STUDY ON CONTAMINANTS IN CANNABIS AND ITS CBD-RICH PRODUCTS IN THE PERIOD 2018 TO 2023

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INTRODUCTION

This study presents the statistical results obtained from the identification and quantification of possible contaminants (microbiological load, heavy metals and pesticides) in cannabis and derived or processed cannabis-based products rich in cannabidiol (CBD) for each of the different matrices during the years 2018 to 2023.

MATERIALS AND METHODS

The matrices under study are plant samples, oils, extracts and CBD isolates.
Microbiological analysis was performed by plate culture on selective media for each microorganism studied, and the parameters analyzed are fungi and yeasts, enterobacteria, coliforms, *E.coli* and total aerobes at 30ºC. The limit of quantification (LOQ) for moulds and yeasts and total aerobes at 30ºC is100 colony-forming units per gram of product (cfu/g) while for the others it is 10 cfu/g.
The analysis of the heavy metals arsenic (As)cadmium (Cd) and lead (Pb) is carried out by inductively coupled plasma mass spectrometry (ICP-MS) and of mercury (Hg) by atomic absorption spectroscopy(AAS). For arsenic, the limit of quantification is 0,05 mg/kg and for the others it is 0,01 mg/kg.
For the analysis of pesticides, gas chromatography and liquid chromatography techniques, both with mass/mass spectrometry(GC-MS/MS and LC-MS/MS) are used . The limit of quantification for each of the identified and quantified pesticides is 0.01 mg/kg.

RESULTS

Microbiological load

Of the total analyzed samples of plant (N=63), extracts (N=61), oils (N=422) and isolates (N=46), the proportion of samples showing one or more contaminants with values above LOQ was 95.24%, 11.48%, 15.17% and 8.70%, respectively.

Of the total number of samples showing one or more contaminations, the proportion of each of the contaminants is shown **image 1.**

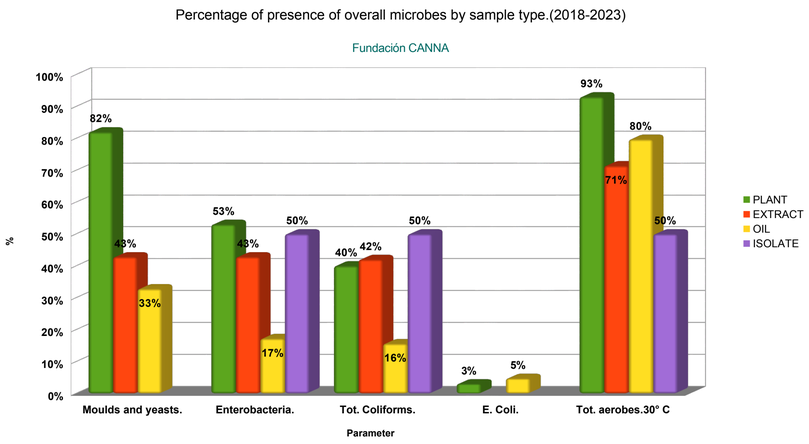

Image 1. Proportion of each of the contaminants according to sample type in the total number of samples showing any contamination.

Maximum values, average and standard deviation for each matrix and contaminant type are presented in **image 1.**

Table 1. Maximum values, average and standard deviation for each matrix and contaminant type.

		Moulds and yeasts. (cfu/g)	**Enterobacteria** (cfu/g)	**Tot. Coliforms** (cfu/g)	**E. Coli** (cfu/g)	**Tot. aerobes 30°C** (cfu/g)
Plant *(N=60)*	MAX	9,40E+05	1,50E+05	1,60E+04	3,10E+03	4,30E+06
	AVERAGE	3,37E+04	1,25E+04	6,84E+03	1,60E+03	2,45E+05
	SD	1,34E+05	2,54E+04	5,76E+03	1,50E+03	6,29E+05
Extract *(N=7)*	MAX	7,50E+02	2,60E+03	2,50E+03	/	8,80E+03
	AVERAGE	3,17E+02	1,09E+03	9,80E+02	/	2,72E+03
	SD	3,07E+02	1,08E+03	1,08E+03	/	3,14E+03
Oil *(N=64)*	MAX	1,00E+05	2,70E+05	1,60E+05	1,20E+03	6,90E+06
	AVERAGE	8,63E+03	2,95E+04	2,02E+04	5,47E+02	1,87E+05
	SD	2,11E+04	7,63E+04	4,69E+04	4,72E+02	9,60E+05
Isolate *(N=4)*	MAX	/	6,10E+03	5,40E+03	/	1,00E+04
	AVERAGE	/	5,85E+03	5,30E+03	/	5,15E+03
	SD	/	2,50E+02	1,00E+02	/	3,88E+03

