biomass, re-valorization

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Nunziottavio Parrilli

1. INTRODUCTION

Hemp is a valuable ally in the environmental challenges facing humanity. Not only because it can be crucial in paradigm change from the unrestrained use of petroleum derivatives, such as plastics, or for the huge reduction of CO_2 emissions or to reduce deforestation but also because it might help us in sustainably clean up some former industrial sites through phytoremediation. The plant with a thousand uses in fact, as told in a dedicated chapter of the book "*Cannabis, Il futuro è verde canapa*" [1], has the potential to remove from the soil organic pollutants and heavy metals by storing them inside its body. According to what is explained in the book, the highest concentration of heavy metals was in the leaves of the plant. The overall quality of hemp fibre was not affected by contamination by heavy metals", but is not suitable for human consumption and is "more suitable for industrial purposes or for energy production" [1]. Today it is at the center of medical-scientific research. It contributes to the healing of economies that have been disrupted by billions of dollars and is becoming an almost inexhaustible resource that can replace oil derivatives and be perfectly compatible with the principles of the circular economy. Hemp is not just a medicine or substance used at a spiritual or recreational level. Considered it as the "vegetable pig", because it can be used in all its parts, cannabis is a nutraceutical food, raw material with excellent quality for paper (thus avoiding deforestation), a material for the green building houses with reduced consumption, a performing tissue, resistant and natural, biodegradable bioplastics, as well as a futuristic material for the cars of tomorrow and to store energy, thus contributing to remove pollutants and heavy metals from soils and removing huge amounts of CO_2 from the atmosphere.

1.1 BRIEF HISTORY OF THE PLANT

In the eighth millennium BC, hemp grew in wild areas of central Asia, particularly on the slopes of Himalaya. There is no other novelty of this plant species until 2700 BC, when hemp appeared in a text of the Chinese pharmacopoeia, in which it is indicated as the main textile plant used by those populations.

In the fifth century BC, hemp reached the most prestigious moment in the religious sphere with Buddha: the legend (or faith) says that the Enlightened fed himself only with hemp seeds.

In the 3rd century BC, Romans and Carthaginians fought for the monopoly of the spice and hemp route in the Mediterranean Sea.

Always the Chinese, in 770 AD, made the first book printed on hemp paper: *Dharani*.

In medieval times, Germans, Franks and Vikings used hemp massively, which was soon replaced by cotton.

The first example of a work printed on hemp paper in Europe was the Gutenberg *Bible* in 1455.

In 1484, Pope Innocent VIII issued a papal bull prohibiting its use by the faithful: for the first time in its history hemp is defined as a demonic medium through which to live mystical experiences outside those "divine" and in any case not authorized by God. Despite the ban by the Catholic world, hemp continues to be cultivated for centuries to come.

In 1492, Cristoforo Colombo managed to sail the Atlantic Ocean thanks to the 3 caravels, whose sails were made of hemp.

In 1776 the declaration of independence of the United States of America was written on hemp paper.

Even the canvases used by Van Gogh, Rembrandt and many other painters were made of hemp, and thanks to the extraordinary resistance of its fiber they resisted the erosion of time.

The lamps that illuminated the world worked with hemp seed oil.

In the modern era, with the appearance of new machines for harvesting and processing, cotton was easier to process, even if the quality was lower. With the use of bleaching agents such as chlorine and sulphites, for the extraction of lignin, the production of paper from wood fibers was increasingly favored.

The USA demonstrated an unclear vision of the problem of marijuana and industrial hemp throughout the 20th century. In 1937, the "Marijuana Tax Act" was published, which taxed cannabis growers in an unsustainable way.

In the 60s the definitive consecration of cannabis took place as a symbol of an alternative culture, ranging from hippies to Vietnam veterans: this however accentuated the perception of the use of the hemp plant useful for the sole purpose of “getting high”.

In 1976, Netherlands introduced a certain tolerance towards psychotropic varieties, legalizing and controlling their sale. In the 90s, finally, we talk again about industrial hemp and the EU subsidizes the cultivation of non-psychotropic hemp varieties [2].

In 1998, the Canadian Government provided enabling legislation allowing for the planting and processing of industrial hemp, but it remains highly regulated and monitored by Health Canada. For the first time in 60 years, farmers were able to grow it for food, fiber manufacturers were able to process it and exporters were able to ship processed products outside Canadian borders.

In the United States, it is still illegal to grow industrial hemp for commercial purposes. The U.S. Drug Enforcement Administration (DEA) remains strongly opposed to the importation of viable hemp seed citing the “Controlled Substances Act”. This stance is frustrating the production of industrial hemp even though the *2014 U.S. Farm Bill, Section 7606*, explicitly states that industrial hemp is approved for research by state agriculture departments and universities. But individual state governments are at odds with the DEA and are aggressively supporting the research and varietal development in their respective states to inject economic activity and commerce. Replacing the shrinking tobacco industry is high on the agenda for states like Kentucky and Tennessee [3].

1.2 SOCIAL ACCEPTANCY

The word "cannabis" is used in various ways. In its broadest sense, it refers to the cannabis plant (*Cannabis sativa*), especially its psychoactive chemicals (employed particularly as recreational and medicinal drugs), fiber products (such as textiles, plastics and dozens of construction materials), edible seed products (now in over a hundred processed foods), and all associated considerations. In short, cannabis is a generic term referring to all aspects of the plant, especially its products and how they are used. Italicised, *Cannabis* refers to the biological genus name of the plant (of which only one species is commonly recognized, *C. sativa* L.). Non-italicised, "cannabis" is a generic abstraction, widely used as a noun and adjective, and commonly (often loosely) used both for cannabis plants and/or any or all of the intoxicant preparations made from them.

Cannabis is widely classified as a "narcotic," a term which is most often used as an arbitrary juridical category. A narcotic is frequently defined as a substance or preparation that is associated with severe penalties because of real or alleged dangerous (usually addictive) properties. "Folk taxonomy" refers to the spontaneous ways people have traditionally described, named and organized (or classified) objects, thoughts, events, or indeed any aspect of human experience. The name "hemp" can be confusing. It usually refers to *C. sativa*, but the term has been applied to dozens of other species representing at least 22 genera other than Cannabis, often prominent fiber crops. Montgomery (1954) listed over 30 "hemp names" [4]. Nowadays, European Commission has listed 71 hemp plant species [5].

1.3 THE ITALIAN SITUATION

In Italy, currently, the law in force establishes the legal limits of the production, marketing and consumption of *Cannabis sativa* L. (law nr. 242 of December 2nd, 2016). It represents an exception to the Italian legislation of reference, the so-called *Single Text* on the subject of narcotic substances (Presidential Decree nr. 309/90): it deals with regulating, among other things, the authorizations necessary for the cultivation, sale and possession of psychotropic substances. Also, to the Presidential Decree 309/90 are annexed the tables (periodically updated) which contain the psychotropic substances. Specifically, the table concerning hemp is that indicated by the Roman number I, where the main active ingredient of cannabis appears, namely tetrahydrocannabinol (THC). The art. 14 subparagraph 6 says: «cannabis indicates, the products obtained from it; tetrahydrocannabinols, their natural analogues, substances obtained by synthesis or semi-synthesis which are traceable to them by chemical structure or pharmaco-toxicological effect» [6] [7]. The Ministerial tables provide for m.d.q., i.e. the maximum detainable quantity expressed in mg, below which the personal use of the narcotic drug is presumed. In essence, therefore, the 242/2016 represents a *lex specialis*, compared to the *lex generalis* constituted by the Presidential Decree 309/90. Since the first applies only to seeds authorized by the European Union [5], it will mean that all the rest will be governed, in fact, by the 1990 single text. The art. 4 subparagraph 5 of Law 242/2016 says: "if the total THC content of the crop is more than 0.2 % and within 0.6 % at the end of the check, no liability is charged of the farmer who has complied with the provisions of this law". The art. 7 of Law 242/2016 says: "public research institutions, universities, regional agencies for development and innovation, including by stipulating protocols or agreements with cultural associations and consortia dedicated specifically to canapiculture, they can reproduce for a year the seed purchased certified in the previous year, using it for the creation of small productions of demonstrative, experimental or cultural character, after communication to the Ministry of Agricultural, Food and Forestry Policies" [6] [7]. This is in general, on National scale: then there are some differences among the Italian Regions. For instance, the law nr. 1 of February 2nd 2017, in Lazio Region, establishes "Interventions to promote the cultivation of hemp (Cannabis Sativa) for production, food and environmental purposes and related supply chains" [8]. In the Art. 1 is stated that "The Region, in compliance with European and State regulations as well as in the context of multifunctional and sustainable rurality, promotes the cultivation, transformation, marketing and supply chain of hemp as well as the structuring of the related production chains aimed at supporting the competitiveness and productive diversification of the agricultural enterprises and to encourage, within the context of its agricultural, environmental and industrial policies, the integration between agricultural processes and industrial processes, containing the environmental impact in agriculture, the consumption of soil and protecting biodiversity" [8]. The main Italian stakeholders are Federcanapa (https://www.federcanapa.it/); AssocanapaGroup (https://www.assocanapagroup.it/) which is formed by CNC Coordinamento Nazionale per la Canapicoltura, Assocanapa Srl, PROCAIT SCA Cooperativa Produttori Canapa Italiana; UCI (Unione Coltivatori Italiani) (https://www.uci.it/ancica). These are also among the main promoter of Expo-fests - like IndicaSativa (Bologna), CanapaMundi (Roma), 420 Hemp Expo (Milan) – in which are highlighted the complete business eco-system of this plant, its many varieties, derivatives and uses, from agricultural, industrial and medicinal, to production, wholesale and retail. The production of hemp in Italy is growing, at a rate that was unimaginable 10 years ago. In 5 years it has gone from about 400 hectares cultivated in Italy in 2013 to over 4.000 in 2018. But this is not enough, thinking at golden era hemp has lived in Italy: at the beginning of the 20th century Italy was among the first world producers. In 1940, Italy devoted 90.000 hectares of its territory to the cultivation of hemp. "We produced more hemp than is produced worldwide today, with 85,000 hectares globally (2011)", said Loredana Lupo, agronomist, member of Chamber of Deputies. Fortunately, the hemp production chain, after a long period of freezing production, is arousing new interest as a result of the increase in the price of oil and greater attention for the protection of the environment, as well as the huge effort researchers are putting into the field. The global market for hemp is predicted to double from the year 2016 to 2020. At present, hemp is cultivated for commercial or research purposes in at least 47 countries, and it is utilized by indigenous populations for textiles in another two countries. Canada, China, Chile, France, and North Korea are currently the largest producers of hemp, while The USA is the largest importer of hemp products [9].

1.4 CONTAMINATED SITES IN EUROPE

The European Union (EU) member states (27) have a combined area of 4.233.255,3 km^2 [10]. In Western Europe, over 300.000 potentially contaminated sites have been identified [11]. In Eastern Europe, the most serious risk is represented by soil contamination around abandoned military bases [11]. For the EU-28, an estimate of around 2.8 million sites where polluting activities took/are taking place was obtained [12]. 694.000 sites are already identified and registered in national and/or regional inventories [12]. Since 2011, more than 76.000 new sites have been registered Austria, Belgium (Flanders), Croatia, Estonia, Finland, the former Yugoslav Republic of Macedonia, Italy, Lithuania, Malta, Norway, Serbia, and Switzerland. A reduction in the number of registered sites where polluting activities took/are taking place can be observed for several respondents, such as Cyprus, France, Germany, the Netherlands, Hungary, Slovakia and Spain. This difference can be explained because over the past decade there have been changes in the criteria for defining an activity as potentially polluting to include a site in the national inventory [12]. In Italy, although there is no list of potentially polluting activities in the current legislation used for CS identification, the identification procedure is associated with refineries, chemical plants, steelworks and asbestos production or extraction sites. In addition, a preliminary investigation begins when an event occurs that may cause contamination of soil and/or groundwater or when historical contamination is discovered. It is important to highlight that the regional inventories in Italy include sites in need of investigation and remediation, where management procedures have already been initiated, but do not take into account sites where polluting activities took/are taking place, since there it is not legally required to register sites where polluting activities took place, but only sites under management [12]. In Italy, according to JRC report [12], the approach for assessing contaminated sites is the Screening Values for assessing the need for investigation and on-site-specific risk assessment for assessing the need for intervention and it is regulated by the Legislative Decree n. 152/2006 approving the Code on the Environment [12]. The area that has been remediated is reported in table 4 below [12].

Country	Total area remediated (ha)	Area remediated *off-site* (mill. tonn)	Area remediated *on-site* (mill. tonn)	Area remediated *in situ* (ha)
Denmark	-	2.5	-	-
Estonia	53	-	-	3.5
Finland	-	1.5	-	1015
France	14500	1.1	-	-
Hungary	97	1.0	1.5	8.8
Luxembourg	-	0.2	-	-
Portugal	27.21	0.7	-	-
Switzerland	600	2.0	0.1	70

Table 1 - Total remediated surface and area treated with different remediation techniques

According to the Italian Ministry of the Environment and Protection of the Territory and the Sea, the state of contamination in Italy includes 41 SIN (Siti di Interesse Nazionale https://www.minambiente.it/bonifiche/documentazione-sin). Different contaminants have different effects on human health and the environment, depending on their properties, for example: their potential for dispersion, their solubility in water or fat, their bio-availability, carcinogenicity, etc. The distribution of the different contaminants is similar in the liquid and the solid matrix. The main contaminant categories are mineral oils and heavy metals. Contamination with mineral oil is especially dominant in Belgium (solid matrix: 50 %) and Lithuania (solid matrix: 60 %), while heavy metals are the dominant contaminants in Austria (solid matrix: 60 %) and the former Yugoslav Republic of Macedonia (solid matrix: 89 %) [13]. In figures 1 and 2 below, are reported data summarizing the average contaminants affecting soil and groundwater in Europe [13].

