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CHEMICAL CHARACTERIZATION OF SOME AQUEOUS LEACHATES FROM CROP RESIDUES IN "CELSS"

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Special thanks are given to Barbara Peterson who willingly relinquished a corner of the Analytical Laboratory to me and the patience that she showed when my mess encroached on her side of the sink is much appreciated. Barb's enthusiasm and persistent smile made each day enjoyable even when a malfunctioning instrument caused frustration. The summer was too short which suggests that I stayed busy in a productive and enjoyable manner and that frequent interactions with very special people were part of the daily routine.

ABSTRACT

Aqueous leachate samples prepared from crop residues that are produced as a component of the Controlled Ecological Life Support System program designed to support long duration space missions have been compared and general chemical characterization has been accomplished. Solid phase extraction and high performance liquid chromatography were used to accomplish comparisons based on chromatographic and ultraviolet absorption properties of the components that are present. Specific compounds were not identified, however, general composition related to the presence of phenol-like compounds was explored.

SUMMARY

Aqueous leachate samples prepared from potato, soybean and wheat crop residues that are produced as a component of the Controlled Ecological Life Support System program designed to support long duration space missions have been compared and general chemical characterization has been accomplished. Solid phase extraction and high performance liquid chromatography were used to accomplish comparisons based on chromatographic and ultraviolet absorption properties of the components that are present. Specific compounds were not identified, however, several compounds known to be present in these plant residues were used for comparison purposes. General composition related to the presence of phenol-like compounds was explored. The leachates are highly colored and an aqueous fraction of the leachates exhibited chromatographic properties similar to tannin-like materials. Microbial treatment of leachate samples removes or converts most of the tannin-like and phenol-like constituents. An exhaustive characterization is not a feasible undertaking because of the complexity associated with leachate composition.

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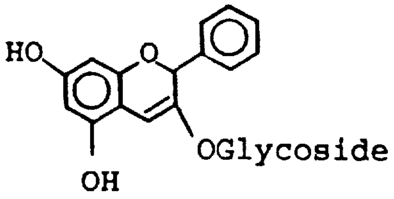
I. Introduction

The Controlled Ecological Life Support Systems(CELSS) program has as a main objective the construction of a life support system that will provide food, water and oxygen for long duration space missions and will allow for recycling of some of the wastes produced(1-4). An ongoing project involves growing plants under hydroponic conditions. The plants of current focus include wheat, soybeans, lettuce and potatoes. It is desirable to recycle non-edible portions of the plants to minimize waste product buildup and to minimize the need for additional materials to support the plant growth cycle.

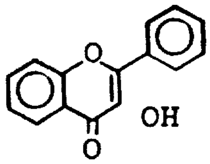
One current problem involves the characterization of crop residues, specifically the root, stem and chaff portions of these plants. The water soluble leachate of these dried residues contain both inorganic and organic constituents. The inorganic constituents have been characterized and can be recycles as fertilizer in the growth chambers(5). The aqueous leachate can be treated microbially to decrease the organic matter content (Richard Strayer, private communication), however, a brown color persists to some extent and increases in intensity as the leachate is used to replenish the hydroponic solution. Preliminary investigations have shown that the organic composition of the leachate can be subdivided into broad categories with complex chemical makeup. It is not unusual for a natural product material to contain in excess of 100 different chemical species. Specific fractions might include carbohydrates, organic acids, polyphenols, tannins and humic materials. A variety of analytical techniques are typically employed to derive solutions to complex chemical problems of this nature.

This report describes attempts to obtain a basic understanding of the chemical makeup of the organic composition of water leachate solutions derived from crop residues with specific emphasis on compounds that have exhibited phytotoxicity. Potentially useful techniques include liquid chromatography, spectrophotometry, gas chromatography and mass spectrometry. The presence and persistence of phytotoxic substances in a variety of crop residues has been recognized for some time (6). Many of these substances exhibit significant water solubility and have been shown to inhibit seed germination and growth of various crops. The separation and identification of specific compounds from soils and crop residues suspected to be phytotoxic have been attempted using a variety of techniques. These include combinations of column, paper and thin-layer chromatography and more recently gas chromatography and high-performance liquid chromatography (HPLC) (7). For example the presence of vanillic, p-hydroxybenzoic, protocatechuic, p-coumaric, syringic and ferulic acids (8, 9) were reported as phytotoxic compounds in weeds and subtilled soils. These compounds and others of similar structure can readily be detected using ultraviolet spectroscopy. The utility of HPLC in the chemical characterization of plant products can be demonstrated by studies such as reported by Lee, et al (10) where separation, identification and quantification of sugars, non-volatile acids, flavones, flavone glycosides, carotenoids and anthocyanidins were reported. A photodiode array detector was used to characterize spectral properties of each class of compounds. Other examples of studies that relate to measurement of specific compounds in plant crops include the determination of phenolic acids in potato tubers (11, 12), non-volatile constituents including gallic acid in tea (13), and phenolic constituents in rhubarb (14). New methods of analysis for tannins has been the subject of a recent review (15). The chemical makeup of tannins is extremely complex. Broad groups of water soluble compounds include hexahydroxydiphenic acids, gallic acid esters with molecular weights less than 3000, proanthocyanidins with molecular weights less than 20,000 and additional compounds with molecular weights near 200 are also present.

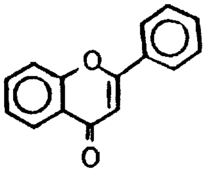
The yellow-brown color associated with aqueous systems that contain dissolved organic matter is typically associated with the presence of tannic and humic acids. Color can also be associated with the presence of flavonoid compounds. Anthocyanins, flavonols and flavones constitute this flavonoid group of compounds. The basic structure of each group is shown below (16):



Anthocyanins



Flavonols



Flavones

Various sugars may be attached to the ring system, typically at the phenyl ring which increases the water solubility of these compounds. The anthocyanins are intensely colored and account for the brilliant colors associated with many flowers. Substituted flavones and flavonols tend to exhibit yellow-brown colors that deepen as concentration increases.

This study has progressed from an initial investigation where leachate fractions and subfractions from potato, soybean and wheat were compared by HPLC to determine if major similarities or differences exist. Each fraction represents a very complex combination of chemical compounds. Progress made to provide general and specific makeup of these fractions is reported below.

