Cannabinoid Profile and Elemental Uptake of *Cannabis sativa* L. as Influenced by Soil Characteristics[1]

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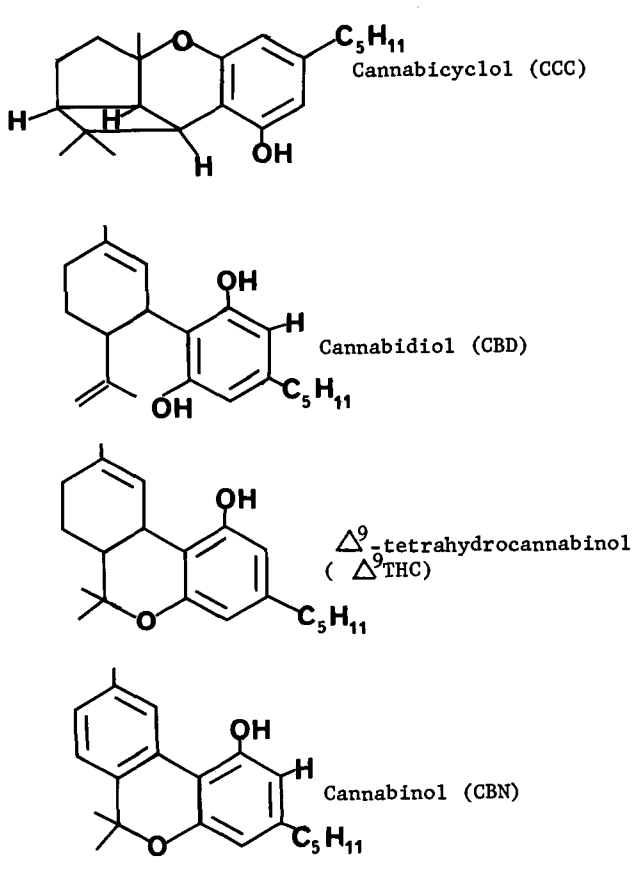
ABSTRACT

The consumption of Cannabis products (marihuana) derived from domestic and foreign sources persists in the United States despite its illegality and health hazards. The objectives of this investigation were: 1) to evaluate relationships between soil and plant elements, cannabinoids, and growth of *Cannabis sativa* L., and 2) to evaluate the practicality of using chemical analysis of Cannabis products to determine their geographic origin. Knowledge of geographic origin is useful to governmental agencies investigating illicit narcotic traffic.

Cannabis sativa L. was grown on 11 different soils for 45 days in the greenhouse. Soils differed significantly in 15 measured elements and pH. Plants were grown from seed of Afghan origin. The following cannabinoids were extracted and measured from leaf tissue: cannabicyclol (CCC), cannabidiol (CBD), Δ^9-Tetrahydrocannabinol (Δ^9THC), and cannabinol (CBN). Fifteen elements were measured in leaf tissue and correlated with soil and cannabinoid measurements. Soil pH was negatively correlated with leaf concentrations of Mn, Fe, Zn, and S. Extractable soil Mg was negatively correlated with N, Δ^9THC and CBD concentrations in leaf tissue ($p < 0.05$). Plant height was negatively correlated with Δ^9THC concentration, suggesting enhancement of the narcotic principle of marihuana when grown under stress. Extractable soil P_2O_5 was negatively correlated with CBD concentration while extractable soil Zn was positively correlated with CCC concentration. Several correlations between soil and plant characteristics having potential value for determination of geographic origin of marihuana were elucidated. However, environmental, harvesting, and analytical procedures used by different workers which do not conform to one another could result in changes in the soil-plant correlations reported herein. Thus, additional studies are required before determination of the geographic origin of Cannabis products by foliar analysis becomes feasible.

Additional index words: Marihuana, Hemp, Narcotic plants.



CANNABIS sativa L. continues to be consumed in the United States despite its illegality and health hazards (Nahas, 1973). The plant is found in wild and cultivated environments in the United States and can be destroyed by chemical and cultural techniques. Determination of the origin of confiscated derivatives of *C. sativa* remains difficult. One purpose of this study was to evaluate procedures that might enable narcotics officials to more readily determine the geographic origin of marihuana and related materials.

Cannabinoids are non-nitrogenous compounds associated with the narcotic character of *C. sativa*. Cannabinoids discussed in this paper are as follows (Mechoulam et al., 1970; Mechoulam, 1973):

C. sativa extracts are being evaluated for their medical value, and Δ^9THC has been studied for treatment of glaucoma. Krejci (1970) stated that cannabidiolic acid has antibiotic activity, and Mechoulam et al. (1970) reported similar activity for CBD.