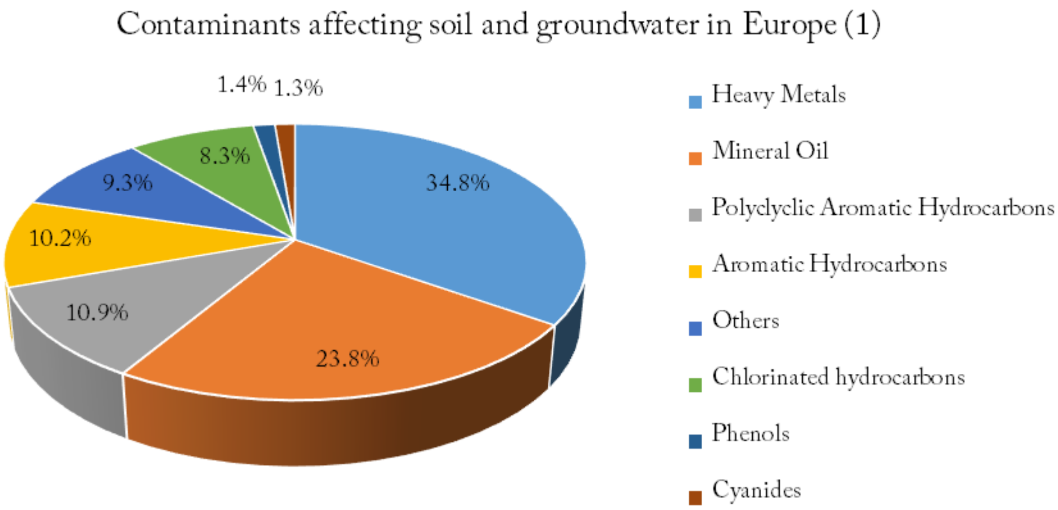

Figure 1 - Contaminants affecting the solid matrix (soil, sludge, sediment) as reported in 2011 [13]

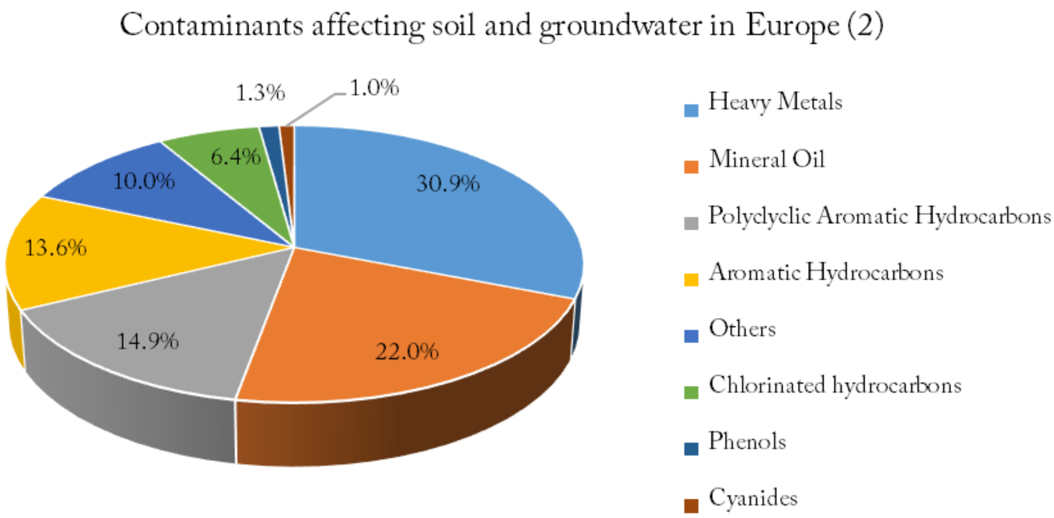

Figure 2 - Contaminants affecting the fluid matrix (groundwater, surface water, leachate) as reported in 2011 [13]

The data suggests that phenols and cyanides make a negligible contribution to the total contaminant loading. For this reasons, this study is focused on the Remedium of sites contaminated by heavy metals and hydrocarbons xenobiotic compounds.

2. BOTANY AND ANATOMY

Hemp is an annual plant that adapts to almost all types of soil but prefers soft, deep and permeable ones. It grows well in temperate climates, and requires temperatures slightly above zero for germination, is sensitive to the photoperiod and needs about 20°C for the flowering plant and about 13°C for the seed maturation [14].

Botanically speaking, cannabis is a dioecious species, meaning it produces both male and female plants. However, there are also cases of hermaphroditic plants with male and female sex organs. These reproduce through self-pollination, forming flowers that contain seeds, and have the ability to pass on their hermaphrodite genes [15].

Figure 3 – male specimen of Cannabis sativa L (left), and female (right)

2.1 TAXONOMY

Cannabis is a dicotyledonous angiosperm plant belonging to the *Cannabaceae* family, which also comprises hops.

Domain: *Eukaryote*

Kingdom: *Plantae*

Division: *Tracheophytes*

Class: *Magnoliopsida*

Order: *Urticales*

Family: *Cannabaceae*

Genus: *Cannabis*

Species: *Cannabis sativa* L.; *Cannabis indica* Lam.; *Cannabis ruderalis* Janisch [16].

According to the "Hemp and Diseases Pets" publication [17], Cannabis sativa can be further classified into: *Cannabis sativa* (=*Cannabis sativa* var. sativa) [17] up to 5 meters high, and present in Europe, Africa and the tropical areas of America and Asia [18]; *Cannabis indica* (=*Cannabis sativa* var. indica) [17] lower and thicker, native to Asia and then widespread in sub-tropical areas [18]; *Cannabis ruderalis* (=*Cannabis sativa* var. spontaneous) [17] bush-like in shape, capable of living wildly in the harshest climates of Russia and northern China [18].

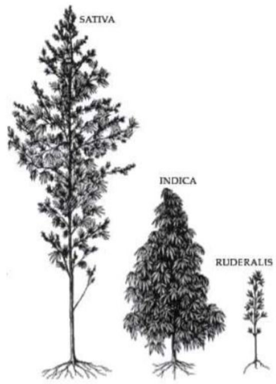

Figure 4 - varieties of cannabis plants

2.2 PHYSIONOMY

Roots

The roots grow below the substrate surface - soil or otherwise - and have various functions, ranging from providing the plant with firmness to absorbing the nutrients necessary for growth. The root system consists of a main root from where many other secondary roots grow, forming a fibrous mass. These are part of the plant's vascular system, which using xylem (the tissue that carries sap), transport water and nutrients up and down the plant.

Figure 5 - germinated seeds and roots system

Main stem

The central stem grows upright from the roots. It allows for vertical growth and is the plant's major vascular tissue, transporting water and minerals from the roots to the leaves and flowers. Under normal conditions, the main stem develops nodes that allow for secondary growth.

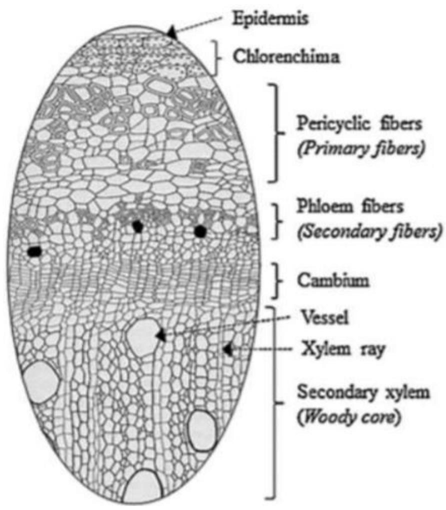

Figure 6 - stem cross section

Leaves

Leaves are a vital part of every plant. In the case of cannabis, they tend to be fan-shaped and symmetric, growing in pairs from the main stem and the branches. A good way to differentiate between cannabis strains is to observe the leaves. While indica's leaves are dark and large, sativa's leaves exhibit lighter, more slender leaflets (each of the structures that make a compound leave). Hybrid leaves are a combination of both. It is through the leaves that plants absorb the light available from sun. Internally, the phloem (conducting tissue) transports energy from the leaves to other parts of the plant, just as an electrical wiring system would.

Figure 7 - leave of cannabis sativa (left), leave of cannabis indica (right)

Flowers

Prior to flower formation, plants develop floral primordiums at the point where the branches join the stems. These formations, also known as preflowers, vary according to plant gender. Female plants produce a tiny, pear-shaped structure known as the calyx that is made up of pistils. These contain two hair-like stigmas that are connected to the ovaries of the flower, which are generally white in colour. Designed to contain the pollen grains, the calyx of male flowers is formed by five sepals that support the petals, containing no stigmas. As flowering progresses, male flowers form hanging clusters, while female flowers are grouped into the plant's buds and colas.

<u>Trichomes and chemical components</u>

The importance that *Cannabis inflorescences* can have in the medicinal field is due to the content of numerous secondary metabolites, including phytocannabinoids, terpenes and phenolic compounds. These compounds are localized largely in the plant's trichomes, especially in female flowers. Trichomes present as mushroom-shaped epidermal bumps. More than 110 cannabinoids have been described in the literature. The most important are:

- Tetrahydrocannabinol (THC)
- Cannabidiol (CBD)
- Cannabinol (CBN)

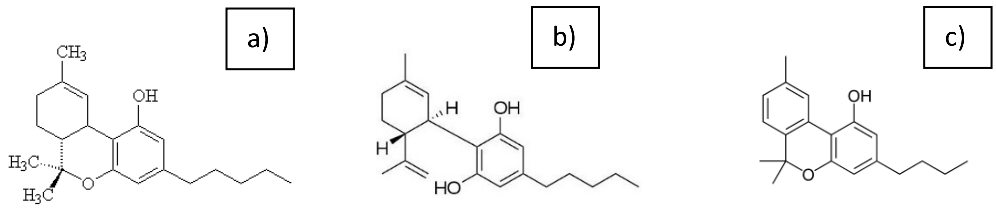

Figure 8 - chemical structure of cannabinoids contained in trichomes: from left towards right, a) THC, b) CBD, c) CBN.

Figure 9 – trichomes

2.3 NEW HEMP CULTIVARS

There is a phylogenetic controversy regarding considering three distinct cannabis species (*Cannabis sativa*, *Cannabis indica* and *Cannabis ruderalis*) or a single species with multiple varieties. Many botanical scholars believe that it is a single species, having all the cannabis plants similar morphological, chemical and growth characteristics, which vary their phenotype according to the areas in which they grow, altitude, soil characteristics etc. . In addition, all cannabis strains can be crossed with each other, obtaining fertile and healthy individuals. It is estimated that there are more than 1000 different species nowadays. A recent study was carried out in order to check the variability in fiber quality within the hemp species. 123 hemp accessions were grown in: Rovigo, at CRA in Italy; Chèvrenolles, Neuville-sur-Sarthe, at FNPC in France; and Westerlee, at VDS in Netherlands [19]. Fiber quality traits were strongly affected by the environment, such as photoperiod and temperature regimes and probably water availability [19]. Some accessions displayed characteristics that are highly appreciated by the hemp industry. Such traits included large contents of bast fiber and cellulose, low contents of lignin and pectin, fine fiber bundles (high PL and PH values), and late flowering time: IWRNZ-902 (Beniko) and MH-WU-111 (Kompolti Sargàszàru) [19]. In general, hemp cultivated in northern latitudes showed a larger plant vigor while earlier flowering was characteristic of plants cultivated in southern latitudes [19].

2.4 ENVIRONMENTAL BENEFITS OF HEMP CULTIVATION

Hemp is not only one of the best sources of renewable energy available on the Planet: it is one of the best weapons to combat pollution, reduce the devastating effects of man on the climate and in general contribute to creating a sustainable model of economic development:

- **High biomass yield**: the concept of bio-refinery can be easily applied to the production of hemp crops, which can be understood as an integrated system that serves the production of energy and chemicals from biomass;

- **CO_2 sequestration**: when growing, hemp captures 4 times the CO_2 stored on average by trees. The entire production chain of hemp is *negative carbon*, i.e. it removes more CO_2 from the environment than would be injected by processing it;
- **Limiting deforestation and use of fossil-derived energies**: according to the United States Department of Energy, hemp is the biomass fuel producer that requires less specialization in both the cultivation and processing of all plant products. Hemp hydrocarbons can be transformed into a wide range of biomass energy sources, from pellets to liquid and gas fuels. The growing demand for trees led to deal with the problems related to deforestation and above all to the awareness that, unlike a tree, hemp matures in much shorter times. The difference between a mature tree ready to cut for obtaining paper and hemp, in fact, is that the former needs twenty years to grow, the latter only one year;
- **Conserves water usage**: on average, hemp requires from 3 to 10 litres per plant per day, much less than other crops (industrial or non-). For instance, cotton plants need about 50 percent more water per season than hemp;
- **Preventing pollution by pesticides and herbicides**: toxic chemical compounds go into groundwater and target not only insects, but all organisms, including humans. Cotton cultivation is probably the largest pollutant on the planet in terms of release of pesticides into our environment since, occupying only 3% of the world's agricultural land, it requires 25% of the pesticides used in total. Hemp fiber is longer, more absorbent, resistant and insulating than cotton fiber.