II. **Experimental**

2.1 CHEMICALS

Acid and phenol derivative samples were purchased from Aldrich Chemicals except that Naringin and Morin were from J. T. Baker and Quercetin was from Eastman Chemicals. Methanol used to prepare samples and as HPLC eluent were HPLC grade purchased from Fisher or J. T. Baker. Water was obtained from a Hydrodyne Ultrapure/Picopure purification system which provided 18 megohm water filtered through a 0.45μ filter. All HPLC mobile phase solvents were filtered through Nylon 66 0.45μ filters prior to use. All aqueous based mobile phases were prepared fresh at three to four day intervals. Standard solutions of the various analytes were prepared in methanol at concentrations of 200-1500 ppm. All analyte solutions at these concentrations were stable during the last eight weeks of the study when stored in clear glass vials at room temperature.

2.2 INSTRUMENTATION

Routine HPLC was performed using a Perkin-Elmer HPLC that included a PE Series 4 LC Microprocessor Controlled Solvent Delivery System, Rheodyne 7125-075 rotary injection valve with 50 μl sample loop, reverse phase columns and LC 85B Variable Wavelength Spectrophotometric Detector. Chromatograms were obtained using a LCI-100 Laboratory Computing Integrator. Typical injection volumes of 10-20 μl were used in most applications and were delivered to the injector using a 100 μl syringe. Full scale absorbance of 5.0 units (5.0 AUFS) was provided from the LC 85B. An attenuation of 1024 on the Computing Integrator is required to display the entire 5.0 unit absorbance scale on the integrator printout. A typical attenuation setting of 16(0.078 AUFS) was determined to be acceptable for the display of most chromatograms in this study.

Several different mobile phase compositions and two different columns available in the Life Support Laboratory Analytical Laboratory were used in this investigation. Descriptions of the mobile phase compositions and columns are included in Table 2 as part of the presentation of results.

A PC controlled Varian Instruments HPLC located in the University of Central Florida Chemistry Department was used to partially characterize selected leachate fractions. This system consists of a Model 9010 Pump Module, rotary injection valve equipped with a 25 μl injection valve, 225 x 4.6 mm Brownlee ODS column with ODS guard column and Model 9065 Photodiode Array Spectrophotometer Detector.

Ultraviolet absorption spectra were obtained using either a Perkin-Elmer 552 UV-Visible spectrophotometer with Hitachi Model 52 X-Y recorder for output or a Beckmann Model DU-64 microprocessor controlled UV-Visible spectrophotometer. Samples were diluted in 40:60 methanol:water that contained 0.13% phosphoric acid.

2.3 LEACHATES

Preliminary studies were performed on samples of untreated plant leachates labelled LS-2, LP-3 and LW obtained from Dr. Jay Garland as were all subsequent leachate samples. Fresh portion of leachate were prepared on June 16, 1992 by mixing 5g of several different dry leachate samples with 100 ml of deionized water. After standing for approximately 2 hr. the solutions were filters under suction through Whatmann No. 1 filter paper. A second filtration of each sample was performed using 0.45 μ Nylon 66 filters. Small portions of samples identified as LP-1, LP-2, LS-1, LS-2 and LS-3 were frozen until subsequent use. A sample identified as MS-T was also obtained. This sample represents a mixed soybean leachate that had been microbially treated to reduce organic matter content. An additional sample identified as LWHS represented treated wheat leachate that had been periodically added to a growth chamber hydroponic solution over a 60 day period.

2.4 SOLID PHASE EXTRACTION (SPE)

Most leachate sample were partitioned into fractions prior to HPLC investigation. Partitioning was accomplished using Sep-Pak Bond-Elute-CH cartridges from Analytichem International. Cartridges were conditioned prior to use by washing with 5-0.8 ml aliquots of methanol and 5-0.8 ml aliquots of water. Flow was controlled at less than 1.0 ml/min by vacuum applied to the cartridge. Accurately measured aliquots of leachate sample and eluent were used to selectively partition components and elute leachate fractions from the Sep-Pak. Leachate not subjected to SPE were filtered through 0.45μ Nylon 66 filters before injection. Eluents from the SPE process were not filtered prior to injection. Treatment of samples by SPE resulted in several eluent fractions depending on the specific scheme employed. Table 1 summarizes the general fraction labelling scheme that was used. It was possible to increase the amount of leachates subjected to SPE by increasing the amount of cartridge packing material. When necessary this was accomplished by removal of packing material from up to six cartridges and dry packing into a single cartridge.

Table 1. Solid Phase Extraction Elution Scheme and Fraction Identification Scheme

SepPak-CH Conditioning	
4 x 0.8 ml Methanol	
4 x 0.8 ml Water	
Leachate Application	
1 or 2 x 0.8 ml Sample*	Fraction 1
Fraction Elution	
1 x 0.8 ml Water	Fraction 2A
1 x 0.8 ml Water	Fraction 2B
etc.	
1 x 0.8 ml 25% Methanol/Water	Fraction 3A1
1 x 0.8 ml 25% Methanol/Water	Fraction 3A2
etc.	Fraction 3A3
1 x 0.8 ml 60% Methanol/Water	Fraction 3B1
1 x 0.8 ml 60% Methanol/Water	Fraction 3B2
etc.	Fraction 3b3
1 x 0.8 ml Methanol	Fraction 4
etc.	
1 x 0.8 ml 0.1% HCL/Methanol	Fraction 5

* The contents of multiple SPE cartridges were combined to increase sample capacity in selected experiments.

2.5 SAMPLE STORAGE

All leachate samples were stored frozen at -4 °C. Fractions obtained from SPE were also stored at -4 °C. Samples were warmed to room temperature prior to use in any experiment.

III. RESULTS AND DISCUSSION

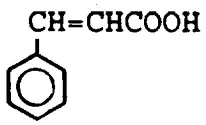
3.1 REPRESENTATIVE COMPOUNDS

Several compounds have been studied in the past in relation to phytotoxic effects that they exhibit on plant growth. Preliminary experimentation in this study focused on the HPLC and SPE behavior of several compounds that were easily obtainable. These compounds are presented in Figure 1 along with their respective chemical structures. Included are several benzoic acid derivatives, cinnamic acid derivatives and flavonoid compounds. Chromatograms that demonstrate the separation possible for mixtures of related compounds are presented in Figure 2. HPLC retention times obtained for several of these compounds under various chromatographic conditions are presented in Table 2. These observations suggest that the separation of fairly complex mixtures of these types of compounds is feasible and practical.

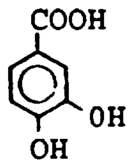
The SPE behavior of six different compounds is summarized in Table 3. The partitioning behavior of compounds with quite similar structure is drastically different. For example salicylic acid demonstrates a relatively large water solubility by predominant elution in f1/2 while ferulic acid which has methoxy groups in the 3 and 5 positions instead of hydroxy groups shows no affinity for the aqueous eluent.