Heavy Metals

Of the total analysed samples of plant (N=83), extracts (N=74), oils (N=402) and isolates (N=59), the proportion of samples showing one or more contaminants with values above LOQ was 100.00%, 90.54%, 93.53% and 91.53%, respectively.

Of the total number of samples showing one or more contaminations, the proportion of each of the contaminants is shown **in image 2.**

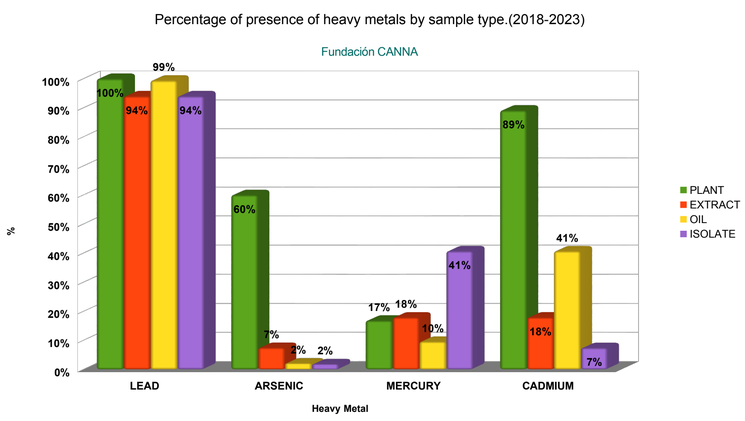

Image 2. Proportion of each of the contaminants according to sample type in the total number of samples showing any contamination.

Maximum values, average and standard deviation for each matrix and contaminant type are presented in **image 2.**

Table 2. Maximum values, average and standard deviation for each matrix and contaminant type.

		Lead (mg/kg)	**Arsenic** (mg/kg)	**Mercury** (mg/kg)	**Cadmium** (mg/kg)
Plant *(N=83)*	MAX	5	1.10	0.06	0.62
	AVERAGE	0.40	0.16	0.02	0.09
	SD	0.69	0.17	0.01	0.10
Extract *(N=67)*	MAX	3.30	0.19	0.32	0.06
	AVERAGE	0.14	0.13	0.06	0.02
	SD	0.44	0.06	0.09	0.09
Oil *(N=376)*	MAX	21	0.29	0.12	2.80
	AVERAGE	0.13	0.13	0.02	0.25
	SD	1.21	0.08	0.02	0.74
Isolate *(N=54)*	MAX	3.20	0.08	0.25	0.07
	AVERAGE	0.09	0.08	0.05	0.03
	SD	0.44	/	0.07	0.03

Pesticides

Of the total analyzed samples of plant (N=69), extracts (N=76), oils (N=309) and isolates (N=53), the proportion of samples showing one or more contaminants with values above LOQ was 42.03%, 60.53%, 30.10% and 13.21%, respectively.

Of the total number of samples showing one or more contaminations, the most common contaminants and the proportion of each of them is shown **in image 3.**