3. PHYTOREMEDIATION TECHNIQUES

Phytoremediation is a technology that uses various plants to degrade, extract, or immobilize contaminants in soils and water. This type of bioremediation is suitable when the pollutants are within the root zone of the plant. This technology has been receiving attention lately as a cost-effective alternative to the more established microbial based treatment methods used in hazardous waste contaminated sites. Most physical and chemical methods (such as encapsulation, solidification, stabilization, electrokinetics, vitrification, vapour extraction, and soil washing and flushing) are expensive and alter the physico-chemical and biological features of the soil along with its fertility [20]. The remediation techniques are normally classified in:

- **Ex-situ**, which requires removal of contaminated soil for treatment on- or off-site, excavation, detoxification (but also stabilization, solidification, immobilization, incineration or destruction), and then returning of the treated soil to the former site;
- **In-situ**, in which the remediation is carried out without excavation of the soil, which is treated on place, at the non-saturated zone or the saturated one.

Current technologies resort to soil excavation and either landfilling or soil washing, followed by physical or chemical separation of the contaminants. The cost of soil remediation is highly variable and depends on the contaminants of concern, soil properties, and site conditions. Cost estimates associated with the use of several technologies for the cleanup of contaminated soil are shown in Table 1.

Treatment	Cost ($/ton)
pyrolysis	170
vitrification	75-425
landfilling	100-500
chemical treat.	100-500
electrokinetics	20-200
bioslurry	40-80
landfarming	40
soil washing	30

stabilization	50-100
phytoextraction	5-40
phytoremediation	15-30

Table 2 – Average costs of soil treatment

In addition, because it remediates the soil *in situ*, phytoremediation avoids dramatic landscape disruption and preserves the ecosystem. Despite these advantages, several disadvantages and constraints restrict the applicability of phytoextraction: low plant tolerance to contaminants, pollutants beneath root zone, need for disposal of contaminated plant waste, risk of food chain contamination, etc.

Phytoremediation of contaminated soils is generally effective through one or more of the following mechanisms or processes, which will occur simultaneously: phytoextraction, phytostabilization, phytodegradation, phytovolatilization, and rhizodegradation [21].

For instance, some classes of organic compounds are relatively more rapidly degraded in the rhizospheric region than in others: these include polychlorinated pesticides (PCP), polychlorinated biphenyls (PCBs), petroleum hydrocarbons, nitroaromatic compounds, explosives, surfactants and organophosphate insecticides [22].

Phytoextraction

Phytoextraction is the uptake of contaminants by plant roots and translocation within the plants. Contaminants are generally removed by harvesting the plants. Phytoextraction is primarily used for the treatment of contaminated soils. To remove contamination from the soil, this approach uses plants to absorb, concentrate, and precipitate toxic compounds from contaminated soils into the above ground biomass (shoots, leaves, etc.). It is the best approach to remove contaminants from soil, sediment and sludge [23].

Advantages

- phytoextraction is fairly inexpensive;
- the contaminant is permanently removed from the soil.

Disadvantages

- hyperaccumulators are generally slow-growing with a small biomass and shallow root systems;
- plant biomass must be harvested and removed, followed by metal reclamation or proper disposal of the biomass. Hyperaccumulators may accumulate significant metal.

<u>Phytostabilization</u>

Phytostabilization, also referred to as in-place inactivation, is primarily used for the remediation of soil, sediment, and sludges. It is the use of plant roots to limit contaminant mobility and bioavailability in the soil. Phytostabilization can occur through the sorption, precipitation, complexation, or metal valence reduction. It is useful for the treatment of lead (Pb) as well as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu) and zinc (Zn) [24].

Advantages

- soil removal is unnecessary;
- it has a lower cost and is less disruptive than other more-vigorous soil remedial technologies;
- disposal of hazardous materials or biomass is not required [23].

Disadvantages

- the contaminants remain in place. The vegetation and soil may require long-term maintenance to prevent rerelease of the contaminants and future leaching.
- application of extensive fertilization or soil amendments, mandatory monitoring is required [25].

<u>Rhizofilteration</u>

Rhizofilteration is primarily used to remediate extracted groundwater, surface water, and wastewater with low contaminant concentrations. It is defined as the use of plants, both terrestrial and aquatic, to absorb, concentrate, and precipitate contaminants from polluted aqueous sources in their roots. Rhizofilteration can be used for Pb^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, and Cr^{6+}, which are primarily retained within the roots [26]

Advantages

- either terrestrial or aquatic plants can be used. Although terrestrial plants require support, such as a floating platform, they generally remove more contaminants than aquatic plants. This system can be either *in situ* (floating rafts on ponds) or *ex situ* (an engineered tank system).
- an *ex situ* system can be placed anywhere because the treatment does not have to be at the original location of contamination [23].

Disadvantages

- the pH of the influent solution may have to be continually adjusted to obtain optimum metals uptake;
- a well-engineered system is required to control influent concentration and flow rate.

Phytovolatilization

Phytovolatilization involves the use of plants to take up contaminants from the soil, transforming them into volatile forms and transpiring them into the atmosphere. With this method, the contaminant may be transformed into a less toxic substance. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations. Phytovolatilization has been primarily used for the removal of mercury: Hg ion (Hg^{2+}) is transformed into less toxic elemental Hg^0 [27].

Advantages

- contaminants could be transformed to less-toxic forms, such as elemental mercury and dimethyl selenite gas;
- contaminants or metabolites released to the atmosphere might be subject to more effective or rapid natural degradation processes such as photodegradation [23].

Disadvantages

- the contaminant or a hazardous metabolite might accumulate in vegetation and be passed on in later products such as fruit or lumber;
- low levels of metabolites have been found in plant tissue [23].

<u>Phytodegradation</u>

In phytoremediation of organics, plant metabolism contributes to the contaminant depletion by transformation, break down, stabilisation or volatilisation of contaminants from soil and groundwater. Phytodegradation is the breakdown of organics, taken up by the plant to simpler molecules that are incorporated into the plant tissues [28]. Plants contain enzymes that can breakdown and convert ammunition wastes, chlorinated solvents such as trichloroethylene C_2HCl_3 and other herbicides. The enzymes are usually dehalogenases, oxygenases and reductases.

Advantages

- plants are able to grow in a sterile soil and also in a soil that has pollutant concentration levels that are toxic to microorganisms [23].

Disadvantages

- toxic intermediates or degradation products may be asked formally;
- the presence or identity of metabolites within a plant tissue might be difficult to determine; thus contaminant biodegradation could be difficult to confirm [23].

These mechanisms are briefly described in Table 2 [21].

Mechanism	**Description**
Phytoextraction	Plants absorb contaminants and store in above-ground shoots and the harvestable parts of roots.
Phytostabilization	Roots and their exudates immobilize contaminants through adsorption, accumulation, precipitation within the root zone, and thus prevent the spreading of contaminants
Phytodegradation	Plant enzymatic breakdown of organic contaminants, both internally and through secreted enzymes
Phytovolatilization	Contaminants taken up by the roots through the plants to the leaves and are volatized through stomata where gas exchange occurs

Rhizodegradation	Plant roots stimulate soil microbial communities in plant root zones to break down contaminants

Table 3 - Phytoremediation mechanisms for treatment of contaminated soils

The phytoremediation methods are often taking place in parallel in the same plant or can be combined by using specific crops simultaneously. However, phytoextraction is the prevailing method of phytoremediation used for treatment of heavy metal and aromatic hydrocarbons polluted soils. It ensures the complete removal of the pollutant. Normally microbes occurring in the soil around roots contribute to the biological removal of the pollutants. Combining both plants and microorganisms in treatments, the efficiency of the remediation increases. Both mycorrhizal fungi and other PGPR (Plant Growth-Promoting Rhizobacteria) have been successfully incorporated in various phytoremediation programmes [20]. In conclusion, the success of phytoextraction, as an environmental cleanup technology, depends on several factors including the extent of soil contamination, xenobiotic availability for uptake into roots (bioavailability), and plant ability to intercept, absorb, and accumulate pollutants in shoots [29].

3.1 BIOAVAILABILITY OF METALS IN SOIL

The following definitions for bioavailability were compiled in Technical Reports published by the EU Centre for Ecotoxicology and Toxicology of Chemicals and the United State National Research Council: I) *the ability of a substance to interact with the biosystem*; II) *the fraction of the contaminants in the environment that is potentially available for biological action*. In soil, metals are associated with several fractions: (1)the water phase, in soil solution as free metal ions and soluble metal complexes; (2) adsorbed to inorganic soil constituents at ion exchange sites; (3) bound to soil organic matter; (4) as precipitate such as insoluble oxides, hydroxides, and carbonates; and (5) embedded in structure of the silicate minerals. For phytoextraction to occur, contaminants must be bioavailable, i.e. available in the water phase where they can be absorbed by roots. Bioavailability depends on metal solubility in soil water phase. Only metals associated with fractions 1 and 2 (above) are readily available for plant uptake. Some metals, such as Zn and Cd, occur primarily in exchangeable, readily bioavailable form. Others, such as Pb, occur as soil precipitate, a significantly less bioavailable form. Soil pH affects not only metal bioavailability, but also the process of metal uptake into roots. In table 3 are summarized the concentrations of metals toxic for plant tissues [30].

Metal	Normal	Toxic
Zn^{2+}	20-100	> 200
Cu^{2+}	5-15	> 25
Ni^{2+}	15-25	> 50

Table 4 - Cu, Ni and Zn levels in plants [$mg.kg^{-1}$ dried plant matter]

In plants, the most apparent symptom of Cd toxicity is the chlorosis of leaves. Cd is suggested to destruct chlorophyll by replacing Mg in chlorophylls, suppress enzymes of the chlorophyll biosynthesis pathway, and damage the structure of chloroplasts, as a result, leading to chlorosis of leaves. Thus, the pigment contents should be indicators of damages to photosynthetic system induced by Cd [31].

3.2 BIOAVAILABILITY OF AROMATIC COMPOUNDS IN SOIL

Polycyclic aromatic hydrocarbons (PAHs) are the world's largest class of carcinogens known to date, not only because of their ability to cause gene mutation and cancer, but due to their persistency in the environment. They are particularly recalcitrant due to their high molecular weight, hydrophobic nature and thus, accumulate in various matrices in the environment. Many factors have been known to affect PAH bioavailability, which are:

- Physical and chemical properties of PAHs;
- Aging of PAHs contamination in soil;
- Soil properties (soil organic matter, dissolved organic matter, moisture content, etc.).

Bioavailability is influenced by the molecular weight (MW) and size of PAHs molecules. LMW PAHs are removed faster by physico-chemical and biological processes due to their higher water solubility, volatility and the ability of many microorganisms to use them as sole carbon sources in comparison to the HMW PAHs. Bioavailability changes with time and weathering. Aging is a central part concerning availability and refers to the process of organic compounds in soil becoming less susceptible to degradation and extraction.

3.3 HOW PLANTS UPTAKE POLLUTANTS

To grow and complete the life cycle, plants must acquire not only macronutrients (N, P, K, S, Ca, and Mg), but also essential micronutrients (such as Fe, Zn, Mn, Ni, Cu, and Mo). Metal movement, across biological membranes, is mediated by proteins with transport functions, and it is an energy consuming process. The uptake mechanism, of both metals and organic compounds, is selective, depending upon the structure and properties of membrane transporters.

In non-accumulating plants, accumulation of these micronutrients does not exceed their metabolic needs (<10ppm). In contrast, metal hyperaccumulating plants can accumulate exceptionally high amounts of metals (in the order of thousands of ppm). Hyperaccumulating plants do not only accumulate high levels of essential micronutrients, but can also absorb significant amounts of nonessential metals, such as Cd and Pb. Accumulating species have evolved specific mechanisms for bioaccumulation of extremely high concentration of metals. However, for phytoextraction to occur, metals must also be transported from the root to the shoot. Movement of metal-containing sap from the root to the shoot, termed *translocation*, is primarily controlled by two processes: I) hydraulic gradient and II) leaf transpiration. From the rhizosphere, water is absorbed by roots to replace water transpired by leaves. Water uptake from rhizosphere creates a hydraulic gradient directed from the bulk soil to the root surface. Some ions are absorbed by roots faster than the rate of supply via mass flow. Thus, a depleted zone is created in soil immediately adjacent to the root. The storage of certain conjugates in plant organelles such as vacuoles is a common mechanism, whereupon the compound is no longer capable of interfering with cell function. The xenobiotic conjugates can also be incorporated into biopolymers such as lignin, where they are characterized as non-extractable/bound residues, whereas in aquatic/wetland plants they can be excreted for storage outside the plant.