3.2 LEACHATE SOLID PHASE EXTRACTION AND CHROMATOGRAPHY

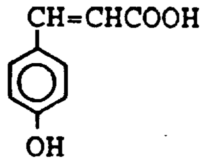
The results of HPLC on four leachate samples are presented in Figure 3. An indication of the complexity of the problem is illustrated by the chromatograms obtained directly from potato, soybean and wheat leachates. An abbreviation scheme as described in the experimental section of this report was employed to identify leachate samples. There are similarities in the measured chromatograms but substantially different specific details are present. Each has a region corresponding to elution times of less than 10 minutes where components with significant water solubility elute. These could be tannin type compounds. In the region corresponding to 10 to 40 minute elution times for compounds with greater organic-like character compared to the earlier eluting components are observed. The general broad band elution pattern with superimposed peaks suggests complex sample makeup.



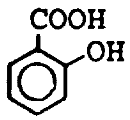
Cinnamic Acid(FW 148.16)



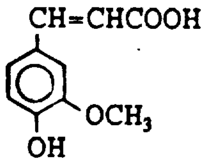
Protocatechuic Acid(FW 154.12)



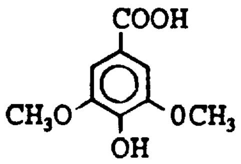
Coumaric Acid(FW 164.16)



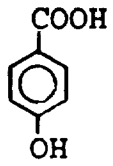
Salicylic Acid(FW 138.12)



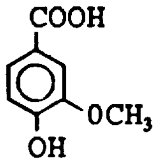
Ferulic Acid(FW 194.19)



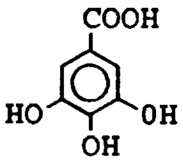
Syringic Acid(FW 198.17)



p-Hydroxy-Benzoic Acid(FW 138.12)

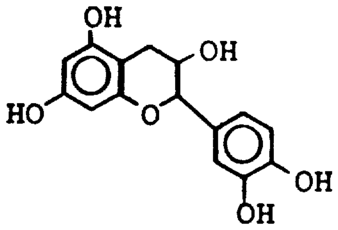


Vanillic Acid(FW 168.14)

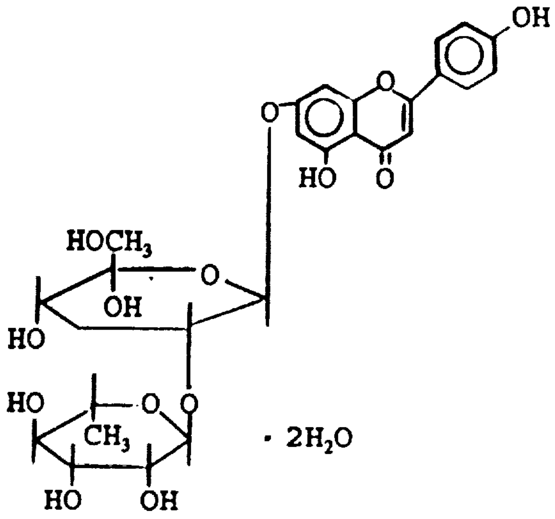


Gallic Acid(FW 170.12)

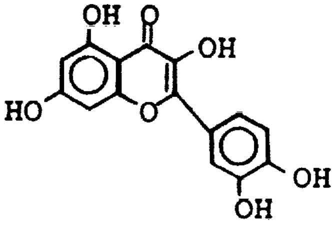
Figure 1. Chemical Structures of Representative Compounds.



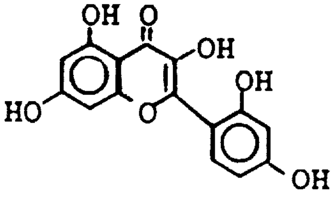
Catechin Hydrate(FW 290.28)



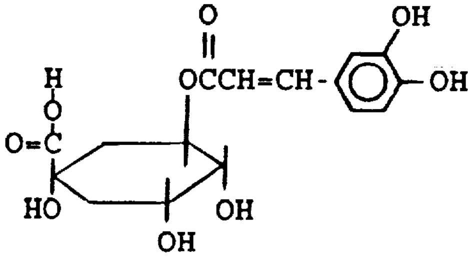
Naringin(FW 616.58)



Quercetin(FW 302.24)



Morin(FW 302.24)



Chlorogenic Acid(FW 354.81)

Figure 1. (Continued)