Hively (1966) indicated that soil factors probably influenced the relative abundance of several cannabinoids, although climate presumably had the strongest influence. Doorenbos et al. (1971) indicated that heredity had greater effect on the cannabinoid profile than environment. However, Maunder (1970) reported modification of the CBN content of English *C. sativa* grown in warm environments. Farnsworth (1969) reported that *C. sativa* grown in short days or temperate climates was higher in CBD than in Δ^9THC, whereas plants grown in the tropics or subtropics were higher in Δ^9THC than in CBD. Fetterman et al., (1971) reported that a Mexican drug variant and a

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Turkish fiber variant of *C. sativa* did not change cannabinoid profiles when the variants were grown in different environments. Latta and Eaton (1974) and Haney and Kutscheid (1973) reported significant relationships between cannabinoids of *wild* plants in Kansas and Illinois and several soil elements.

Many scientific studies of *C. sativa* growth have been oriented toward fiber production. Stearn (1970) reported that *C. sativa* escapees from previously cultivated hemp fields were found more often in fertile, light, well-drained soils than in infertile, heavy, poorly drained soils. According to Haney and Bazzaz (1970), N content of soils influenced the sex ratio of wild plants. Wilsie, Black, and Randall (1944), indicated that hemp plants grew taller and had coarser stalks when adequately supplied with N. They also found that hemp plants grown on Iowa peat soils were more branched than plants grown on mineral soils. Jordan, Lang, and Enfield (1946) associated high soil N levels with hemp that had low fiber strength. Wilsie et al. (1944) reported slight increases in hemp yield with additions of P and K and added that hemp grew best at neutral pH. Tibeau (1936) used solution culture techniques and found that vegetative growth of *C. sativa* was positively influenced by the following elements (arranged in order of decreasing effect): N, K, Ca, and Mg.

The objectives of this study were 1) to evaluate interrelationships between soil and plant elements, cannabinoids, and growth of *C. sativa,* and 2) to evaluate the practicality of using chemical analysis of *C. sativa* products to determine their geographic origin.

Table 1. Soil type, classification, parent material, and laboratory number of 11 Maryland soils.

Soil type	Classification	Parent material	Lab no.
Collington fine sandy loam	Typic Hapludult	*Glauconitic sands*	1
Gilpin loam	Typic Hapludult	Acid, gray shales	2
Lakeland loamy sand	Typic Quartzipsamment	Unconsolidated sands	3
Myersville loam	Ultic Hapludalf	Chloritic metabasalt	4
Pocomoke loam	Typic Umbraquult	Unconsolidated sediments	5
Penn loam	Ultic Hapludalf	Acid, gray shales	6
Bladen fine sandy loam	Typic Albaquult	Acid clay beds	8
Hagerstown loam	Typic Hapludalf	Limestone	9
Christiana loam	Typic Paleudult	Red clay beds	12
Manor loam	Typic Dystrochrept	Mica schist	13
Iredell loam	Typic Hapludalf	Basic meta-igenous rocks	15

* A horizons. All soils were either cultivated or pasture fields except Manor, Lakeland, Iredell, and Pocomoke, which were from idle fields or woodlands.

MATERIALS AND METHODS

Soils derived from varied geologic materials were collected from the Coastal Plain, Piedmont, and Appalachian provinces of Maryland (Table 1). County soil survey reports were used to locate each site. Surface horizons of each soil were air-dried, mixed for homogenity, and passed through a 2-mm sieve.

C. sativa seeds of Afghan origin (P.I. 378939), a drug variant, were planted in 12.7 cm pots and covered lightly with soil. Pots were arranged in a randomized complete-block design with five replicates, one plant per pot. Plants were grown for 45 days at Beltsville, Md. in a greenhouse maintained with a 12-hour period of light.

The aboveground plant parts were harvested, stems were discarded, and leaf tissue was rinsed with distilled water. Tissue was oven-dried at 65 C for 16 hours before grinding and chloroform extraction for cannabinoid analysis by the methods of Coffman and Gentner (1974) and Turner and Hadley (1973).