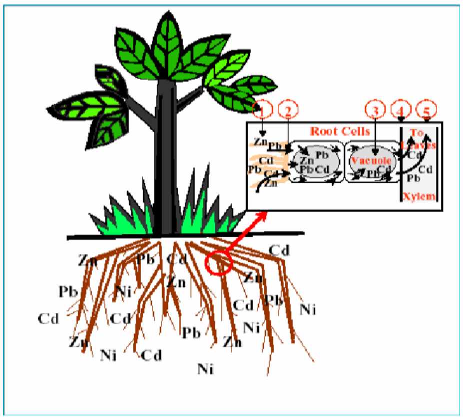

Figure 10 - metal uptake and accumulation: 1) A metal fraction is sorbed at root surface. 2) Bioavailable metal moves across cellular membrane into root cells. 3) A fraction of the metal absorbed into roots is immobilized in the vacuole. 4) Intracellular mobile metal crosses cellular membranes into root vascular tissue (xylem). 5) Metal is translocated from the root to aerial tissues (stems and leaves). [32]

Subsequent to uptake into the root, three processes govern the movement of pollutants from the root into the xylem: (1) sequestration of metals inside the root cells, (2) symplastic transport into the stele and (3) the release of these xenobiotic compounds into the xylem. The transport from root to shoot has been observed to primarily take place through the xylem via a specialized membrane transport processes. This membrane provides a strong electrochemical gradient for the inward movement of the metal ions [25].

3.4 IMPORTANCE OF MYCORRHIZAE

Mycorrhizae are fungal associations between plant roots and beneficial fungi. The term mycorrhiza refers to the role of the fungus in the plant's rhizosphere, its root system. The fungi effectively extend the root area of plants and are extremely important to most wild plants. Mycorrhizae play important roles in plant nutrition, soil biology and soil chemistry. Chemically, the cell membrane chemistry of fungi differs from that of plants: they may secrete organic acids that dissolve or chelate many ions, or release them from minerals by ion exchange. Mycorrhizae are especially beneficial for the plant partner in nutrient-poor soils. Fungi also have been found to have a protective role for plants rooted in soils with high metal concentrations, such as acidic and contaminated soils. Mycorrhized plants are often more competitive and more tolerant of environmental stress than non-mycorrhized plants thanks to the possibility of:

- acquiring nutrients present in forms normally not available for plants (for example N in organic compounds), and accumulation of them;
- the ability to reduce the presence of toxic phenolic compounds and toxic metals in the soil;
- protecting against water stress;
- protecting against parasitic fungi and nematodes;
- At the ecosystem level, all this positively affects:
- the cycles of nutrients; the microbial populations of the rhizosphere, through qualitative and quantitative modifications of the radical exudates;
- the soil structure, which is being improved;
- the primary and secondary succession of plant species. [33]

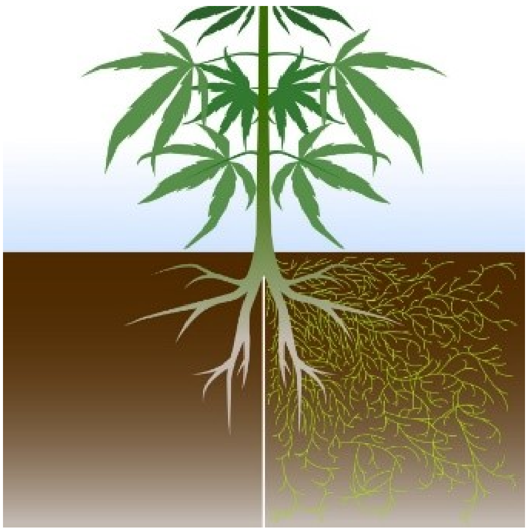

Figure 11 – effect of mycorrhization in cannabis root system

The impact of the fungal root symbiosis *Arbuscular Mycorrhiza* (AM) on PAHs degradation was assessed in France, at University of Nancy [34]. At the final harvest

(26 weeks), the amounts of PAHs extracted from nonplanted pots were higher than the initial concentrations. In parallel, in planted pots, PAH concentrations decreased as a function of proximity to roots. The most polluted soil showed higher initial PAH dissipation (25% during 13 weeks), but at the final harvest PAH concentrations had increased to values between the initial concentration and those at 13 weeks. Mycorrhiza enhanced PAHs dissipation when plant effects were observed [34].

The same occurred in an Italian experiment, performed with *Cannabis sativa* on a heavy metal polluted soil. The use of *Glomus mosseae* helped plants to phytoextract an higher percentage of metals (Cd, Ni, Cr) from root to shoot [35]. The possibility to increase metal accumulation in shoot is very interesting for phytoextraction purpose, since most high producing biomass plants, such as non-mycorrhized hemp, retain most heavy metals in roots, limiting their application [35].

3.5 MAJOR LIMITATIONS IN THE USE OF PHYTOTECHNOLOGIES

Phytoremediation technologies, however natural, still presents complexities, as well as limits: it requires long times and a deep knowledge of contaminants and plants capable of eliminating them. Furthermore, the selected specie has to be kept alive in the face of the high quantity of contaminants contained in the soil to be reclaimed. This shows that this remediation technique cannot be improvised, because a detailed evaluation of the soil supported by specific laboratory analysis is necessary. Some other limits can be:

- Root depth: The range of action on the contaminant depends on the extension of the root system of each plant;
- Applicability: The application of phytotechnologies is often limited to sites with medium-low contamination levels;
- Duration of treatment: The application of phytotechnologies is relatively slow, compared to other remediation processes;

- Seasonality: The efficiency of deciduous plants is drastically reduced during the non-vegetative period;
- Potential contamination of the trophic chain: There is the possibility that the contaminant enters the trophic chain through the ingestion of plant tissues by animals.

In this context, *Cannabis sativa* can be chosen, demonstrating its phytoremediation potential (Cap. 4) and its cost-effective benefits in the re-valorization of biomass (Cap. 5).

With appropriate measures it is possible to improve the effectiveness of the physiological mechanisms of treatment. Generally the impact on public opinion is favourable as in most cases phytorimediom is a passive, silent, aesthetically pleasing and ecological technique. Costs can be competitive, and less maintenance is required. It is probably the most economically and technically feasible approach for widespread contamination over a large area. The use of phytotechnologies requires careful planning, providing for integration with other techniques in order to guarantee compliance with CSR / CSC in short-term exposure risk situations, especially in the presence of high concentrations of rapidly spreading contaminants [36].

The effect of phytotechnology is twofold: i) remedying ecological crimes by safeguarding the health of environment and humans; ii) and as a possibility for economic development starting from critical conditions so that those lands that are now unusable for agri-food use can be converted into biomass production for industrial use [37]. In this framework, the Ministerial Decree, nr. 46 of March 1st 2019, plays an important role regulating remediation, environmental restoration and safety, emergency, operational and permanent interventions of the areas destined for agricultural production and livestock farming, pursuant to Article 241 of Legislative Decree April 3rd 2006, n. 152. (19G00052) [38].

For this reasons, the phytomanagement is recognised as an effective approach to carry out a risk management strategy [39]. Specifically, phytomanagement relies on the choice of suitable plant species for the purpose of the management of the contaminated sites [39]. Among polluted sites, agricultural areas represent a particular concern for safe food production [39], forcing local administrations to restrict their exploitation with relevant loss of income for farmers. Because of its biological characteristics, such as rapid growth, high biomass production, wide root system, high genetic variability, remarkable ability to adapt to different environmental conditions, and low susceptibility to disease and pests, hemp (*Cannabis sativa L.*) is a plant species of notable interest for phytomanagement [39].

4. HOW HEMP CAN PHYTOREMEDIATE

In this chapter will be briefly described how *Cannabis sativa* can help in order to bioremediate contaminated soils. As seen in chapter 3, the phytoremediation process is a combination of different mechanisms (phytoextraction, phytostabilization, phytodegradation, phytovolatilization, and rhizodegradation) often occurring synchronously, taking place in parallel in the same plant. Hemp is recognized as one of the plants that could be used for land reclamation due to its intense and fast growth, its dense root system and for the high yielding fiber varieties [40].

4.2 RECOVERY OF HEAVY METALS

The study about hemp ability in soil restoration began in 1998 when the Phytotech company, along with Ukraine's Institute of Bast Crops, planted Cannabis sativa for the purpose of removing contaminants (radioactive Cesium-137 and Strontium-90) near the Chernobyl site, a 10 km radius around the reactor. *Cannabis s.* provided to be one of the best phyto-remediative plants tested at the site. In 2001, a team of German researchers confirmed the Chernobyl results by showing that hemp was able to extract Lead, Cadmium and Nickel (Pb^{2+}, Cd^{2+}, Ni^{2+}) from a plot of land contaminated with sewage sludge. In 2009, scientists from Belarus also experimented with hemp in areas polluted by Chernobyl. The disaster contaminated nearly 30 km around the site. The Belarusian scientists noted that one added benefit of industrial hemp over other phytoremediation plants is that it can also be used to produce biofuel, potentially adding a second use for the crop after it removes toxins from the soil.

Hemp phytoremediation has also been used in Italy to clean up the city of Taranto (Puglia), where the ILVA steel plant has been leaking dioxin into the air and soil (since 2017, +917%, from 0.77pg up to 7.06pg in just 1 year) [41]. According to press reports (Espresso weekly magazine of 5 April 2007), as far back as 2002 the ILVA steel plant in Taranto — in breach of European standards for dioxin emissions, according to data from EPER, the European Pollution Emission Register

— produced 71.4 grams of dioxin, or 32.1 % of all the polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofuran (PCDF) produced by industry in Italy [42]. The project CANAPA, promoted by *Canapuglia* in 2014 and designed together with the ABAP (Associazioni Biologi Ambientalisti Pugliesi) and the CREA (Consiglio per la Ricerca in agricoltura e l'analisi dell'Economia Agraria), planned to create a "green belt", consisting of hemp crops, around the industrial pole. Inspired by the experience of Taranto, the CANOPAES project, in Sardinia (IT), promoted by the Region with an appropriate law since 2015, for the urgent reclamation of the former industrial and mining areas of Sulcis Iglesiente, is currently underway. Since then, 150.000 € have been allocated each year for experimentation activities, until today. In the laboratory, the absorption mechanisms of plants are being carefully evaluated, starting from the roots. In fact, hemp has proven to be capable of growing in soils highly polluted by metals and "remedying" Cadmium, Chromium, Nickel, Lead, Copper and Zinc.

4.2.1 MATERIALS AND METHODS

Few documents were found in the literature about field scale applications of the plant, but the results indicate great potential for the approach. The conditions of soil and climate of the polluted land have a key role in the process rate and efficiency, and they have to be:

- in the average temperature: 25.9±3.8°C (daytime) and 21.7±3.6°C (night) in Huaibei (China) [31], while min 11-20°C, max 18-32°C [43], in the Italian experiment conducted in the Botanical Garden in Milan;
- relative humidity: 61.5±5.3% (daytime) and 67.4±6.9% (night) [31];
- soil type and pH: gravel blackland (sand/silt/clay: 23.8%:20.3%:55.9%), which is characterized as pH 7.24, organic matter 1.12%; electrical conductivity 23.6 μs cms^{-1}; Cd 0.126 mg kg^{-1} [31]; organic C = 20% (wt% on dry product), Organic N = 1%, organic matter = 35%, pH: 6–7 [43].

The Chinese study was focused on the evaluation of Cd tolerance and accumulation, and there were used 18 different hemp cultivars, also for identifying the best species for bioremediation use. The experiment was performed in laboratory scale, where 25 mg Cd^{+} kg^{-1} soil (DW) were added into pots. The results showed that almost all cultivars were able to cope with 25 mg Cd^{+} kg^{-1} soil stress with the biomass reduction less than 50% [31]. By contrast, the plant's species with high biomass were more tolerant to Cd stress than the low-biomass ones. Therefore, these species are good candidates for biodiesel production by cultivating hemp in Cd-contaminated soils. It was found that some cultivars, such as Longxi and Lu'an, had higher total Cd in shoot (13.74 and 12.77 μg $plant^{-1}$ DW, respectively), because of their high biomass. These cultivars are still showing great potentials for phytoextraction combined with bioenergy production. Some others, such as Yunma 1, Yunma 2, Yunma 3, Yunma 4, Xingtai, Qujing, Lu'an, and Longxi, exhibited high total Cd in roots, reach up to 17.2–34.1 μg plant−1 (DW). Considering the plants of hemp are able to develop extensive root systems and result in a high root biomass, it was concluded that these hemp species may be more suitable for phytostabilization. The low Cd concentration in shoot of these plants indicates a less risk of Cd entering the food chain if the plants are consumed [24]. In table 4 are summarized the 2 plant species with the highest Cd content and total Cd in plant tissues, after 45 days [31].

	Cd content in tissues (μg g^{-1})		Total Cd in tissues (μg g^{-1})	
Cultivar	**shoot**	**root**	**shoot**	**root**
LX Longxi	22.8±1.1	249±21	13.74±0.82	17.2±2
U31 USO-31	33.3±1.1	481±12	1.69±0.07	4.7±0.5

Table 5 - Cd content and total Cd in plant tissues [31]

In the Botanical Garden in Milan, the experiment was performed always in controlled conditions, but with different concentrations of pollutants: soil treatment S1 (25 μg g^{-1} Cr + 25 μg g^{-1} Ni + 25 μg g^{-1} Cd), while soil treatment S2 (50 μg g^{-1} Cr + 100 μg g^{-1} Ni + 100 μg g^{-1} Cd). The growth rate of plants grown in S1 soil was not significantly different from that of plants grown in control (CTR) soil. On the contrary, a slight slowdown in development and a slight late ripeness were observed for plants grown in S2 soil containing higher heavy metal concentrations. Control and S1 plants reached the ripeness about 128 day after germination whereas most of S2 plants one week later. In table 5 are reported the concentrations of cadmium, nickel and chromium in the leaves, stems and roots of ripe plants grown in control (CTR) and contaminated soils (S1, S2) [43].