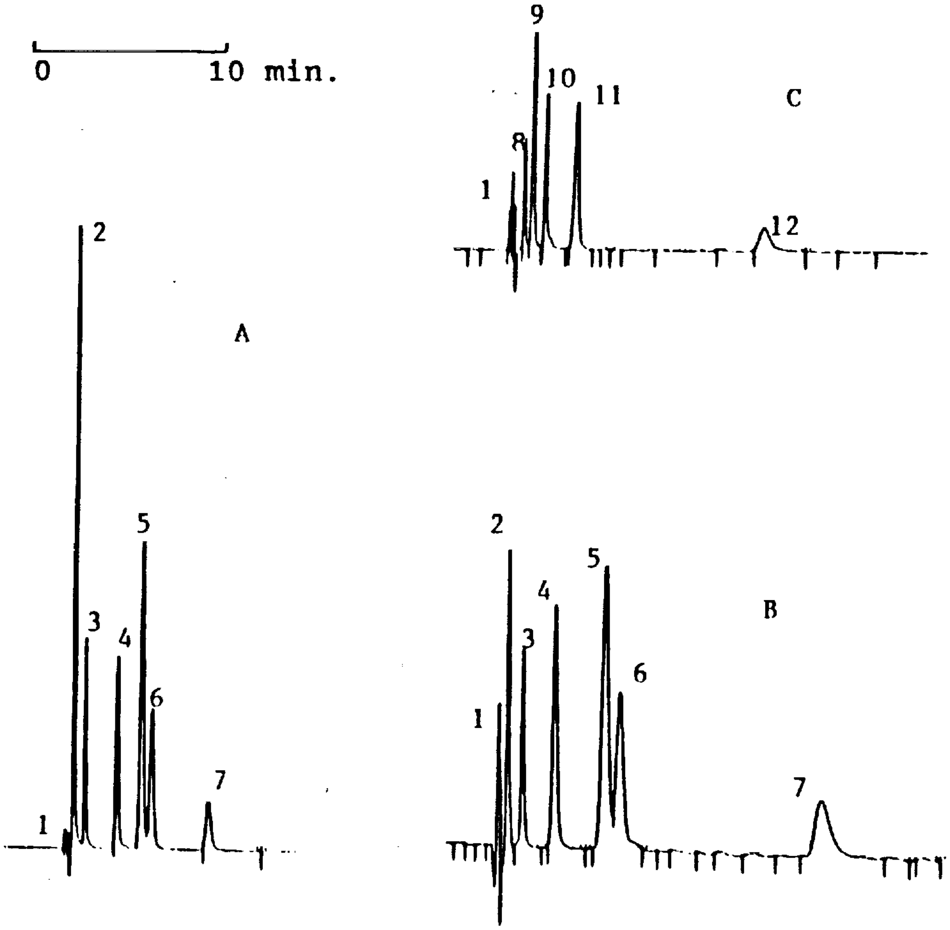


Figure 2. Chromatograms of Some Representative Compounds Measured at 280 nm. Compounds: 1 - V_o, 2 - gallic acid, 3 - protocatechuic acid, 4 - syringic acid, 5 - coumaric acid, 6 - ferulic acid, 7 - salicylic acid, 8 - catechin, 9 - chlorogenic acid, 10 - p-hydroxybenzoic acid, 11 - naringin, 12 - quercetin. A: Column A, mobile phase MP1; B: Column A, mobile phase MP1; C: Column C, mobile phase MP2.

Table 2. Chromatographic Retention Behavior for Representative Compounds

Column	A		B	C				
Mobile Phase Composition								
% Methanol	40	35	40	40	35	30	20	20
0.22% $H_3PO_4:H_2O$	60	65	60	60	60	70	60	65
% Acetonitrile							20	15
Mobile Phase Designation	MP1							MP2
	Retention Times, minutes**							
Gallic Acid	3.4	3.6	2.4	3.3	3.6	3.9	3.2	3.0
Protocatechuic Acid	4.0	4.4	3.0	4.4	5.0	6.1	4.0	3.6
Syringic Acid	5.8	6.6	4.6	7.0	9.3	13.2	5.7	4.5
Coumaric Acid	7.2	9.1	6.9	11.0	15.4	23.3	8.3	5.9
Ferulic Acid	7.6	10.2	7.5	11.9	17.4	27.8	9.1	6.3
Salicylic Acid	10.8	13.6				35.7	19.8	13.0
Cinnamic Acid	25.4							30.5
Naringin	9.0				27.6			9.1
Quercetin	29.5							
Catechin	4.0				4.8			3.9
Chlorogenic Acid	4.8				6.1			3.9
p-HydroxyBenzoic Acid	5.7							5.5

(A) Zorbax C8, 250 x 4.6 mm, 1.0 ml/min, $V_o = 2.7$ ml
(B) Brownlee C18, 225 x 4.6 mm, 1.0 ml/min, $V_o = 2.2$ ml
(C) Altex Econosil C18, 250 x 4.6 mm, 1.0 ml/min, $V_o = 2.2$ ml

** Reported retention times are approximate and will vary by 10% due to variation in flow rates, temperature, etc.

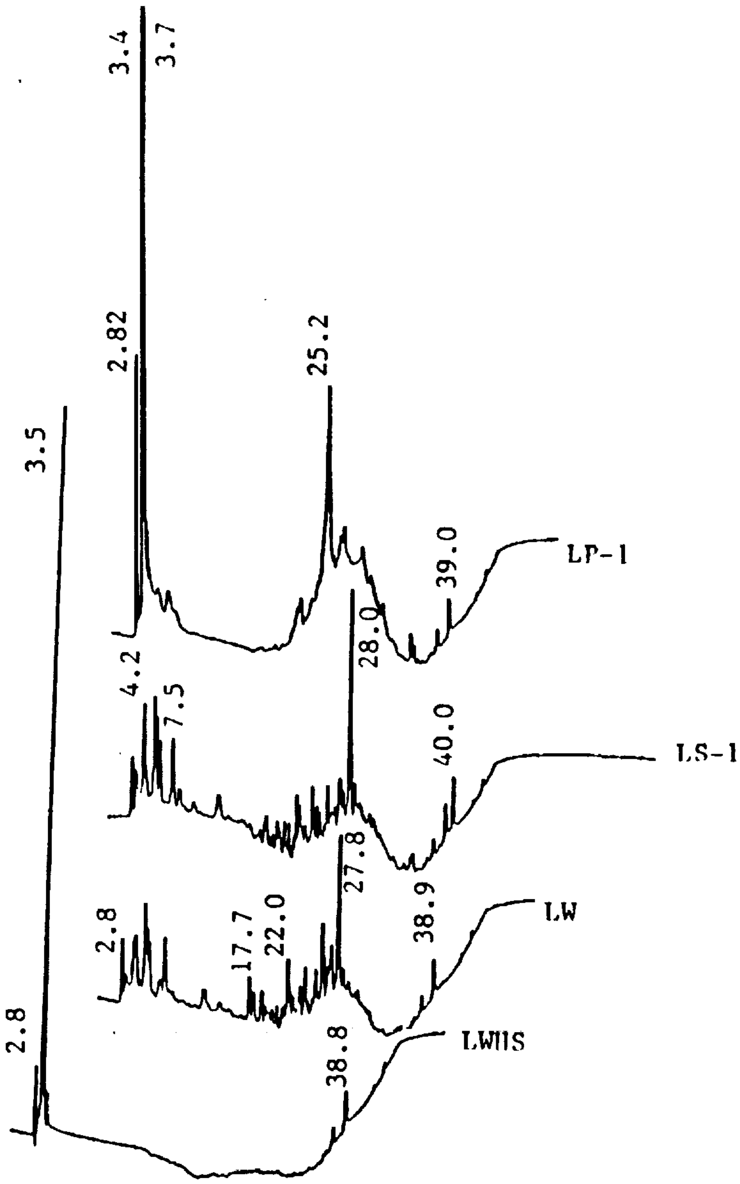


Figure 3. Chromatograms for Potato, Soybean and Wheat Leachates Measured at 280 nm using Mobile Phase Gradient Elution. Column A, mobile phase: 0-10 min. 0.22% aqueous phosphoric acid; 10-40 min. linear ramp 0% to 100% methanol:0.22% aqueous phosphoric acid; 40 min. and longer 100% methanol.

Table 3. Partitioning Behavior for Some Representative Compounds During Solid Phase Extraction.