Elements of leaf tissue were analyzed by X-ray fluorescence with a Diano-X-ray Milliprobe[3] with a He path and by optical spectroscopy with a 3.5 Ebert arc Jarrell-Ash Spectrograph.[3] Total N in leaves was determined by the semimicro Kjeldahl method, with N measurement by Technicon Autoanalyzer.[3]

Soil pH, Mg, Ca, K, P, Mn, and B were determined by the University of Maryland Soil Testing Laboratory (Method for Testing Soil Samples at the University of Maryland Soil Testing Laboratory, University of Maryland, Mimeo. No. 37). Al was measured by the aluminon method (McLean, 1965). The Soil Testing and Plant Analysis Laboratory, University of Georgia, analyzed other soil components by atomic absorption spectroscopy by using 0.025 *N* H_2SO_4 plus 0.05 *N* HCl extracts. Total soil N was determined by standard AOAC Kjeldahl procedure and total S by the method of Jones and Isaac (1972).

Statistical analyses were performed by the National Agriculture Library Computer Center, Beltsville.

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by USDA, and does not imply its approval to the exclusion of other products that may also be suitable.

Table 2. Chemical characteristics of 11 Maryland soils.*

Soil	Mg	P_2O_5	K_2O	Ca	Mn	Fe	Cu	Al	Zn	Na	B	Sr	Ba	S†	N†	pH
	ppm													%		
1	131a*	180a	61e	942c	2h	62b	0.75cd	125a	7.8cde	11de	0.22f	19de	16ab	0.04b	0.09cde	5.0e
2	34d	15d	35f	1,345ab	19efg	8g	0.50d	ND‡	25b	14cd	0.34b	19de	11bc	0.03d	0.23b	6.3ab
3	24d	16d	18g	90f	10fgh	12f	0.50d	ND	3.5e	10de	0.03h	4h	6d	0.01f	0.07de	5.6cd
4	123a	65c	84d	872cd	105a	8g	1.00bc	ND	4.8de	24b	0.26d	23cd	10cd	0.02de	0.20b	6.0bc
5	81bc	19d	51e	560de	5gh	80a	0.75cd	110b	24.2b	18c	0.16g	40a	9cd	0.05b	0.36a	4.5f
6	104abc	174a	218a	1,345ab	22ef	10fg	1.00bc	ND	6.2cde	13cde	0.24e	26c	12bc	0.02e	0.14c	6.6a
8	78bc	15d	22fg	1,118bc	7gh	50c	1.00bc	5d	14.0c	45a	0.29c	34b	16ab	0.03de	0.14c	6.4ab
9	115abc	68c	164c	422ef	38bcd	25d	1.00bc	ND	7.0cde	12cde	0.24e	17e	13bc	0.09a	0.14c	5.4de
12	116ab	132b	194b	1,415ab	40bc	83a	1.50a	5d	11cde	16cd	0.62a	28c	14bc	0.02de	0.14c	6.7a
13	14d	10d	20fg	130f	3h	8g	0.50d	20c	4.8de	8e	0.03h	9fg	6d	0.01f	0.04e	6.0bc
15	131a	8d	32fg	1,600a	26de	16c	1.00bc	10d	32.8a	13cde	0.24e	40a	6d	0.04c	0.22b	6.8a

* Values within an element not followed by the same letter differ significantly at the 5% level. † S and N values are total. ‡ None detected.

Table 3. Mean element content of *Cannabis sativa* leaf tissue.

Soil	Mg	P	Mn	Fe	Cu	Al	Zn	Na	B	Sr	Ba	K	Ca	S	N
	ppm											%			
1	5,867bc*	2,467ab	179bc	87c	7d	1,103abc	112b	133b	220a-d	9c	9f	2.7b-d	2.6e	0.31b	1.8b
2	2,900d	1,967bc	152cd	97bc	11bcd	747c	83cd	100b	190b-d	9c	25d	2.4b-d	8.3a	0.30b	2.5a
3	5,600c	1,267c	180bc	195ab	7d	1,357abc	92c	120b	192b-d	33bc	46b	2.6b-d	3.8d	0.29b	2.0ab
4	5,533c	2,267b	199b	106bc	16ab	1,000abc	75cd	100b	247a-d	40b	77a	2.6b-d	5.6b	0.31b	2.3ab
5	4,100c	1,550bc	602a	216a	9cd	1,685ab	312a	135b	210a-d	85a	36c	2.0c-d	2.4e	0.54a	---
6	2,700d	3,267a	139d	114bc	15abc	1,500abc	78cd	100b	230a-d	13c	22de	3.7ab	4.3cd	0.30b	1.7b
8	5,733c	2,000bc	80f	84c	10bcd	1,170abc	76cd	100b	303a	77a	15ef	1.8c	5.0bc	0.26b	1.8b
9	7,233ab	1,733bc	162bcd	106b	7d	1,307abc	72d	100b	273a-c	47b	35c	2.9a-d	3.8d	0.31b	1.8b
12	2,533d	2,233b	99ef	117bc	18a	933bc	80cd	100b	150d	23bc	13f	4.1a	3.4de	0.28b	1.9ab
13	4,450c	3,750a	132de	107bc	9cd	1,725a	76 cd	220a	172cd	25bc	15ef	3.3a-c	3.4de	0.27b	2.4ab
15	7,433a	1,967bc	76f	92bc	7d	1,450abc	71 d	143b	288ab	9c	9f	1.8d	4.1cd	0.26b	1.7b

* Values within an element not followed by the same letter differ significantly at the 5% level.