Soil	Leaf	Stem	Root
		Cadmium (μg g^{-1})	
CTR	0.3±0.2	0.4±0.4	0.6±0.4
S1	18.1±8	9.4±5	109±65
S2	58.8±26.9	73±44	1368±1106
		Nickel (μg g^{-1})	
CTR	2.7±0.9	nd	3.4±3.1
S1	7.1±1.4	14±3.9	35.8±18.9
S2	31.4±8.1	52±13	322±242
		Chromium (μg g^{-1})	
CTR	1.8±0.8	nd	2.3±2
S1	1.4±0.8	nd	6.2±4.2
S2	1.2±0.8	nd	9±9.9

Table 6 - Heavy metal concentrations in ripe hemp organs (μg g^{-1} dry matter). Mean values ± standard deviations [43]

Most of the Cd was accumulated in roots and only 14 and 66 μg were found in shoots of S1 and S2 plants. Cd was about 3 times more concentrated in S2 than in S1 soil. Only 10% of all the Cd found in plants was transported to the shoot of the S2 plant compared to the 24% calculated for the shoot of the S1 plant. Cr is not readily bioavailable for plants: one hypothesis is that Cr uptake can be limited by the presence of other soil metals competing for the plant transport systems.

According to the authors "the order of accumulation by *Cannabis sativa* was Cd>Ni>Cr and hemp cannot be considered a hyperaccumulator plant but a metal tolerant organism that has evolved mechanisms allowing it to cope with high metal concentration in soil". For Ni, the concentration relation was leaves > seeds > hurds > fibres, whereas it was leaves > fibres > hurds > seeds for Pb. For Cd, the relation was leaves > seeds > fibres=hurds [44]. Since hemp harvesting can be carried out by cutting the plants 2–3 cm above the ground or by pulling up the entire plant, the total uptake was also assessed considering the mean metal concentration of the entire plant and the entire plant harvest. A big difference between the amounts of the metals extracted can be observed when only the shoots or the entire plants are harvested. In fact, the experiments showed that hemp accumulates most of the metals in its root system.

Cannabis has been also applied under field conditions for the phytoremediation of metals contaminated site near Kohi Noor Textile mills in Rawalpindi, Pakistan. The metals occurring in the industrial area were Pb, Zn, Cu, Co, Ni, Cr, and Cd [45]. *Cannabis* leaves a final treatment displayed a higher concentration of Cu (1530mg kg^{-1}), Cd (151mg kg^{-1}), and Ni (123mg kg^{-1}), whereas all other heavy metals were not significantly adsorbed (table 6) [45].

Heavy metal	leaves
Cd	151±19
Pb	39±5
Cu	1530±19
Zn	4.5±1.2
Ni	123±13.2
Cr	25.3±1.75

Table 7 - Accumulation of heavy metals in leaves of Hemp plant in mg kg^{-1}

Cannabis sativa has been also adopted in a preliminary study in Italy, in Lazio region, in the so-called “Valle del Sacco”. This is a site of National Interest contaminated by metals due to agricultural activities. The experiment was performed on the actual soil but in microcosms established with the soil under controlled greenhouse conditions where the soil was seeded to allow the plant to grow; the plants were harvested at the end of the incubation. In Table 6, the contamination plant components after 90 days of growth on the soil [39] are reported.

	As	Pb	Zn	V
Sandy loam	22.6	115	92.8	106.7
Leaves	nd	nd	35.2	nd
Stem	nd	nd	9.5	nd
Roots	0.8	3.6	14.1	4.1
Inflorescence	nd	nd	62.7	nd

Table 8 - metal content in the organs of hemp. Values in ppm. nd= not detected.

Cannabis did display the ability to extract toxic metals (As, Pb, V), which are not appearing in the vascular tissues [39]. On the other hand, Zn was taken up in all above-ground parts of the plant. This investigation indicated that hemp plant cultivation could not efficiently remediate heavy metal contaminated soils [39].

In 2015, an area of 0.25 close to Taranto (Italy), on a site used for many years as disposal site for batteries, electronic devices and metal waste by the Italian Navy, other phytoremediation experiments were performed. In table 7, data on hemp effects in the removal of heavy metals from the polluted soil are reported [46].

Metal		Initial	Final
Cd	Total	0.2	0.2
	Extractable	0.1	0.1
Cu	Total	38.3	34.7
	Extractable	10.1	9.5
Ni	Total	31.9	28.1
	Extractable	0.4	0.2
Pb	Total	220.9	188.3
	Extractable	40	21.4
Zn	Total	131.7	107.5
	Extractable	41.3	36.5

Table 9 - total and extractable concentrations of metals in soil at the beginning and the end of the trial

Data suggest a very slow restoration of heavy metal contaminated soil by hemp. On the other hand, compared to many wild plant species tested for phytoremediation purposes, hemp has a greater adaptability to different soil and climatic conditions. It has deep roots, is an industrial crop with multiple non-food uses and is an excellent rotation and companion crop for improving soil quality [43].

4.3 RECOVERY OF AROMATIC COMPOUNDS

Polycyclic Aromatic Hydrocarbons (PAHs) can result from the incomplete combustion of organic matter. PAHs can also be produced geologically when organic sediments are chemically transformed into fossil fuels such as oil and coal. PAHs are considered ubiquitous in the environment and can be formed from either natural or manmade combustion sources. The dominant sources of PAHs in the environment are thus from human activities: wood-burning and combustion of other biofuels such as dung or crop residues contribute more than half of annual global PAH emissions, particularly due to biofuel use in India and China. They are often the most hazardous components of oil spills. According to NOAA (National Oceanic and Atmospheric Administration), there are two things which harm the environment and wildlife when an oil spill occurs: i) the first is the toxic oil itself. When swallowed by fish and wildlife, or when the ecosystem is coated with it, the oil is deadly. For some wildlife, the coating of oil can restrict movement, and for other wildlife, it can clog breathing passages; ii) the second is the chemical agent(s) used to 'help clean up' the oil spill. For example, the product Corexit is billed by oil companies as being useful in cleaning up the oil spills. However, chemicals like Corexit just make the environmental problem worse. Hemp is a versatile plant: not only can it be used to replace fossil fuel, it can also be used to help clean up the mess that goes along with fossil fuel related disasters. There is one big reasons why hemp isn't already being used to help with the problem: money. It is more expensive to produce the hemp than the chemical agents. Essentially, it would take a tremendous amount of core fibers from hemp to be able to clean up some of these large oil spills. There is an old saying that "*when an oil spill occurs, it's a disaster. But when a hemp bio-fuel spill occurs, it's fertilizer*". Several PAHs are considered to be carcinogenic, including chrysene ($C_{18}H_{12}$) classified as very weakly active and benzo[a]pyrene ($C_{20}H_{12}$) classified as very active. They have been found to be present in many industrial sites such as manufactured gas plants and harbor sediments. Being products of incomplete combustion of hydrocarbon fuels, PAHs are present in many types of foods and smoke flavoring agents.

4.3.1 MATERIALS AND METHODS

Also in this case, it was very difficult to find articles of already performed tests in field or laboratory conditions. The article presented the recovery yield of *Cannabis sativa L* on a land contaminated by aromatic compounds containing Benzo[a]pyrene and Chrysene [47]. The work focused on the phytoremediation of laboratory-contaminated soil with benzo[a]pyrene and chrysene with a unique crop, industrial hemp (*Cannabis sativa L*). All the experimental analysis were performed in Honolulu. Hawaii presents a unique growing environment for industrial hemp: lack of cold winter season and high levels of sunlight throughout the year enable the maturation of hemp at a very high rate. Thus, the plants can go through several flower cycles in one year. The soil used in this study is a silty clay, with irregular size and shape of the clumps. Several batches of spiked soil were prepared at different levels: 25, 50, 75, 100, and 200 μg/g.

The standards chrysene (98%) and benzo[a]pyrene (98%) were purchased from Aldrich (Milwaukee, Wisconsin) and Sigma (St. Louis, Missouri) respectively. After 60 days the average recovery of chrysene was 80 ± 14%, while the average recovery of benzo[a]pyrene was between 79 and 86% for the two soils after 1-day aging or 60 aging. So, hemp can tolerate high levels of chrysene and benzo[a]pyrene. It is noted that at least 50% of all the planted pots showed a large base root ball at harvesting time, some pots even becoming root bound. This suggests that the pot size may restrict plant growth at the later stage of the experiment. Table 5, below, shows the degradation of benzo[a]pyrene and chrysene [47].

Initial Concentration [μg/g]	Benzo[a]pyrene [μg/g]		Chrysene [μg/g]	
	control (no plant)	treated	control (no plant)	treated
25[a]	22±1	18±2	10±1	9±1
50[a]	22±1	23±3	15±1	13±1
75[a]	44±5	41±1	118±5	150±3
50[b]	32	28	35	35
100[b]	104	78	70	78
200[b]	216	133	212	174

Table 10 - degradation of benzo[a]pyrene and chrysene [47]

[a]Plants harvested after 45 days.

[b]Plants harvested after 49 days.

The control plants were not damaged during the experiment and their data are averaged. The analysis of the soil samples after 49 days of growth indicated a general decrease of the concentration of both PAHs. The largest decrease of benzo[a]pyrene concentrations was observed for the 200 μg/g benzo[a]pyrene-contaminated soil treatment: 133 μg/g compared with 216 μg/g for the average of control pot (contaminated soil-no plant). Table 5 also shows the results of the soil analysis after 45 days. The levels of PAHs were greatly reduced in the control pots as well as in the planted pots. However, the concentrations of chrysene for the 75 μg/g set were 118 μg/g and 150 μg/g respectively. This may be due to uneven fortification and distribution of chrysene in the pots for that concentration. However, the standard deviations for the entire set of data, including controls and treatment samples, varied from 1 to 5% with an average of 2%. The relatively small difference of the PAH levels between pots with and without hemp after 45 days indicates that degradation of chrysene and benzo[a]pyrene take place in watered soil regardless of the presence of hemp. This study showed that industrial hemp (*Cannabis sativa*) has a very high tolerance to benzo[a]pyrene and chrysene, but it does not completely extract and remove pollutants from the contaminated area. Hemp would be a prime candidate for remediation of PAHs-contaminated tropical areas (due to the higher growth rate than in other areas of the Earth), or be a candidate having high tolerance to PAHs for research interests, due to the fact that it grows in such environment [47].

Cannabis sativa has been also used in 2005 by German researchers in order to bioremediate a soil - in a controlled environment - contaminated with PHCs (Petrol Hydrocarbons 23200 mg kg^{-1}) and PAHs (Polycyclic Aromatic Hydrocarbons 2194 mg kg^{-1}). In table 10, concentrations at beginning and at the end (after 68 days) of the experiment are summarized for hemp specie [48].

	Conc. 0 days	Conc. 68 days
PHC	687	215
PAH	2203.4	1544.8
Naphthalene	55.5	22.5
Benzo(b)fluoranthene	78.5	65.6
Benzo(a)pyrene	94.5	76.8
Dibenzo(ah)anthracene	19.1	16.4

Table 11 - Concentrations of contaminants in unplanted petrol hydrocarbon-polluted (PHC) soil and PHC soil planted with field crops. Values in mg kg^{-1} [48]

Hemp has fostered the biodegradation of petrol hydrocarbons reaching final TPH concentrations that were 17.9% and 16.4% lower than in unplanted soil [48], and also stimulated PAH removal from soil so that final TPAH concentrations were 17.6% and 26.9% lower than in bare soil [48]. The ability of *Cannabis s.* plants to increase both the total number of bacteria and the abundance of aromatic ring-cleaving bacteria in its root zone has certainly contributed to the phytoremediation effect [48]. Bacteria are the major players in the degradation of aliphatic and aromatic hydrocarbon contaminants → RHIZODEGRADATION [23, 49].

Finally, it can be concluded that *Cannabis sativa L.* is a very good crop for the remediation of contaminated sites, both for heavy metals and PAHs. Obviously, the rate of capturing xenobiotic compounds also depends by plant species: hemp for fiber better allocates pollutants in roots or leaves, while hemp for oil does it inside seeds. Moreover, studying the quoted papers, a relevant problem is emerged: none of those researches has investigated how the biomass coming from phytoremediation process can be valorized. Studies addressed to such an objective have been reviewed in the following sessions.

5. INTRODUCTION TO RE-VALORIZATION

Cannabis sativa L., as stated in previous chapter (4), is promising tool for accelerating the removal of persistent organic pollutants, such as PAHs from long-term contaminated soils, as well as heavy metals accumulated due to industrial activities. Hemp cultivations produce huge amount of biomass (depending on climate conditions: for instance in Mediterranean environment the yield of hemp biomass is roughly 13 ton/ha DM) that has to be processed in order to obtain useful products.

The fate of such biomass is the center of interest of this study, since a circular approach to economy must be adopted to achieve sustainable development. If this is possible, biomass, previously just considered as waste, will get extremely value.

This will be explained in detail in the following chapters.