	Fraction Number		
Components	1/2	3a	3b
Gallic Acid	93%	6%	1%
Protocatechuic Acid	77%	23%	
Syringic Acid		83%	17%
Coumaric Acid		100%	
Ferulic Acid		100%	
Salicylic Acid	87%	13%	

One sample of microbially treated wheat leachate was also chromatographed and the result for this sample is displayed at the bottom of Figure 3. The microbial treatment has substantially reduced the numbers and concentrations of components previously observed to be present in the untreated wheat leachate. It is possible that the chromatograms displayed in Figure 3 do not detail the entire water soluble composition. Only components that exhibit an ultraviolet absorption at 280 nm and that elute from the column during the 1 hr. run will be detected. For example sugars and carbohydrates do not absorb radiation at 280 nm and their presence will not be detected.

Because leachate composition is very complex attempts were made to obtain simpler fractions of the various leachates by SPE. The use of the available SPE-CH cartridge allows for fractionation by removal of nonpolar or moderately polar components from the aqueous sample upon passage through the cartridge. Removal is based on preferential solubility of each considered component in water or the nonpolar cyclohexyl(-CH) chemical moiety. The SPE-SAX cartridge will interact to remove negatively charged ionic species from aqueous solution based upon ion exchange interaction. The chromatograms shown in Figure 4 are obtained from potato leachate after passage through the identified SPE cartridge. Chromatogram A represents eluent from the -SAX and represents non-ionic water soluble constituents. Chromatogram B represents eluent from the -CH and will include anions and highly polar components that will not be retained by the -CH. Chromatogram C represents eluent that is devoid of ionic, moderately polar and non-polar constituents. Each fraction includes a broad band that elutes in less than 6 minutes and each probably consists of a mixture of compounds with similar chemical properties.

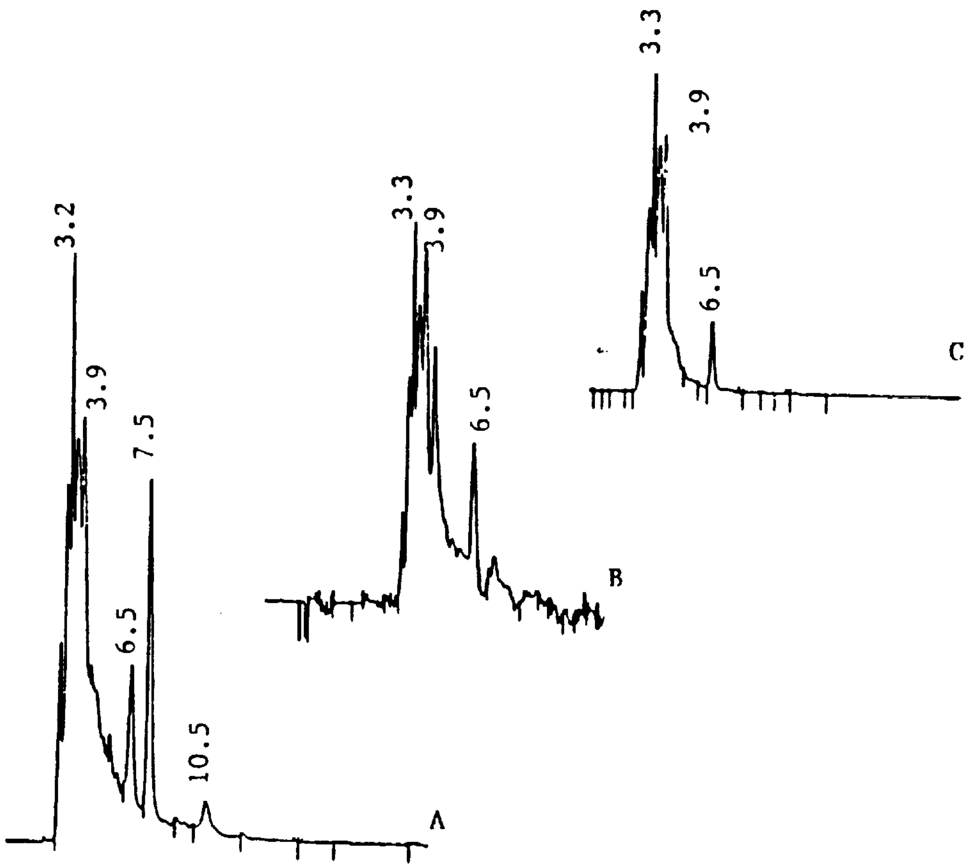


Figure 4. Comparison of Chromatogram Fractions(f1/2) from SPE -CH and -SAX Treatment of Potato Leachate(LP-1) at 280 nm. A: eluent from -SAX; B: eluent from -CH; C: eluent from -CH and -SAX in series. Column A, mobile phase MP1.

Some representative chromatograms for potato and soybean leachate fractions are presented in Figure 5. The two different potato leachates yield nearly identical chromatograms for each fraction but different than those for the one soybean sample shown in Figure 5. Each potato fraction yields one major peak and considerable band background indicative of a complex mixture of compounds which are probable of related structure or similar relative solubility between water and a non-polar organic solvent. The fractions identified as f3a and f3b that correspond to the soybean sample each yield one major peak.

The ability of organic molecules to absorb UV radiation is a function of chemical structure and bonding considerations. Each compound will absorb over a somewhat characteristic range of wavelengths. Chromatogram appearance can therefore be influenced by selection of monitoring wavelength. Chromatograms displayed in Figure 6 demonstrate this effect. Potato leachate fractions were chromatographed and monitored at three different wavelengths. The changing amplitude of the peaks appearing at approximately 6.0 minutes in f1/2, at approximately 6.3 minutes in f3A and the peak at approximately 5.2 minutes in f3B demonstrate how selection of wavelength can influence chromatogram appearance. At the same time it can be possible to derive some information about compound identity from peak amplitude changes that are observed. For example, the peak at 4.5 minutes in f3A corresponds closely to that of protocatechuic acid and protocatechuic acid absorbs more strongly at 254 nm than at 280 nm and does not absorb at 320 nm. The UV absorption spectrum for this compound and other compounds is presented in Figure 7. Chlorogenic acid also has a retention time of approximately 4.5 minutes, however, it absorbs very strongly at 320 nm. Chlorogenic acid can be ruled out as a possible candidate for this peak based on UV absorption properties. Protocatechuic acid while exhibiting UV absorption consistent with that seen in the chromatograms is primarily partitioned into f1/2 and should not be seen in chromatograms of f3A.

The UV spectra presented in Figure 7 were obtained in methanol-0.13% aqueous phosphoric acid to approximate mobile phase composition used for most HPLC separations. Spectra represent various classes of compounds. For example, spectra A-E are from benzoic acid derivatives, Spectra L and M are from cinnamic acid derivatives, spectra N and P are tannic acid and gallic acid, respectively and spectra G and H are for pentahydroxyflavones. Additional reference to these spectra will be presented later in this report.