RESULTS AND DISCUSSION

Elemental Analyses of Soils and Plants

Results of elemental and pH analyses of soils are presented in Table 2. Significant differences between soils were found for all measurements. Soils 6 and 12 were highest in overall fertility and yielded the greatest quantity of plant material.

Results of elemental analysis of *C. sativa* leaf tissue are presented in Table 3. Plants grown in soil 5 had the highest concentrations of Mn, Fe, and Zn in their leaf tissue (these elements are readily available in acid soils such as soil 5 (Table 2)). Continued metal uptake without growth dilution resulted in high metal concentrations in leaf tissue and in leaf chlorosis and stunted growth.

Soil pH was negatively correlated with leaf content of Mn, Fe, Zn, and S (Table 4). Extractable Al was positively correlated with leaf Mn, Zn, and S (Table 4). Zn, Mn, and Fe concentrations were highest for plants grown on acid soils. Antagonisms between Mn and Fe have been cited as causing various deficiency or toxicity symptoms in plants. Mn toxicity of tobacco in acid soils was found by Abruna-Rodriguez et al. (1970). Peas grown in acid soils showed Mn toxicity with 550 ppm Mn in tissue (White, 1970). Jones (1967) reported > 250 ppm Mn in upper mature trifoliate leaves of soybeans as an excess level. *C. sativa* grown on soil 5 contained 602 ppm Mn in leaves and showed leaf chlorosis and stunted growth. Leaf tissue of these plants also contained 216 ppm Fe, 312 ppm Zn, and 0.54% S. Jones (1967) reported that > 100 ppm Zn in the top 15 cm of alfalfa before bloom was excessive.

Extractable soil B was positively correlated with leaf Cu but negatively correlated with leaf Al and Na (Table 4). Extractable B was also positively correlated with extractable Cu ($P < 0.01$), but was not correlated with extractable Al or Na.

Extractable soil Cu was positively correlated with leaf Cu, and extractable K was positively correlated with leaf K (Table 4). Tibeau (1936) indicated that K was more important for vegetative hemp growth than either Ca or Mg. Thus, *C. sativa* may respond under certain conditions to increased quantities of available soil K by increased growth and mineral uptake.

Soil Mg was negatively correlated with plant N (Table 4). Ahmad and Tulloch-Reid (1968) reported reduced N accumulation by okra fruit with soil Mg applications greater than 56 kg/ha. Other workers (Burris, 1959; Broyer and Stout, 1959) have reported antagonistic interactions between ammonium N and soil Mg when oats and celery have been used as test species. Agboola and Corey (1973) reported a positive relationship between soil Mg and N in maize plants but could find no relationship between the two elements in plant tissue. Embleton (1966) indicated that Mg-deficiency symptoms may be reduced with increased levels of soil or plant N. No significant relationship was found between Mg and N in *C. sativa* leaf tissue.

Soil Na and plant Sr were positively correlated (Table 4). No significant relationships existed between soil Na and soil Sr. Extractable soil Ca and Sr were positively related (r = 0.79**), but the two elements were not significantly related in leaf tissue. Soil Mn and leaf Ba were highly correlated (Table 4), but the cause-and-effect relationship was vague.

Sulfur concentrations in leaf tissue were positively correlated with leaf Fe, Mn, and Zn (Table 5). The metallic elements were also positively correlated in leaf tissue (Table 5). Latta and Eaton (1974) speculated that Mn or Fe may regulate the enzyme systems responsible for cannabinoid synthesis. Agboola and Corey (1973) reported positive correlations between Zn and Mn in maize tissue. Leaf Zn was also positively correlated with leaf Sr, and leaf Mn was positively correlated with leaf Ba (Table 5). Lindsay (1972) reported that Sr reduced Zn uptake by wheat over a wide range of soil Zn concentrations.