5.1 BASICS OF CIRCULAR ECONOMY

Unlike the traditional linear economic model, based on a *'take-make-consume-throw away'* pattern, a circular economy is based on sharing, leasing, reuse, repair, refurbishment and recycling, in an (almost) closed loop, where products and materials are highly valued. This production and consumption model is based on two complementary loops:

- one for “biological” materials (which can be decomposed by living organisms);
- one for “technical” materials (which cannot be decomposed by living organisms).

In both cases, the aim is to limit the leakage of resources as much as possible.

In practice, a circular economy implies reducing waste to a minimum. When a product reaches the end of its life (EOL), its materials are kept within the economy wherever possible. These can be productively used again and again, thereby creating further value. The amount of waste generated in the European Union (EU) appears to be declining. According to the European Environment Agency (EEA), between 2004 and 2012 waste generation from manufacturing and services sectors in the EU-28 and Norway declined by 25% and 23% respectively, despite respective increases of 7% and 13% in sectoral economic output.

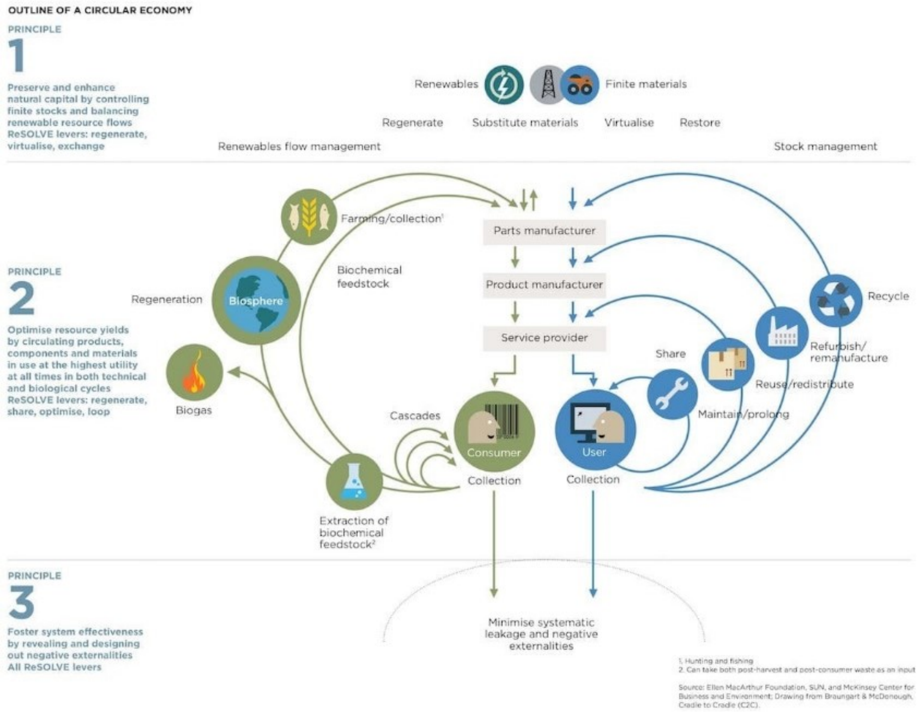

Figure 12 - Outline of a circular economy [50]

Moving towards, a more circular economy has both an environmental and economic rationale.

Potential opportunities include:

- Reduced pressures on the environment: a circular economy would significantly reduce greenhouse gases (GHGs) emissions through better waste management and reduced use of resources (such as energy, water, land and materials) in manufacturing, with positive impacts on the climate.
- Enhanced security of supply of raw materials: a circular economy would mitigate risks associated with the supply of raw materials, such as price volatility, availability and import dependency.
- Increased competitiveness: a circular economy could bring savings to businesses and consumers through improved resource efficiency.
- Innovation: a circular economy could trigger a large innovation drive across sectors of the economy because of the need to redesign materials and products for circular use.

- Growth and jobs: a circular economy could strengthen growth and create new jobs. It is estimated that the transition would increase GDP by 1 to 7 percentage points by 2030.

The European Commission put forward an initial circular economy package in July 2014, but withdrew the legislative proposal on waste included in the package in February 2015, in order to make way for new proposals. On 2 December 2015, the European Commission presented its new circular economy package containing a communication (action plan for the circular economy, together with a list of measures in annex) and four legislative proposals on EU waste policy:

1) the Waste Framework Directive (2008/98/EC);

2) the Landfill Directive (1999/31/EC);

3) the Packaging and Packaging Waste Directive (1994/62/EC);

4) the Directives on end-of-life vehicles (2000/53/EC), on batteries and accumulators (2006/66/EC), and on waste electrical and electronic equipment (2012/19/EU) [51].

The European Commission has adopted in March 2020 a new Circular Economy Action Plan, one of the main blocks of the European Green Deal, Europe's new agenda for sustainable growth. The new Action Plan announces initiatives along the entire life cycle of products, targeting for example their design, promoting circular economy processes, fostering sustainable consumption, and aiming to ensure that the resources used are kept in the EU economy for as long as possible [52].

The action plan for the circular economy aims to "close the loop" by complementing the measures contained in the legislative proposals and to contribute to meeting the United Nations Sustainable Development Goals (SDG), in particular Goal 12 on sustainable and responsible consumption and production. The action plan presents measures to a) make sustainable products the norm in the EU; b) empower consumers; c) focus on the sectors that use the most resources and where the potential for circularity is high (electronics and ICT, batteries, vehicles, packaging, textiles, construction, food); d) ensure less waste [52].

Figure 13- Sustainable Development Goals

5.2 HEMP PRODUCTS APPLICATIONS

An article published in Popular Mechanics in 1938 claimed hemp as the New Billion-Dollar Crop: "*American farmers are promised a new cash crop with an annual value of several hundred million dollars, all because a machine has been invented that solves a problem more than 6,000 years old*" [53]. Claiming to have thousands of potential applications, there seemed to be no limit on how and where hemp could be used. Yet, not even a year after this publication, the government passed restrictions on hemp that essentially killed the entire industry. For the next 80 years, hemp and its history has largely been forgotten and misunderstood.

As stated in chapter 1.1, hemp was widely used in any field of industry along centuries. Nowadays, thanks to technological improvements, better knowledge of the matter, further studies on its application in industry as well as for human consumption and medicine, hemp can be transformed in products that were unimaginable in 1938.

<u>Food and Drinks</u>

- Seeds: popular among vegetarians and athletes, easily digested by the body. Can sustain our dietary needs, even without consuming any other healthy food. Contains essential fatty acids (Omega-3 and Omega-6), helping with immune system and cholesterol levels [54].

- Seed oil: when the oil is unrefined, it is commonly considered "*Nature's most perfectly balanced oil*". It contains a perfectly balanced 3:1 ratio of Omega 6 and Omega 3 essential fatty acids. Great source for Vitamin A & E. Includes number of minerals such as calcium, magnesium, sulfur, potassium, phosphorus, iron, and zinc. Has amazing skin care properties [54].
- Protein powder: derived from seeds, hemp protein promises an average of 15 grams of protein per serving - that is the highest vegan source of simple protein available [55].
- Flour: contains 33% protein and is second only to soy in protein content, and is completely gluten-free [54].
- Hemp tea, coffee, healthy flavored water, milk, vodka, beer [54, 55, 56].
- Energy bars, burgers, hot dogs, granola [54].

<u>Clothing and Accessories</u>

- Shirt: it is typically a 60% Hemp / 40% Cotton mix. They are some of the most durable shirts [54, 56].
- Jeans, yoga pants, sunglasses, hats, wallets, backpack, totes, belts, scarfs, ties, bracelets, robes, shoes, sandals [54, 56].
- Jacket and coat: Hoodlamb offers a wide array of jackets and coats made from hemp. They are obviously not made with 100% hemp fiber, but using hemp allows them to make a more durable product while also being more sustainable than other brands [54].

<u>Beauty and Skin</u>

- Body lotion: free of any harmful chemicals, it can be used for eczema, psoriasis, contact dermatitis, and other auto-immune skin disorders, burns, bug bites, athlete's foot, ringworm, and dry-damaged skin [54, 56].
- Balms, lip balms, facial cream, cleanser, sunscreen [54, 55, 56].
- Shampoo, body wash [54, 56].

Health

- Hemp extract CBD: Dr. Raphael Mechoulam from Israel is the leading scientist who pioneered the research behind these extracts: "Cannabinoids have been found to have antioxidant properties, unrelated to NMDA receptor antagonism". This newfound property makes cannabinoids useful in the treatment and prophylaxis of wide variety of oxidation associated diseases, such as ischemic, age-related, inflammatory and autoimmune diseases. The cannabinoids are found to have particular application as neuroprotectants, for example in limiting neurological damage following ischemic insults, such as stroke and trauma, or in the treatment of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and HIV dementia [US Health and Human Services] [54].
- CBDP (cannabidiphorol): it is a recent discover from an Italian study (2019) guided by Dr. Cannazza [57]. Their current work is focused on testing its anti-inflammatory, anti-oxidant and anti-epileptic activity, which are typical of CBD.
- Essential oil, massage oil, aromatherapy candles, heat muscle rub, vape juice [54, 55, 56].

Pets

- Dog toys, dog collar, dog leash [54, 56].
- Animal bedding: hemp is extremely absorbent as it can hold 4X its own weight, and lasts much longer than pine or straw bedding. Hemp bedding is very low dust, which is great for horses with respiratory issues. It also reduces odour better than straw or wood shavings [54, 56].

Automobiles

In 1941, Henry Ford built a car out of hemp. It was not only built out of hemp plastic, but it also ran on hemp fuel. Hemp plastic was tested to be much stronger than steel: they tried denting the hemp car with a hammer, but the car didn't even budge.

- Sports car, thermoset compression molding: the process involves taking a sheet of the hemp fiber matting, injecting it with thermoset resin, placing into a mold, heating it to high temps for a set amount of time, then popping the panel out of the mold for cleanup and final drilling/cutting. Currently, Mercedes, BMW, and Audi are few of the auto companies that are using this in their vehicles.
- Biofuel. [54, 55, 56].

Home and Office

- Pens, hemp sheets, towels, curtains, laundry detergent, crafts, chairs, blankets, covers for mobile phone and tablet [54, 56].
- Paper: 0.4 hectares of hemp can produce as much paper as 1.6-4 hectares of trees over a 20 year cycle. Trees are made up of only 30% cellulose, requiring the use of toxic chemicals to remove the other 70%. Hemp, on the other hand, can have up to 85% cellulose content. Hemp paper is more durable than trees. Unlike regular paper, hemp paper does not get yellow, crack, or deteriorate over time. Wider use of hemp paper can help sustainability efforts to reduce deforestation [54, 55, 56].

Farming and Gardening

- Hemp growing mats: typical growing mats are made from plastics or treated materials [54].

Industrial and Others

- Ropes [54, 55, 56].
- Plastics: the biggest problem using hemp plastic commercially is that it is cost prohibitive. Since farming is still largely regulated, hemp is still in short supply. Yet, it is important to know that hemp plastic is much more durable and sustainable [54, 56].
- Hempcrete: building homes with hemp is one of the most promising applications of hemp. There are several benefits that hempcrete can offer. First, it acts an amazing thermodynamic insulator, so it helps to reduce the energy costs by 50–70% annually for families (especially those in extremely cold or hot climates).

Hempcrete also continuously absorbs CO_2 as it ages, so it reduces your carbon footprint. As it absorbs CO_2, the material actually becomes stronger. Essentially, a building made with hempcrete will become stronger over time. When it comes to construction, hempcrete homes have zero construction waste [54, 55, 56].

- Hemp batteries: hemp fibers have been found to be more conductive than graphene, making it a superior source for supercapacitors and batteries. Dr. D. Mitlin found that hemp "*works just as well as graphene. And it costs a fraction of the price at $500 to $1,000 a tonne*" [54]. In table 12 below are summarized all hemp industrial and commercial applications.

Food – Drinks	Seeds, oil, protein powder, flour, tea, coffee, milk, beer, energy bars, burgers, hot dog, granola
Clothing – Accessories	Shirt, jeans, coat, shoes, sandals, scarf, wallet, hat, belt, tie, bracelet, sunglasses, robe, tote, backpack
Beauty – Skin	Body lotion, balm, facial cream, cleanser, sunscreen, shampoo
Health	Essential/massage oil, aromatherapy candles, heat muscle rub, vape juice, Hemp extract CBD
Pets	Dog toys, dog collar, dog leash, animal bedding
Automobiles	Sports car, thermoset compression molding, biofuel
Home – Office	Pens, hemp sheets, towels, curtains, laundry detergent, crafts, chairs, blankets, covers for mobile phone/tablet, paper
Farming – Gardening	Growing mats
Industrial – Others	Rope, plastic, hempcrete, battery

Table 12 - industrial and commercial applications of hemp products

All these findings are not coming from one species of *Cannabis sativa* only but from both fiber and oil varieties. Tall plants are usually used for fiber production and derivates (e.g. Carmagnola var.); small varieties are instead used to obtain seeds, for oil and derivates production (e.g. Futura 75 var.).

6. *CANNABIS SATIVA* BIOMASS VALORIZATION

The aim of this research is to combine phytoremediation with a crop of commercial interest (*Cannabis sativa* L.), with the view of achieving low price decontamination of soil by the production of a commercially usable resources. Since the selected plant is capable of extract and immobilize pollutants (both heavy metals and aromatic xenobiotic compound), now the questions are:

1) Are the fiber material, from contaminated biomass suitable for commercial use? If so, how do the contaminants influence fiber quality?
2) Are biopolymers (PP, PLA, P(3HB), HDPE), produced from such contaminated biomass, good enough for industrial/medical purposes?
3) Can hemp be used for biofuel production? What about its quality?
4) Is burning for making energy the best solution?