The use of UV spectral properties for identification is limited in a positive sense because these spectra are devoid of spectral fine-structure that is present in other techniques such as infrared and nuclear magnetic resonance spectroscopy and mass

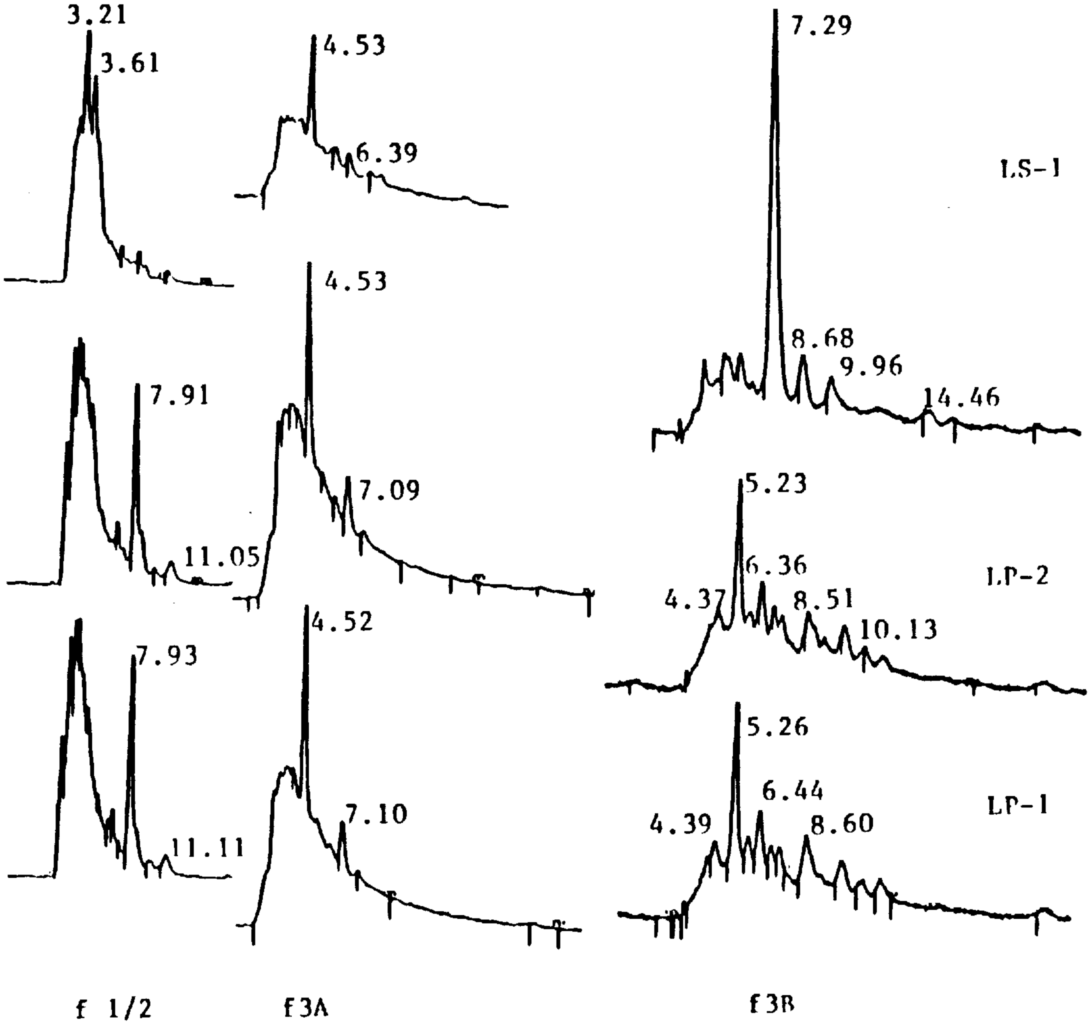


Figure 5. Chromatograms for Aqueous and Aqueous-Methanol SPE Fractions of Leachates Measured at 280 nm. Column A, mobile phase MP1.