Leaf Cu was positively correlated with leaf K, but negatively correlated with leaf Mg (Table 5). Gilbert (1952) reported greater yields of tobacco when Cu and K were both applied to the soil than when K was applied without Cu.

Leaf B was negatively correlated with leaf K, but positively with leaf Mg (Table 5). Bradford (1966) reported that B deficiency in some crops was eccentuated by increased soil K applications.

Leaf Na was significantly correlated with leaf Al (Table 5). These elements were highest in plants grown on soils 5 and 13. Plants grown on soil 13 had the highest concentration of both elements (Table 5).

Physical Characteristics of *C. sativa*

Significant differences in *C. sativa* growth on different soils were expressed in height, numbers of leaflets and nodes, dry weight, and cannabinoid profile (Ta-

Table 4. Simple correlations of several soil parameters with *C. sativa* leaf elements.

Soil variable	Tissue variable			
	Mn	Zn	S	Fe
pH	-0.78**	-0.72**	-0.69*	-0.56*
Al	0.64*	0.71*	0.64*	
	Cu	Al	Na	
B	0.66*	-0.68*	-0.58*	
Cu	0.60*			
	K			
K	0.62*			
	N			
Mg	-0.64*			
	Sr			
Na	0.64*			
	Ba			
Mn	0.73*			

*, ** Significant at the 5 and 1% levels, respectively.

Table 5. Simple correlations between several elements in *C. sativa* leaves.

Zn × S	Zn × Fe	Zn × Mn	Zn × Sr	Mn × S	Mn × Fe	Mn × Ba
0.97**	0.74**	0.97**	0.58*	0.99**	0.77**	0.58*
Fe × S	**Cu × K**	**Cu × Mg**	**B × K**	**B × Mg**	**Na × Al**	
0.72*	0.59*	-0.67*	-0.61*	0.71**	0.60*	

*, ** Significant at the 5 and 1% levels, respectively.

ble 6). The tallest plants grew in soils 6 and 12, whereas the shortest plants grew in soils 5 and 13. Extractable B was significantly correlated with plant height ($r = 0.60^*$). Plants grown in soils 4 and 12 increased their average height 2.2 and 2.3 times, respectively, during the 22-day period between the first measurement of plant height (Table 6) and harvest and grew more rapidly than plants growing in other soils.

Numbers of leaflets and nodes differed significantly between soils. Plants growing in soils 5, 8, and 13 differed significantly from plants growing in soils 6 and 12 in number of nodes. This variable is important in *C. sativa* morphology, since most psychoactive compounds are obtained from the leaves and flowers.

Dry weight of *C. sativa* tissue differed significantly between soils (Table 6). Soil 12 yielded the most plant material and soil 5 the least.

Cannabinoid Profile of Leaf Tissue

No significant differences were detected between soils for CCC and CBD concentrations in leaf tissue (Table 6). Significant differences were observed between soils for the psychoactive cannabinoid Δ^9THC and CBN (Table 6). Greatest concentration of Δ^9THC was measured in leaf extract from plants growing in soils 2 and 13. Mean Δ^9THC concentration for leaves of plants growing in soil 2 was 7,828 ppm and for soil 13, 6,952 ppm. Lowest Δ^9THC concentrations were 1,157 ppm for soil 9 and 1,372 ppm for soil 4. Highest CBN concentrations were 596 ppm from leaves of plants grown in soil 3 and 520 ppm from leaves of plants grown in soil 1. CBN concentrations were lowest for plants grown in soil 4 (at 196 ppm) and soil 13 (at 225 ppm). Plants from soil 4 were also very low in Δ^9THC whereas those from soil 13 were very high in Δ^9THC.

Fetterman et al. (1971) stated that the total content of Δ^9THC plus CBN should equal the initial Δ^9THC concentration of the tissue analyzed, regardless of age of material. They developed a formula to determine phenotype (e.g., drug or fiber variety of *C. sativa*):

$$PR = \frac{\%\ \Delta^9\text{THC} + \%\ \text{CBN}}{\%\ \text{CBD}}$$

where PR is the phenotype ratio. They determined that drug varieties of *C. sativa* had phenotype ratios greater than 1, whereas fiber types had phenotype ratios less than 1. From the values presented in Table 6, all plants would have been classified as drug type, since phenotype ratios ranged from 6.1 for soil 6 to 1.1 for soil 9. However, the range in phenotype ratios derived from this study suggested that modifications in the soil environment might result in conversion of a drug phenotype to a fiber phenotype, or vice versa.