6.1 FIBER

Hemp is a renewable resource which grows more quickly and easily than trees and this is making hemp suitable for the production of man-made fiber products. The bark of the hemp stalk contains bast fibers, which are among the earth's longest natural soft fibers, in addition to also rich in cellulose.

Alpha-cellulose	62-67%
Hemicellulose	8-15%
Lignin	4%
Ash	5%
Wax	1%

Table 13 - composition of hemp

Bast refers to the fibers which grow on the outside of the plant's stalk. Bast fibers give the plants strength. The processes required to produce hemp fibers are:

- <u>Retting</u>: harvesting is done with a conventional combine harvester machine. Once cut, the plants, which are composed of two types of fiber - long outer fibers suitable for textiles, and short inner fiber suitable for paper or industrial applications - are left in the field for about 10 to 20 days to ret.

Retting is of two types:

- *Water Retting*: it involves lying the stems in water tanks, ponds or in streams for around 10 days. It is more effective if the water is warm and bacteria-laden.
- *Dew Retting*: it is a natural process that is triggered by dew that falls on the crop each morning. After cutting, the hemp stems were laid parallel in rows to dew ret. The stems need turning at least once per day in order to allow bacteria and fungi to break down the Pectins that bind the fibers to the stem allowing the fiber to be released. Retting is complete when the fiber bundles appear white. Once this process is complete (dry), the stalks are collected and sent to the "decortication" machine.

- Decortication: it is described as breaking the stems by passing through fluted rollers.
- Softening and Cutting: by using a so-called hemp softener, the decorticated fibers are made softer and suppler. Then they are shortened from up to 3 m down to 0.5-0.6 m with a special cutting machine.
- Combing: the short and tangled fibers are combed out, the long fibers are parallelized and smoothed using a hackling machine. In other words "hackling" (combing) means to remove any woody particles and to further align the fibers into a continuous sliver for spinning.
- Spinning: the manufactured slivers are yarned according to quality and desired fineness. The rove produced is then boiled in caustic soda to refine it and most of the yarn is bleached with hydrogen peroxide.

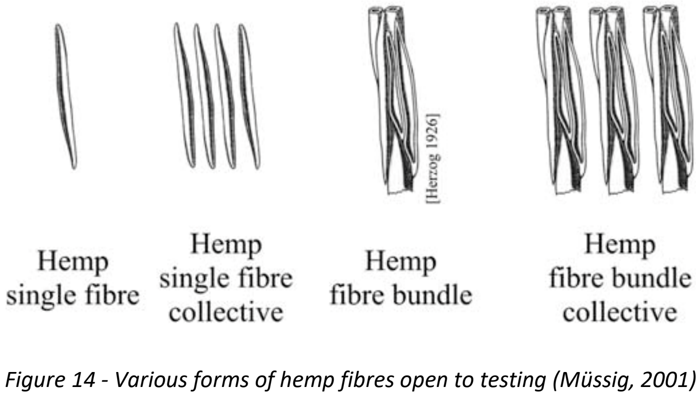

Figure 14 - Various forms of hemp fibres open to testing (Müssig, 2001)

If other processes are added to the previous ones, more products can be done starting from fibers: for instance compacting it can be obtained an insulation batt, grinding it can be obtained a supreme binder.
In the following table (12), products from hemp fiber are collected and compared.

	Hurds (100% hemp)	4.00€/kg
	Natural fiber for insulation (92% hemp, 8% polyester)	5.00€/kg
	Degummed Hemp Fiber (100% hemp)	33.00€/kg
	Degummed Hemp Sliver (100% hemp)	46.00€/kg
	Hemp batt for insulation (92% hemp, 8% polyester) 14x39x122 cm	20.00€/batt

Table 14 - hemp fiber products and related price(€) [58]

Mechanical extractable fiber content after decortication and mechanical separation is usually between 26% and 36%. It seems that heavy metal polluted soil has no significant influence on fiber properties. Heavy metals can be detected in all parts of the plants which are of commercial interest. The contribution of the relatively high metal concentrations found in the seeds to the total mass of metal in the plant is negligible, since the seeds contribute only 8–10% of the plant's total mass. The leaf content accounts for 13–15% w/w, fiber content 25–27% w/w, whereas the dominating part is the hurd content with about 60% w/w. These mass contribution may be slightly different depending on variety, season, and crop region. All this means that the plant's total metal accumulation will be in the hurds, but also in the leaves due to the capacity of *Cannabis* to uptake metal compounds up to the leaves. Further studies are needed for understanding the correlation of polluted hemp fibers and human life. To summarize the results of Linger study [44], hemp fibers were not significant influenced by heavy metal polluted soil. All results seemed to be in natural occurring range. Fibre bundle fineness and strength from the contaminated as well as the non-contaminated hemp are identical within the limits of experimental error. The fibre content was significantly lower in hemp grown on unpolluted soil, however, it is not possible to decide if this is only an effect of the contamination [44].

6.2 BIOPOLYMER (HEMP AS SOURCE OF ADDITIVES)

Every second more than 200 kg of plastic are dumped into the world's oceans and seas, which is equivalent to more than 8 million tonnes of plastic waste per year. Biodegradable or environmental acceptable materials have attained increasing interest over the past decades due to increase in environmental pollution from consumed petroleum materials. Biopolymers from various natural botanical resources such as starch, protein and cellulose have been regarded as alternative materials to conventional plastic because they are abundant, renewable, inexpensive, eco-friendly and biodegradable. Soy protein has been considered as a viable alternative to the petroleum based polymers [59]. The mechanical properties of soy protein plastic can be improved by extrusion cooking with plasticizer and or crosslinking agent such as zinc sulfate or formaldehyde, acetic anhydride. Fiber reinforcement will increase the scope of these materials and their application in various field such as automotive and packaging industries. Hemp fiber was used to reinforce soy based bioplastic to form composite for automotive interior applications [60]. The composites from soy protein plastic and natural fiber are green composites because both of them are biodegradable and environment acceptable. The use of these composites will reduce the dependence on petroleum products, save energy, bring benefit to environment and reduce environmental pollution from petroleum product. Biocomposites from soy based bioplastic and chopped industrial hemp fiber were fabricated using twin-screw extrusion and injection molding process. Soy based bioplastics were prepared through cooking with plasticizer and blending with biodegradable poly(ester amide). Mechanical-thermal properties and fracture surface morphology of the "green"/biocomposites were evaluated with universal testing system (UTS), dynamic mechanical analysis (DMA), Environmental Scanning Electron Microscopy (ESEM). It was found that the tensile strength and modulus, flexural strength and modulus, impact strength and heat deflection temperature of industrial hemp fiber reinforced biocomposites significantly improved [61]. For these reasons, it is easy to affirm that the improvement in mechanical properties is due to the hemp fiber contribution on their strength and modulus and fibrillation of fiber pullouts from matrix.

Another solution is represented by the biotechnologies focused on converting waste materials into useful biomaterials such as poly(3-hydroxybutyrate) P(3HB), starting from hemp hurd biomass. P(3HB), the best known member of the polyhydroxyalkanoates family (PHA), is an energy and/or carbon storing material synthesized and accumulated intracellularly by many microbial strains. It is characterized by its strength, hydrophobicity, inertness, relatively high melting point, optical purity, and thermoplastic process ability similar to those of polypropylene (PP). These properties have made P(3HB) an attractive green alternative to synthetic plastics. This significant advantage of being completely biocompatible and biodegradable within the blood and tissue of mammals has further exploited the high-value of P(3HB) for medical implants. In R. *eutropha*, microbial biosynthesis of P(3HB) starts with the condensation of two molecules of acetyl-CoA to give acetoacetyl-CoA, which is subsequently reduced to hydroxybutyryl-CoA: the carbon source for this process can be pretreated hemp biomass [62].

Thus, hemp biomass coming from contaminated land cultivations can be used for all these purposes. However packaging for food, sanitary implants in human body, and any other goods related with human contact and health cannot have fibers coming from such a biomass. Indeed they can deliver toxic compounds. And in general, the extracted xenobiotic compound cannot be re-put in the environmental and food chains. Maybe, if pollutants are properly immobilized, biopolymers could have important implementations in industrial uses, as well as in components for vehicles, houses and offices.

Figure 15 - hemp plant, pellets and bioplastic spoon

6.3 BIOFUEL

Road transport is one of the activities that consumes the greatest amount of fossil fuels. In 2017, the transport sector accounted for 31% of total final energy consumption in the EU Member States, followed by the households (27%), industry (25 %) and services (15 %) sectors [EEA]. Oil accounted for the largest share of total final energy consumption in the EU, with 37.2%, followed by electricity (22.7%), natural gas (22.6%), other fuels (15.1%) and solid fuels (2.5%) [EEA].

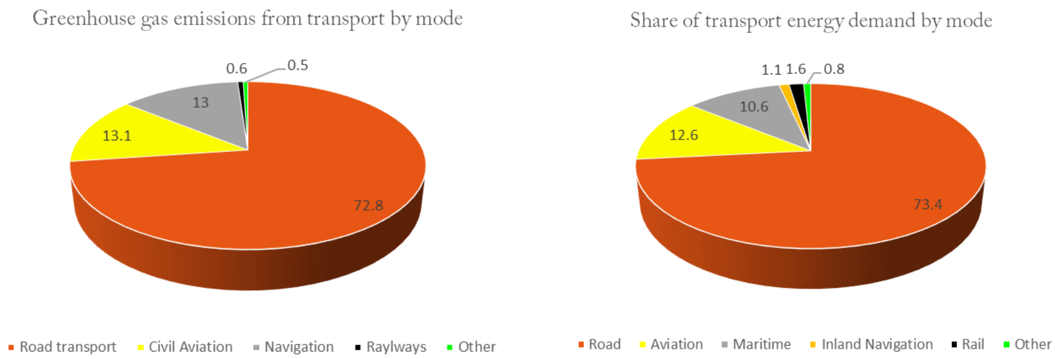

Figure 16 - Greenhouse gas emissions from transport mode (left), Share (%) of transport energy demand by mode (in 2014) [63]

It is necessary to look for alternatives in the form of liquid fuel which adapts best to the combustion engines of the vehicles and which overcomes the current energy dependency. Cultivating energy crops on arable land can decrease dependency on depleting fossil resources and it can mitigate climate change. In this sense, the so-called "RED" Directive (EU) 2015/1513 of the European Parliament and of the Council of 9 September 2015, amending Directive 98/70/EC relating to the quality of petrol and diesel fuels and amending Directive 2009/28/EC on the promotion of the use of energy from renewable sources, states, at point (7), that: "Liquid renewable fuels are likely to be required by the transport sector in order to reduce its greenhouse gas emissions" [64]. Moreover, it is also stated, at point (22), that "Indirect land-use change risks can occur if dedicated non-food crops, grown primarily for energy purposes, are grown on existing agricultural land which is used for the production of food and feed. Nonetheless, compared to food and feed crops, such dedicated crops grown primarily for energy purposes can have higher yields and the potential to contribute to the restoration of severely degraded and heavily contaminated land" [64]. Biodiesel is produced commercially from a variety of crops, mainly from Soybean Oil in the United States, Palm Oil is Southeast and East Asia and Rapeseed Oil in Europe. But some biofuel crops have bad environmental effects: they use too much water, displace people and create more emissions than they save. This has led to a demand for high-yielding energy crops with low environmental impact: industrial hemp is the solution. The quality of fuel depends on a range of characteristics such as heat value, specific gravity, flash point, sulfur content, viscosity, cloud point, pour point, oxidization stability, etc. The flash point is the minimum temperature calculated to a barometric pressure of 101.2 kPa at which the fuel will ignite under specific conditions. The flash point does not affect the combustion directly; higher values make fuels safer with regards to storage, fuel handling and transportation [65]. The cloud point is the temperature at which wax crystals begin to form in a petroleum product as it is cooled. The pour point is the lowest temperature at which a petroleum product will begin to flow. Viscosity is defined as the resistance of a fluid to gradual deformation by shear and tensile stress. Hemp is a cleaner fuel than soybean and rapeseed biodiesel fuels: this is demonstrated by a significantly lower sulphur content. It is also a safer fuel for handling, storage and transport due to its higher flash point. However hemp biodiesel performs poorly when it comes to its kinematic viscosity which is slightly higher than the European *EN 14214* maximum of 5 mm^2/s but still lower the American *ASTM D6751* maximum of 6 mm^2/s.