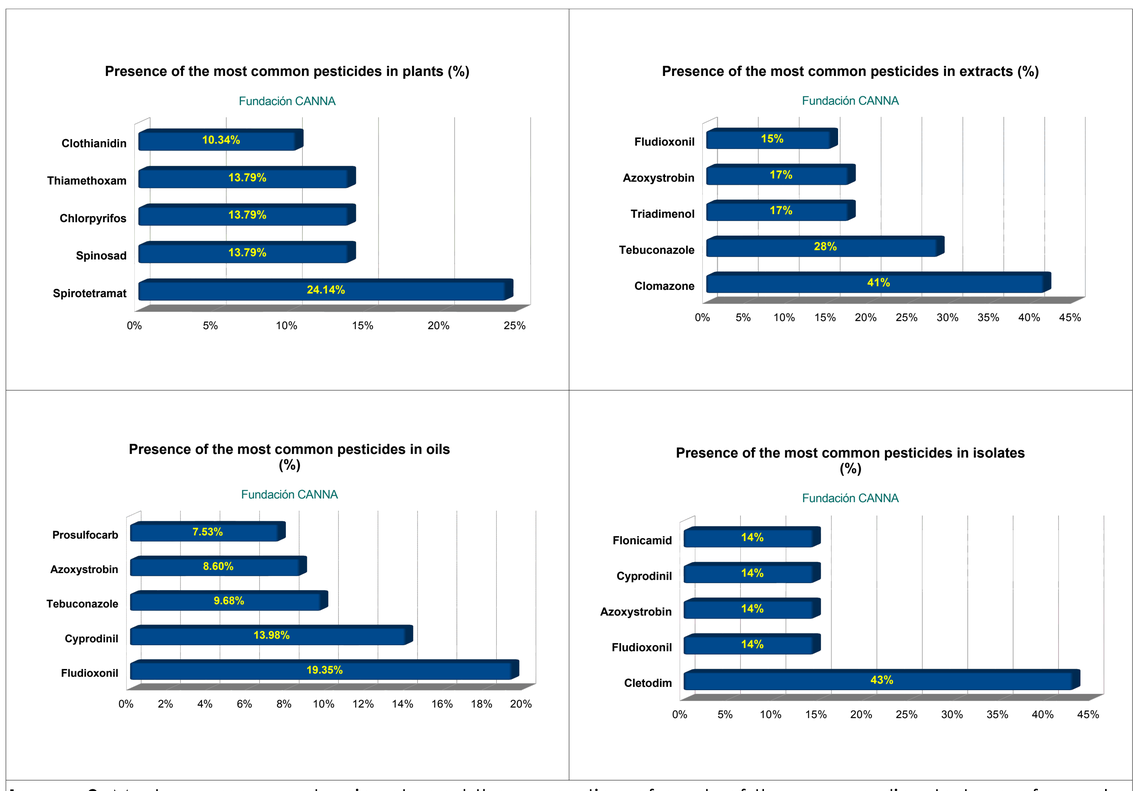

Image 3. Most common contaminants and the proportion of each of them according to type of sample: plants (N=29), extracts (N=46), oils (N=93) and isolates (N=7).

CONCLUSIONS

Most of the plant samples studied had microbiological values above the LOQ for one or more of the parameters studied. This shows that growing conditions, drying and preservation are processes with a high risk of contamination. In addition, some of the products may come from hemp crops that are designated for use as fibre and/or seed and for which therefore the conditions of hygiene are not as strict. For the rest of the matrices, the proportion of samples with microbiological load is much lower than for plants. This seems to indicate that extraction and purification processes have some negative effect on the survival of these micro-organisms. The absence of *E. Coli* in extracts and isolates is notable. However, it has been found in plants and oils. In the case of plants, the use of contaminated utensils or the application of poorly decomposed manure in the late stages of cultivation may be a reason for the presence of this pathogen.

In the case of heavy metals , all matrices have lead as a contaminant. A higher presence of arsenic and cadmium in plant samples compared to the rest of the matrices is notable. This may be due to the great capacity of cannabis to absorb and retain these heavy metals in its tissues. In the case of isolates and extracts , the presence of mercury stands out compared to the rest of the matrices. It is possible that the various extraction and/or purification processes have some selectivity for this metal or that the solvents used contain it. It is therefore desirable to obtain isolates using solvents that have very low levels of this contaminant.

The presence of pesticides in plant samples may result from their application to crops to combat various insect pests. In the case of systemic pesticides, their permanence in the plant is high and may be the reason for their presence in this type of samples. It should be borne in mind that this may also be due to cross-contamination from applications in neighbouring fields with other crops. The pesticides present in the oil matrices can come either from the extract or isolate or from the oil itself, that is from the pesticides used in the cultivation of the oilseed, so it is advisable to use products of organic origin in the production of these oils. The presence of pesticides in the isolates is significant. This indicates that the isolation and purification processes may not remove pesticides that have been used in the cultivation of the raw material and/or are present in the extract used.