As plant height increased, concentration of Δ^9THC in leaf tissue generally decreased (Table 7). Soils that yielded shorter plants usually had lesser amounts of essential nutrients than soils that yielded taller plants (e.g., Tables 2 and 6, soils 4, 6, and 12 vs 2, 5, and 13). Plants grown in soil with less than adequate fertility levels would have been subjected to stress. Thus, Δ^9THC may have been concentrated in plants because of stress or decreased growth. Latta and Eaton (1974) suggested that Δ^9THC production of *wild* Kansas-grown *C. sativa* was enhanced by stress. Similar responses were reported by Haney and Kutscheid (1973) in their study of *wild C. sativa* in Illinois. As competition between *wild C. sativa* plants and other species increased, they reported increased cannabinoid content. Emboden (1972) reported that some groups of Mexican Indians increased the concentration of psychoactvie ingredients of *C. sativa* by subjecting the plants to stress, as by pruning and withholding water, but this increase has not been experimentally verified.

Table 6. Mean morphological and biochemical characteristics of *C. sativa* grown on 11 different soils.*

Soil	23-Day ht	Harvest ht	Leaflets†	Nodes	Dry wt	CCC	CBD	Δ^9THC	CBN	PR‡
	cm		no.		g			ppm		
1	26cd*	48de	5.0bc	8.6ab	0.57d	133a	1,101a	3,150ab	520ab	3.3
2	21d	37e	4.7c	8.3ab	0.37d	195a	1,703a	7,828a	252b	4.9
3	27bcd	50d	5.3bc	9.3ab	0.50d	141a	1,989a	2,257b	596a	1.4
4	30abc	67ab	5.7abc	9.7ab	1.33bc	38a	1,041a	1,372b	196b	1.5
5	20d	30e	5.0bc	7.5b	0.25e	200a	1,129a	2,566b	235b	2.5
6	36a	68ab	6.7ab	10.0a	1.77ab	45a	652a	3,618ab	365ab	6.1
8	26cd	52cd	5.7abc	7.7b	0.60d	130a	1,331a	3,145ab	378ab	2.6
9	31abc	63bc	5.7abc	8.7ab	1.03cd	43a	1,409a	1,157b	341ab	1.1
12	33ab	76a	7.3a	10.0a	2.27a	194a	1,319a	2,458b	308ab	2.1
13	17d	32e	4.5c	7.5b	0.30de	78a	1,642a	6,952a	225b	4.4
15	25cd	52cd	5.7abc	8.7ab	0.67d	273a	1,281a	3,240ab	440ab	2.9

* Values within a column not followed by the same letter differ significantly at the 5% level. † Number of compound leaflets/leaf at uppermost node. ‡ PR = Phenotype ratio = $\frac{\%\Delta^9\text{THC} + \%\text{CBN}}{\%\text{CBD}}$

Table 7. Simple correlations between cannabinoid concentrations and several soil and plant measurements.

Δ^9THC		CBD		CCC		CBN	
			Soil parameters				
Mg	-0.64*	P_2O_5	-0.64*	Zn	0.84**	Mg/B	0.59*
Ca/Mg	0.74**	Mg	-0.72*	Cu/Zn	-0.78**		
Plant parameters		Ca/Zn	-0.76**	Mg/Zn	-0.67*		
		P_2O_5/Zn	-0.67*	K_2O/Zn	-0.66*		
Harvest ht	-0.59*	K_2O/Fe	-0.69*	K_2O/Fe	-0.60*		
N (Total)	0.66*	Mg/Cu	-0.64*	K_2O/B	-0.67*		
Ca/Mg	0.62*	Cu/B	0.58*				
Ca/B	0.64*	Na/B	0.62*				
Ca/Sr	0.67*						

*, ** Significant at the 5 and 1% levels, respectively.

Relation of Cannabinoid Concentrations with Soil and Plant Elements

Soil Mg was negatively correlated with Δ^9THC concentrations in leaf tissue (Table 7). Mean Δ^9THC concentrations greater than 6,900 ppm were found in leaf tissue of plants grown on two soils with <40 ppm extractable Mg. However, one soil with <40 ppm Mg produced plants with a mean Δ^9THC concentration of only 2,257 ppm (Table 6, soil 3). Plant N, previously shown negatively correlated with soil Mg (Table 4), was positively correlated with Δ^9THC (Table 7). Haney and Kutscheid (1973) reported positive correlation of total soil N with Δ^9THC levels in *wild C. sativa* in Illinois. A highly significant positive correlation was found for the Ca/Mg soil ratio to Δ^9THC concentration (Table 7). A balance between Ca and Mg appeared to be required for maximum Δ^9THC accumulation in leaf tissue. As Mg levels increased relative to Ca, Δ^9THC concentrations decreased. These relationships will be examined in future experiments.