Property \ Biodiesel	Hemp	Soybean	Rapeseed	EN14241 requirements
Flash Point [°C]	162	138	96	101
Density [kg/m³]	864	875	882	860-900
Sulfur content [ppm]	0.4	1.1	2.4	10
Kinematic Viscosity at 40°C [mm2/s]	5.13	3.15	4.37	3.5-5
Cloud point [°C]	-4	0	-4.1	Max 0-2 for superior quality
Pour point	-	-	-12	-
Oxidation stability at 110°C [h]	poor	2.35	5.6	8

Table 15 - Properties of various biodiesel fuels [66]

Biodiesel can be blended with petroleum diesel at different percentages. The "*B*" factor is universally used to designate the percentage of biodiesel in the mix (National Biodiesel Board 2015). The most common of these blends are *B100*, *B20, B5* and *B2* which contain 100%, 20%, 5% and 2% respectively. Fuels are blended for various reasons such as environmental compliance. *B20* biodiesel blend is one of the most common blended fuels. It is popular because it represents a good balance of improved performance, lower emissions, materials compatibility, cost and its ability to act as a solvent. Additionally, biodiesel blends of 20% or lower do not require any modification to the diesel engine. Hemp performs well in biodiesel blends. In one comparative study, it is found that hemp *B20* blend provides better thermal efficiency, lower specific fuel consumption, reduced CO and CO_2 emissions in comparison to pure diesel. However, the hemp blend has a higher NO_x emission [67]. In comparison to agricultural lignocellulosic materials like corn stover and wheat straw, which have around 37% (dry mass) cellulose, cellulose content of hemp is relatively high (~75%). The presence of high cellulosic content as well as high biomass yield makes hemp a very potential crop for bioethanol production [68].

The oil content in seeds of hemp, which ranges from 26 to 38%, contains a larger amount of polyunsaturated fatty acids. It has been shown that hemp virgin oil can be converted into hemp biodiesel with a high product yield. Furthermore, hemp biodiesel meets the standards for biodiesel fuel set by *ASTM 6751-09*. The distinct properties of hemp biodiesel are its low cloud point and low kinematic viscosity. These promising cold flow properties make hemp biodiesel attractive and competitive [68].

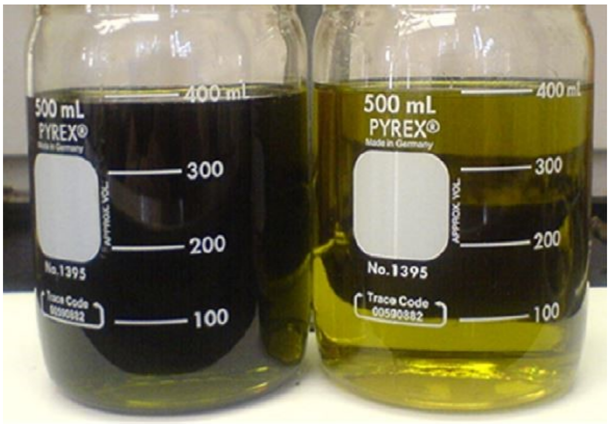

Figure 17 - Dark green liquid at the left hand side is hemp seed oil while the light green liquid at the right hand side is hemp biodiesel.

Nowadays, more effort has been spent on researches focusing on the synergy between hemp ability to phytoremediate polluted soil and related bioenergy production. The biomass produced during phytoremediation could be used for gaining economic benefits such as production of bioenergy (biogas, biohydrogen, bioethanol, biomethanol) [68]. *Cannabis sativa* specie with higher biomass yield (Longxi, Xingtai, Yunma 3, Qujing, Shuyang, Lu'an, Yunma 2, Yunma 1, and Yunma 4) is more tolerant to Cd stress than the low-biomass ones (USO-31, Shenyang, and Shengmu) [28]: therefore these plants are good candidates for remediating Cd-contaminated soils and then producing biodiesel [28]. Generally, pollution concentration in hemp's plant parts are low enough to allow production of a clean end-product (biofuel) [69].

6.4 PHYTOMINING

Phytomining is defined as the cultivation of a crop where the specific purpose is to process metal out of the harvested biomass for sale to the international market. Phytomining can therefore be considered as "farming for metals" and it is regarded as a "green" approach to the environmentally sensitive and energy intensive practice of mining, involving the use of plants to extract valuable metals from both solid and liquid substrates [70]. There are known hyperaccumulator plants for As, Cd, Co, Cu, Mn, Ni, Pb, Se and Zn, of the order of 700 species in all [71]. Phytomining was first described for nickel in 1994, by two scientists from the United States Department of Agriculture (Larry Nicks and Michael Chambers). These scientists grew the nickel accumulating plant *Streptanthus polygaloides* in plots of nickel-rich soil in California, and predicted that a nickel yield of about 100 kg of metal per hectare could be achieved. The trials were carried out at the US Bureau of Mines (Reno, Nevada) on a naturally occurring stand of *Streptanthus polygaloides*, which is a species known to hyperaccumulate nickel. The soil at the site contained about 0.35% nickel, below an economic concentration for conventional mining. It was proposed that a net return of \$513 ha^{-1} to the grower could be achieved, assuming that:

- A minimum of selective breeding produced plants with 1% nickel in dry mass;
- The world price of nickel was \$7.65 kg^{-1};
- The biomass yield after moderate fertilization was 10 t ha^{-1};
- A quarter of the energy of combustion of the biomass could be turned into electricity for a yield of \$131 ha^{-1};
- The return to the grower would be half of the gross yield of \$765 for the metal plus the energy yield of \$131 [70].

Another similar experiment was carried out in Tuscany, on the potential use of the hyperaccumulator *Alyssum bertolonii* in phytomining for nickel in nickel-rich ultramafic soils containing high concentrations of chromium, nickel and magnesium.

While there exists an extensive knowledge base of hyperaccumulating plants, phytomining, as a concept, has received only comparatively recent attention for three reasons:

- increased societal pressure resulting from the environmental performance of conventional mining techniques;
- the inability of current technologies to cost effectively recover metals from ores with low metal contents;
- high metal prices [72].

Phytomining is reported to be less intrusive than traditional mining techniques, which are energy and resource intensive, and require substantial site remediation at the end-of-life of the mine. In comparison, phytomining:

- requires reduced energy inputs;
- uses solar energy to generate a "bio-ore";
- improves the quality of the soil for post-mining applications over the duration of the phytomine.

Furthermore, there is potential to develop industrial synergies with related industries, e.g. by generating renewable energy during the combustion of the plant biomass during metal recovery. This further increases the profit ability and sustainability of phytomining [72]. In table 14 below are reported current price of some metals.

Name	Symbol	Price [USD $ per ton]
Aluminium	Al	1.495,75
Lead	Pb	1.610,85
Copper	Cu	5.249,50
Nickel	Ni	11.949,00
Zinc	Zn	1.997,15
Tin	Sn	15.241,00
Chromium	Cr	7.860,00
Cadmium	Cd	2.600,00

Table 16 - average metal prices (source: https://www.fastmarkets.com/commodities/exchange-data/lme-base-metal-prices-and-charts*)*

The energy content of biomass can be calculated by its heating value. The heating value can be determined in a bomb calorimeter, resulting in the so called higher heating value (HHV): the produced heat by combustion is determined by bringing all products of combustion back to the initial temperature of reactants (25°C), and condensing any produced vapor. HHV is a measure of the theoretical maximum energy to be derived from the biomass by any kind of thermal conversion. The heating value of biomass can also be calculated as lower heating value (LHV) on both a wet and dry basis. LHV is obtained by subtracting the heat of vaporization of the water from the HHV, i.e. treating water as vapor. LHV on a dry basis ($LHV_{d.b}$) shows the maximum energy per mass unit dry matter that can be derived, taking into account the energy needed for vaporisation of water in the biomass and water formed during thermal conversion, e.g. combustion. In table 15, the heating value of some crops are reported [73].

Cultivar	Heating value [MJ/kg]
Cannabis sativa	18.4-19.1
Zea mays	18
Helianthus tuberosus	16.5
Miscanthus sp.	19.8

Table 17 - Heating value of crop species in [MJ/kg]

For a given amount of dry matter, and varying moisture content (MC), $LHV_{d.b.}$ is preferred for calculations.

LHV can be determined empirically thanks the Dulong's Formula (only if an Ultimate Analysis is available).

- Dulong:

$$LHV_{w.b.} = (f_C - \frac{f_O}{2}) * Q_C + \frac{f_H}{2} * Q_H + f_N * Q_N + f_S * Q_S \quad \text{[kcal/kg]}$$

$$LHV_{d.b.} = LHV_{w.b.} * \frac{100}{100+MC} \quad \text{[kcal/kg]}$$

Q_i = -ΔH_i = Heat of Combustion [kcal/mol]

f_i = mass fraction of element i, in dry sample, calculated as:

$$f_i = \frac{wt\%_i}{100 * MW} * 1000$$

MW = molecular weight

Therefore, a low MC is favourable for this application, as the MC influences the energy yield as expressed by the LHV of the material. For hemp, the MC decreases with later harvesting date, increasing the energy yield. In parallel, biomass is lost, e.g. by leaf senescence, decreasing the energy yield. The two factors counterbalance each other, leading to the unchanged energy yield per hectare between September and April. Hemp as a solid biofuel has a number of viable handling and combustion technology options. Herbaceous fuels, such as straw, miscanthus, reed canary grass and industrial hemp, which are chopped to particulate fuels, have particle shapes and sizes which give them poor bulk handling characteristics, i.e. a low bulk density, poor flow properties and a high tendency to bridge over openings. Instead of such fuels being handled in bulk, they are often aggregated to bales or compacted to briquettes or pellets before further handling, transport and combustion. Fuel processing of hemp is likely to require baling of the biomass, since this is a proven, cost-effective system and because the bales function as plugs in the burner openings in some of the newer boilers built for operation on solely straw-like fuels. Use of hemp as a solid biofuel is feasible in several boiler systems. Firstly, hemp can be combusted in boilers built for woody biomass. These boilers are often based on fluidised bed technology, which is normally not suitable for straw due to the low IDT of straw [74]. Secondly, hemp can be combusted in boilers built for firing of straw-like solid biofuels, e.g. boilers with moving or vibrating grates [74]. The high flexibility of hemp applications as a solid biofuel may be attractive to both farmers and potential fuel customers [75]. Hemp also emits comparatively low sulfur compounds and has low ash content [73]. Thus, hemp has the potential to contribute toward renewable energy.

As stated in this chapter, *Cannabis sativa L.* has the potential to enter the production chain, even if the harvested biomass is coming from polluted soils. It can be used for fiber and biopolymer, but only in the case of goods which are not in direct contact with humans (like packaging for food, clothes, medical apparatus etc.); for bioenergy production: in this sense, hemp has the best potential in satisfying both environmental and economical purposes.

7. CONCLUSIONS

Despite phytoremediation is considered a consolidated technology, its use is still limited mainly due to socio-economical barriers. Its efficiency is well demonstrated in multiple applications as discussed in chapter 4 of this Master Thesis. Investigators as well as science administrators have strived to identify and address research needs in an effort to close knowledge gaps and make phytoremediation a more reliable cleanup technology. In order to guarantee their full exploitation, phytotechnologies are now being designed with an approach that looks at the whole process thus preventing from the production of unused waste. In this regard, a circular economy approach based on the re-valorization of feedstocks from contaminated areas would positively contribute to the overall remediation process and would overcome the socio-economic limits by creating virtuous value chains with high market potential. In this field, compared to many wild plant species tested both for phytoremediation and re-valorization purposes, hemp has a greater adaptability to different soils and climatic conditions and it also offer multiple valorization routes.

Briefly, *Cannabis sativa* has deep roots (1.5-2.5m) and produces high quantity of biomass (13 ton/ha) with multiple non-food uses and it displays an excellent rotation rate (depending on local climate) and a high capacity of improving soil quality. During the three month life cycle of the plant, the cannabis fixes carbon dioxide much more rapidly and extensively (10 ton CO_2/ha) than other trees, which makes it a very effective CO_2 scrubber. In addition, its agro-technology and the related biomass industrial processing and valorization are well-known and applied in many countries. In conclusion, the question to be answered is:

Could hemp, grown on contaminated soils, be used as a supplier of raw material for commercial applications?

Cannabis sativa L. showed high tolerance to xenobiotic compounds and also a good ability to extract them from contaminated soils. The high levels of toxic substances extracted by hemp during phytoremediation process would disqualify this plant to be used in food production. The fact that hemp potentially accumulates heavy metals in all plant parts may limit its use as a raw material in clothes as well as in the food chain. However, hemp oil could be utilized in other suitable applications, such as finishing industry or industrial oil production. There are cases in which the use of hemp fibres for clothes production would not be possible as the heavy metal concentrations exceed the Öko-Tex-Initiative (2000), which are 0.1 ppm for Cd, 0.2–1.0 ppm Pb and 1.0–4.0 ppm Ni. The main use of contaminated fibres and hurds might be in composite material, where the fibre are embedded in polymers and could not be set free. However, the high quality of the fibres and hurds, which were not affected by the heavy metal contamination, allows them to be used in special products like composite material. Another possible use of the plant material is for energy production in thermal power stations, thanks to its high heating value (19.1 MJ/kg), as here the plant material falls below the restrictions. In addition, it might be possible to collect and recycle the metals from the ash (phytomining). Also, *Cannabis s.* biomass coming from phytoremediation can be used for biofuel production. Its low sulfur compounds and low ash content makes hemp a more useful plant, in this sense, than conventional rapeseed or soybean. It is also a safer fuel for handling, storage and transport due to its higher flash point.

There is a need to optimize the agronomic practices to maximize the cleanup potential of the *Cannabis sativa L.* Since in many instances metal absorption in roots is limited by low solubility in soil solution, it is important to further investigate the use of chemical amendments to induce metal bioavailability. At the same time, and in order to guarantee a dedicated value chain set up, it is necessary to better characterize the biomass in terms of quality and presence of contaminants.

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