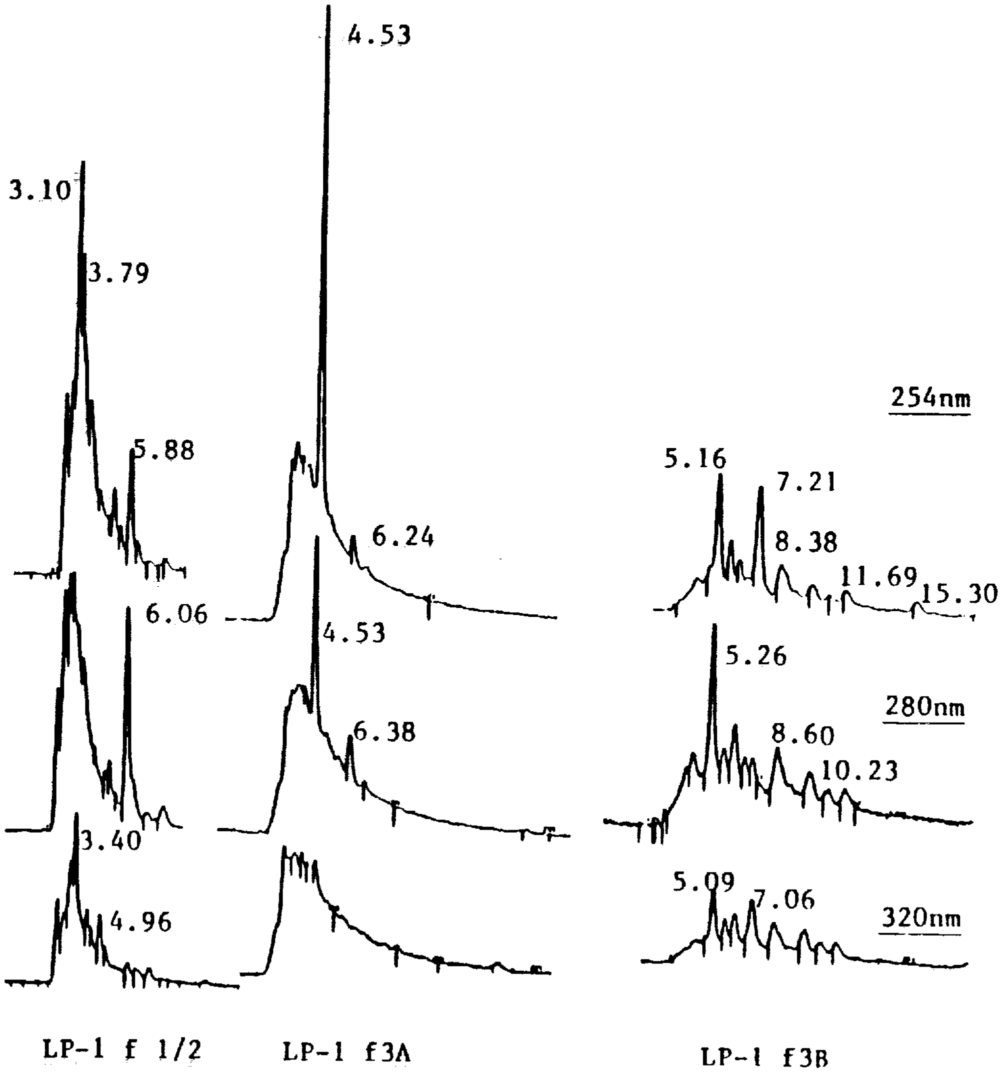
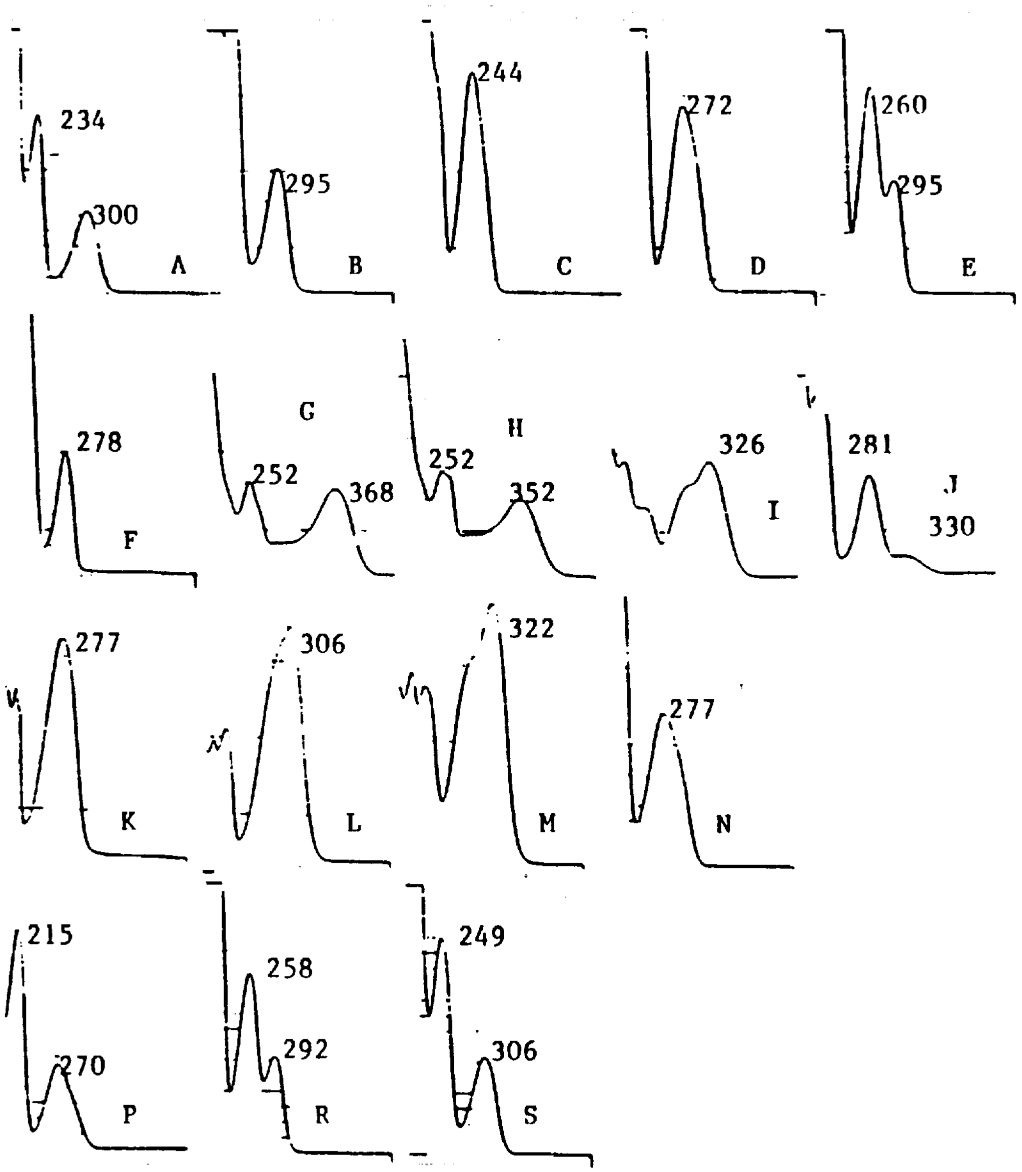


Figure 6. Chromatograms for Potato Leachate SPE Fractions Obtained at Different Wavelengths. Column A, mobile phase MP1.



	Compound	µg/ml		Compound	µg/ml
A	Salicylic Acid	9.5	J	Naringin	10.4
B	m-Hydroxybenzoic Acid	20.4	K	t-Cinnamic Acid	5.4
C	p-Hydroxybenzoic Acid	6.6	L	Coumaric Acid	5.9
D	Syringic Acid	11.8	M	Ferulic Acid	10.5
E	Vanillic Acid	19.2	N	Tannic Acid	10.5
F	Catechin Hydrate	29.1	P	Gallic Acid	5.2
G	Quercetin	4.5	R	Protocatechuic Acid	7.7
H	Morin	4.2	S	3,5-Dihydroxy-Benzoic Acid	19.2
I	Chlorogenic Acid	2.7			

X-axis represents 200nm-450nm.

Y-axis represents 1.0 AUFS.

Figure 7. Ultraviolet Absorption Spectra for Selected Representative Compounds.

spectrometry. It is an ideal choice as a detection technique for HPLC because instrumentation is relative simple and inexpensive and it allows detection to take place at very low concentration levels. Co-chromatography is a technique that can be successfully applied to aid in identification of unknowns in chromatographic separations. Figure 8 shows chromatograms of a soybean leachate fraction without and with addition of two known compounds. Comparison of the two chromatograms clearly show that the peak that is observed at 4.5 minutes is not due to either catechin or chlorogenic acid. The peak at 7.29 minutes in LS-1, f3B(see Figure 5) coincides identically with that of ferulic acid. Co-chromatography of that sample with a second portion of the sample to which ferulic acid had been added resulted in no additional peak. However the peak width had increased slightly. Further chromatography using different mobile phases ultimately resulted in separation of the single peak into two peaks which showed that the sample peak was not due to the presence of ferulic acid. The use of retention time comparisons, co-chromatography and evaluation of UV spectral properties has eliminated any of the compounds identified in Figure 2 as candidates for being present in the leachate samples that were used in this study.

