

Cd, Hg and Pb concentration of finnish lyophilized hemp (*Cannabis sativa* L.) juice

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Abstract

Different hemp (*Cannabis sativa* L.) secondary metabolite based supplement products are being increasingly used, sold and researched. So far the industry lacks a clear regulation for the contaminants of the end products. Hemp is efficient in accumulating heavy metals from the soil. This study shows the Cd, Hg and Pb concentrations of NatureDry[©] lyophilized FINOLA[®] hemp juice grown on fine-sandy moraine soil in central Finland to be negligible in contrast to recommended maximum daily dosages of each heavy metal.

Introduction

The resin which is biosynthesized mostly in the female inflorescence, is the most secondary metabolite condensed part of hemp (Marks et al., 2009; Kleinhenz et al., 2020) and is primarily used for producing supplemental products, which have been researched and produced with accelerating speed but the industry lacks clear regulation to the contaminant contents of the end products. One important class of contamination is heavy metals, which are innate ingredients of the earth's crust and are classified as essential heavy metals, which include Fe, Mn, Co, Cr and Mo, which are important for biological processes (Bhattacharya et al., 2016) and non-essentials include Hg, Pu, Cd and Pb (Bhattacharya et al., 2016; Kumar et al., 2017; Tchounwou et al., 2012), which are harmful even at low concentrations (Bhattacharya et al., 2016; Tchounwou et al., 2012).

Other than natural processes (Ismail et al., 2013; Jadia and Fulekar, 2008), the concentration of heavy metals are increased in the soil by human activities such as using heavy metal containing fertilizers, pesticides (Asgari et al., 2017; Feng et al., 2017) and ash (Nieminen et al., 2005). One of the biggest sources of heavy metal accumulation in the soil is phosphate fertilizers (Bolan et al., 2013; McPartland and McKernan, 2017). Phosphate ions are considered to be the main carrier of heavy metal contaminants and specifically hydroponic fertilizers have been found to have the highest tendency for contamination (Karadjov, 2014). Additionally different manures have been proven to be a source of heavy metal contamination (Singani and Ahmadi, 2012).

Over one-third of the global arable lands are contaminated with heavy metals (Tripathi et al., 2016). For example in Austria, France, Hungary, Slovakia and Sweden there are some 200,000 heavy metal contaminated areas (Yadav et al., 2018).

The bio-availability of heavy metals in the soil can be affected by using different kinds of fertilizers (Barrutia et al., 2009; Nwaichi et al., 2010; Li et al., 2012). This has been documented in studies with oat (*Avena sativa* L.), ryegrass (*Lolium multiflorum* L.), carrot (*Daucus carota* L.), spinach (*Spinacia oleracea* L.) (He and Singh, 1994; He and Singh, 1994) and barley (*Hordeum vulgare* L.) (Singh and Myhr, 1998).

The ability to accumulate heavy metals can be enhanced by adding endophytes (Citterio et al., 2005; Luo et al., 2012; Rajkumar and Freitas, 2008; Wu et al., 2010) and plant growth hormones (Hadi et al., 2010; Hernandez, 1996; Lee et al., 1999; Yasin Ashraf et al., 1989), which is confirmed to increase the accumulation

in hemp (Ahmad et al., 2015). Heavy metal tolerance related genes are being researched for future genetic applications (Ahmad et al., 2015).

When plant accumulates heavy metals or other toxins into their tissues in higher density than the growing medium, it can be called hyperaccumulator (Baker and Brooks, 1989). The hyperaccumulator potential of hemp began to be discovered in Institute of Bast Corps of Ukraine in 1998, where it was planted after Chernobyl nuclear power plant accident to remove contamination from the soil (Ahmad et al., 2015).

The translocation of heavy metals is important to understand as they can concentrate into edible products made from the plant (Abdelsalam et al., 2019). Most of the heavy metals are accumulated into the roots (Ahmad et al., 2014; Ahmad et al., 2015; Citterio et al., 2003; Giovanardi et al., 2002; Shi et al., 2011; Shi et al., 2009), though Cd and Zn are accumulated mostly into the shoots (Löser et al., 2002) or more specifically into the leaves (Arru et al., 2004; Eboh and Thomas, 2005; Giovanardi et al., 2002; Linger et al., 2002). In contrast the fiber does not accumulate heavy metals (Linger et al., 2002). It has been shown that the translocation of heavy metals from roots to shoots increases with higher heavy metal concentrations in soil (Ahmad et al., 2015). The heavy metal concentrations are very different with different hemp cultivars (Shi et al., 2011).

In contrast to accumulation of heavy metals, hemp could be considered to be a Cd excluder (Shi and Cai, 2009; Shi et al., 2009) as its translocation factor (TF), which is the ratio of shoot and root heavy metal concentration $TF = \frac{Cd_{shoot}}{Cd_{root}} < 1$, *i.e.* Cd accumulates mostly into roots, which is suggested to be a property of excluder plants (Baker and Whiting, 2002). This means a lower risk for Cd to concentrate into products for human consumption.

Heavy metal accumulation in the body of the end user is a health concern as they can be toxic and carcinogenic. Especially cadmium, mercury, lead, arsenic and nickel are dangerous heavy metals. The bioavailability of heavy metals depends on the route of administration. For example pyrolysis turns contaminants into toxic compounds (Sullivan et al., 2013). Respiratory administration is more bioavailable than digestion for mercury. When constantly accumulated these toxic metals cause oxidative stress which produces ROS (Reactive oxygen species), cancers, teratogenesis, mutagenesis, immunological syndromes (Hédiji et al., 2015), anemia, cognitive decline, cardiovascular diseases (Iqbal, 2012) as well as brain, nervous system (Sastre et al., 2015), bone, skin and teeth conditions (Luo et al., 2012).