Ratios of Ca/Mg from leaf tissue were positively correlated with Δ^9THC leaf concentrations (Table 7). Thus, the soil Ca-Mg balance was reflected in plants. Ca-Mg balance within plants may influence synthesis and concentrations of Δ^9THC. Other plant element concentration ratios positively correlated with Δ^9THC were Ca/B and Ca/Sr (Table 7). Strontium can be taken up and used much like Ca by many plant species, but it does not completely substitute for Ca in physiological functions (Gauch, 1972). Bradford (1966) reported that plants with high concentrations of Ca usually required high B concentrations. Therefore, increased Ca/B ratios in *C. sativa* may have indicated that Ca-B imbalance relative to cannabinoid biosynthesis requirements contributed to higher Δ^9THC concentrations. However, possible roles of Ca-Sr or Ca-B relationships relative to Δ^9THC concentrations have not been explained.

The pyrolytic conversion of CBD to Δ^9THC has been suggested. Thus, although CBD has no known psychoactive characteristics (e.g., addictive, hallucinogenic) it may transform to Δ^9THC during smoking (Mikès and Waser, 1971). Therefore, concentration of CBD in marihuana must be considered with that of Δ^9THC when the abuse potential is evaluated. Two soil parameters, extractable P (as P_2O_5) and Mg, were negatively correlated with leaf concentration of CBD (Table 7). Higher quantities of CBD were concentrated in leaf tissue of *C. sativa* plants growing in low to medium P levels. As P_2O_5 levels approached optimum (based on general agronomic crop requirements), CBD concentrations decreased. Haney and Kutscheid (1973) reported that soil P was negatively correlated with Δ^8THC, a psychoactive isomer of Δ^9THC. Soils with <40 ppm Mg produced plants with higher CBD concentrations than soils with 70 to 130 ppm Mg.

Negative correlations were found for CBD and several other ratios of soil elements (Table 7). Soil Zn was the denominator in ratios with soil Ca and P_2O_5. High Ca and P_2O_5 levels relative to Zn may have resulted in decreased Zn uptake and affected CBD synthesis and concentration. Soil K_2O/Fe and Mg/Cu ratios were also negatively correlated with CBD concentrations (Table 7). Soil Cu/B and Na/B ratios were positively correlated with CBD concentrations (Table 7).

CCC was positively correlated with extractable soil Zn and negatively correlated with the ratios of soil elements Cu/Zn, Mg/Zn, K_2O/Zn, K_2O/Fe, and K_2O/B. CCC concentrations in leaf tissues were lower than concentrations of other measured cannabinoids (Table 6). CBN was positively correlated with the soil Mg/B ratio (Table 7).

The occurrence of B in ratios significantly related to concentrations of all four cannabinoids strongly implied the involvement of this element in mechanisms associated with cannabinoid synthesis or maintenance of cannabinoid concentrations in leaves. Zn, K, and Mg were likewise involved in several significant correlations with cannabinoid concentrations. Unfortunately, how these various elements affect biosynthesis or degradation of cannabinoids is unknown.

To further evaluate cannabinoid-element relationships, we calculated correlations for ratios of cannabinoids with soil and plant elements and their ratios. A number of significant relationships were found.

Ratios of concentrations of Δ^9THC/CCC were positively correlated with leaf P, P/Fe, and P/Zn ratios (Table 8). Consequently, the P content of plant tissue seemed to affect the Δ^9THC-CCC relationship, which suggested involvement of P in CCC breakdown or Δ^9THC formation. Added involvement of P was shown by the positive correlation between the Δ^9THC/CBD ratio and plant P. Several writers have indicated that CBD may be a precursor of Δ^9THC in *C. sativa* (Küppers, Lousberg, and Bercht, 1973; Farnsworth, 1969). These data suggested that increased concentrations of P in leaf tissue may have enhanced the conversion of CBD to Δ^9THC.

Cannabinoids belong to the chemical class of natural terpenophenols. P may be involved in cannabinoid reactions by interaction of geraniol phosphate and olivetol which may form CBD precursors (Mechoulam, 1973). These, in turn, may transform to Δ^9THC and eventually CBN. Although how Fe and Zn affect cannabinoid biosynthesis is unknown, they may be involved in enzymatic activities that influence mechanisms involving P.