The heavy metal and other contamination concentration of hemp products vary significantly for reasons mentioned above - there are multiple cases of products which have found to contain high concentrations of heavy metals. For example already in 1988 it was discovered that hemp grown in volcanic soil in Hawaii contained Hg per gram of inflorescence concentration of 440 ng/g (Siegel et al., 1988). And more recently an analysis was done in European markets revealing that many of them had traces of heavy metals and other contaminants (Duclos, 2019). Furthermore some medical products have been found to contain possible As contamination (Combemale et al., 2005; Noël, 2001).

This preliminary review and study defines the amount of Cd, Hg, and Pb accumulation in NatureDry(c) lyophilized FINOLA(r) hemp inflorescence and leaf juice, grown in fine-sandy moraine soil type in Central Finland and to clarify the different ratios of biomaterials extractable from the flowers. In order to better plan industrial production of hemp inflorescence juice products.

Cadmium (Cd), mercury (Hg) and lead (Pb) was chosen for the metal analyses because as they are some of the most commonly occurring heavy metals in soil.

Methods

The field experiment was done with hemp cultivar FINOLA(r) in open pollinated field, with males and females. The soil type was fine-sandy moraine with organic matter class being medium and pH 6.7. Soil cultivation included plowing, harrowing, adding Belor Standard NPKS 26-5-5(-2) 2019 fertilizer 280 kg/ha.

Location was in 61deg51'57.1"N 25deg43'48.8"E in Jyvaskyla, Finland. Sowing date for the field experiment was 20.5.2019. Harvest date for the samples were in 3.9.2019, plants had around 60% mature seed, male flowers were already dead and seed harvest was approaching in few weeks. Samples were randomly gathered from the field by hand with scissors and were frozen in regular freezer to -19degC in vacuum sealed bags, with vacuum machine (OBH Nordica 7949 Supreme Food Sealer). Inflorescences and leaves were juiced straight from freezer using a twin gear slow juicer Angel Juicer 7500 ([“Angel Juicer 7500 — Angel Slow Juicers”](#), n.d.) with fine strainer filter. Juice was frozen again in vacuum sealed bags to -19degC. Finally the juice was lyophilized with NatureDry(c) method at Lyotech Oy with in Helsinki. Laboratory analyses for heavy metal concentrations were done by Eurofins WEJ Contaminants GmbH in Hamburg Germany laboratory. Cd and Pb analyses was made with mass spectrometry and ionisation by inductively coupled plasma (ICP-MS). For Hg analysis, reduction of Hg compounds to Hg with Tin(II)-Chloride, then analysis of Hg vapour with atomic absorption spectroscopy (AAS).

Results

As seen from Fig. 1 the end result of lyophilization is green powder like solids dry material that is left after removing the water from the juice.



Figure 1: Lyophilized hemp juice

Produced material quantities of the juicing and lyophilization processes. (Table 1.).

Material	Kg
Inflorescence and leaf	21.23
Juice	10.1
Juice powder	5.24
Pulp	11.3

Table 1: Quantities of materials: Juice is liquid, juice powder is after lyophilization and pulp is the solids left after juicing

Heavy metal concentrations of the lyophilized juice as reported by the Eurofins WEJ Contaminants GmbH in Hamburg Germany laboratory (Table 2.) .

Metal	Concentration $\mu\gamma/\gamma$	\pm
Cd	0.01	0.01
Hg	0.01	0.0
Pb	0.1	0.04

Table 2: Cd, Hg and Pb concentrations

Discussion

In contrast to the usual daily dose of 0.1 - 2 g hemp juice powder samples grown in these conditions were shown to be relatively safe for Cd, Hg and Pb concentrations even for human consumption (Table 3) since in order to reach the safety limit, proposed by The American Herbal Pharmacopoeia (AHP) (Upton and ElSohly, 2014), of Pb in one day, one should consume 60 g of juice powder.

Metal	Safety limit $\mu\gamma/\delta\alpha\psi$	Max dose of powder per day g
Cd	4.1	410
Hg	2	250
Pb	6	60

Table 3: Safety limit of heavy metals (Upton and ElSohly, 2014) and maximum dosages to reach safety limit with current samples

In contrast to recent analyses of hemp based veterinary supplements done in USA, two of 29 products were above the limit human consumption with 2296 $\mu\text{g}/\text{mL}$ of Pb and 2.104 $\mu\text{g}/\text{g}$ of As and two others were in the limit of detection with 0.209 $\mu\text{g}/\text{g}$ of Cd and 0.157 $\mu\text{g}/\text{g}$ of Pb with the others under the limit of detection (Wakshlag et al., 2020).

Discovering the quantities of different biomaterials are useful for further calculations of the production process and costs.

The difference of the soil types in heavy metal accumulation is something to clarify for sustainable cultivation protocol for reduced amount of heavy metals in the biomass and most amount of biomass used.

The translocation of heavy metals directly into the inflorescence is unclear since previous studies are analysing the leaves with inflorescences or just leaves but not inflorescences as such. Outside one study (Husain et al., 2019), in an interview of the author (“Hemp cleans toxic soil and produces clean CBD flower, study finds — Leafly”, n.d.) inflorescence is claimed not to accumulate heavy metals. Meanwhile it is known that heavy metal stress increases the phenolics content (Ahmad et al., 2015) and specifically cannabinoid content (Husain et al., 2019). This could be clarified to further reduce the heavy metal content of the end products.

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