Plant N was positively correlated with the CBD/CBN ratio (Table 8). Plant N was also positively correlated with Δ^9THC (Table 7), but any explana-

Table 8. Simple correlations of soil and plant elements and ratios with cannabinoids and ratios.

Element		r
Soil		
Mg/N	× CBD/CBN	-0.66*
K_2O/Na	× CBN/CCC	0.75**
P_2O_5/Ca	× CBN/CCC	0.63*
Cu/Zn	× CBN/CCC	0.71*
K_2O/Fe	× CBN/CCC	0.74**
K_2O/Zn	× CBN/CCC	0.82**
Plant		
P	× Δ^9THC/CCC	0.88**
P/Fe	× Δ^9THC/CCC	0.71*
P/Zn	× Δ^9THC/CCC	0.83**
P	× Δ^9THC/CBD	0.69*
N	× CBD/CBN	0.91**
N/Cu	× CBD	0.66*
N/Mn	× CBN/CCC	-0.69*

* Significant at 5 and 1% levels, respectively.

tions of these relationships would be speculative at this time.

Ratios of plant N/Cu and N/Mn were significantly correlated with CBD and CBN/CCC ratios, respectively (Table 8). As the N/Cu ratio increased, CBD levels generally increased. When considered with the other cannabinoid ratios related to N or ratios including N (Table 8), these increases suggested an important, though at this time unknown, effect of N in cannabinoid biosynthesis. Thus, the relationship between N and CBD observed for soil ratios was observed also for plant ratios with N.

The correlation of ratios of CBD/CBN with soil Mg/N ratios was significant (Table 8). As soil N increased relative to Mg, CBD increased relative to CBN.

CBN/CCC ratios were positively correlated with the following soil element ratios: K_2O/Na, P_2O_5/Ca, Cu/Zn, K_2O/Fe, and K_2O/Zn (Table 8). CCC was positively correlated with soil Zn and negatively correlated with several ratios of soil elements (Table 7). Increased CBN concentration in leaf tissue relative to CCC was related to increased soil K_2O relative to Na, Fe, and Zn, to increases in P_2O_5 relative to Ca, and to increases in Cu relative to Zn (Table 8). The role of Zn in the ratios significantly related to the CBN/CCC ratio was obvious, based on the Zn-CCC correlation.

Since the mechanism of biogenesis of cannabinoids remains unknown, we can only speculate concerning the use of the elements shown to be significantly related to cannabinoids. However, the possibility that specific elements acting individually or in combination may be involved in biogenesis of cannabinoids has been demonstrated. The specific pathways and mechanisms remain to be resolved.

Determination of Geographic Origin

Relationships between the elemental and biochemical constituents of *C. sativa* and soil chemical characteristics were observed in this study. Determination of geographic origin of *C. sativa* products appeared only slightly feasible. In this study, Δ^9THC content of *C. sativa* leaves was generally less than 4,000 ppm when plants were grown on soils with >60 ppm extractable Mg. Soils with >60 ppm extractable Mg would be considered adequate for most agronomic crops grown in Maryland. Soils with extractable B levels >0.4 ppm produced plants with 1,300 ppm or less CBD in leaves. Other plant-soil relationships were also reported. Relationships between soil and plant elements and cannabinoids could be useful for determination of geographic origin; however, other factors affecting plant-soil relationships must be considered.

Fetterman et al. (1971) stated that Δ^9THC was transformed to CBN in stored plant tissue. Transformation rate varied with time and conditions of tissue storage. Maunder (1970) said Δ^9THC transformation of CBN was decreased when tissues or tissue extract solutions were stored out of direct sunlight. Farnsworth (1969) also reported this alteration, but further stated that CBD could be transformed to Δ^9THC, which could then alter to CBN; the overall rate of transformation was most rapid in tropical environments. Consequently, comparison of Δ^9THC values between *C. sativa* plants grown in various parts of the world would appear to be of little value unless prescribed conditions of harvest, preparation, and storage were followed by all researchers. Climatic and soil variations that could find expression in cannabinoid profiles could be masked and confounded by lack of uniform handling and storage.

Climatic variations, as in temperature and photoperiod, would surely have expression in cannabinoid-soil relationships. Drying time and temperature before cannabinoid extraction and analysis were shown in another study to influence cannabinoid profiles (Coffman and Gentner, 1974). Density of planting, pest control procedures, age of plant at harvest, and part of plant harvested must also be considered as potential confounding factors.

Thus, much remains to be studied about *C. sativa*-soil relationships before definitive statements can be made relative to determination of geographic origin. Results from this study should provide direction for future studies of relationships between characters of *C. sativa* and its environment.

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