Before leachate is used in a plant growth chamber it is treated microbially to reduce the total carbon content. This is accomplished by conversion of carbohydrates and sugars to carbon dioxide which is removed from the system. This treatment changes the organic content of the leachate. A sample of treated soybean leachate was fractionated by SPE and chromatograms of the fractions were obtained. These chromatograms are shown in Figure 9. Relatively clean chromatograms are obtained at 280 nm which demonstrates the effectiveness of the treatment in modifying composition. Chromatograms obtained at 230 nm also show the relative absence of components in f3A and F3B. The chromatogram of fraction f1/2 shows the presence of compounds that absorb at this lower wavelength. These compounds are not likely to be significantly aromatic or unconjugated in nature because aromatic and extensively conjugated systems will absorb consistent with spectra presented in Figure 7.

In an attempt to gain some insight into the chemical makeup of the leachate fractions, chromatograms were obtained from a small number of SPE fractions of LS-2 using a diode-array detector equipped LC. The results of this endeavor are displayed in Figure 10. It is possible to obtain the UV absorption spectrum for components responsible for major peaks in the chromatograms. Several chromatographic peaks are identified and cross referenced to the UV spectrum obtained from each peak. Peaks numbered 2, 3, 6, 7 and 8 provided spectra of reasonable definition. Spectrum 6 is of a shape similar to that of vanillic acid(see Figure 7) and the other four spectra with broad absorption bands in the 350-380

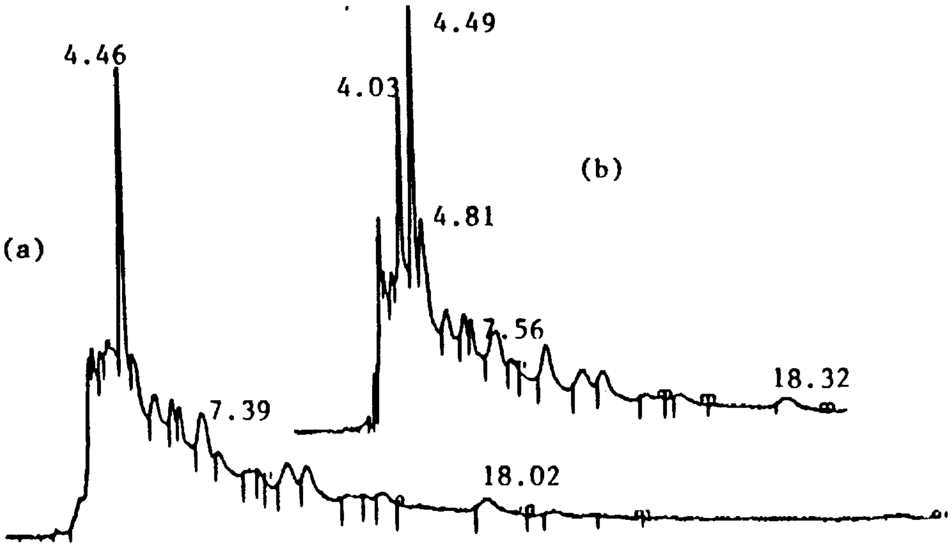


Figure 8. Cochromatography of Catechin and Chlorogenic Acid with the SPE Aqueous Fraction From Soybean Leachate. A: LS-1, 2:3 sample:methanol. B: same as A plus 2.0 ppm catechin(T_r 4.03 min.) and 0.4 ppm chlorogenic acid(T_r 4.81 min.). Column A, mobile phase MP1.

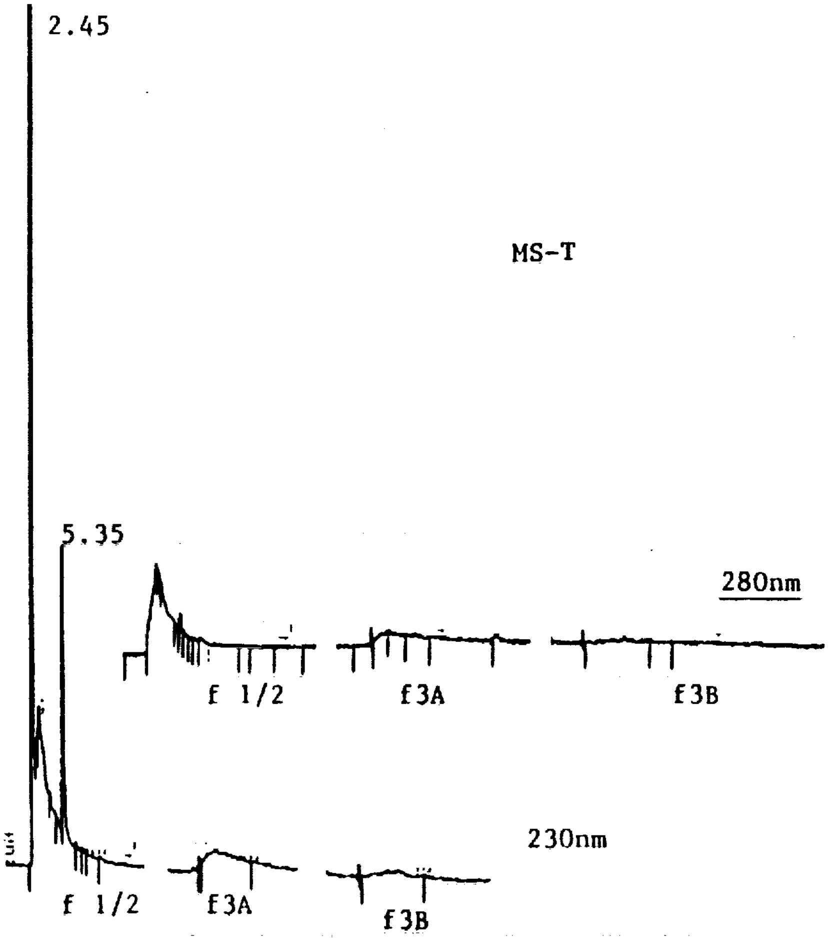


Figure 9. Chromatograms of Microbially Treated Soybean Leachate SPE Fractions. Column A, mobile phase MP1.

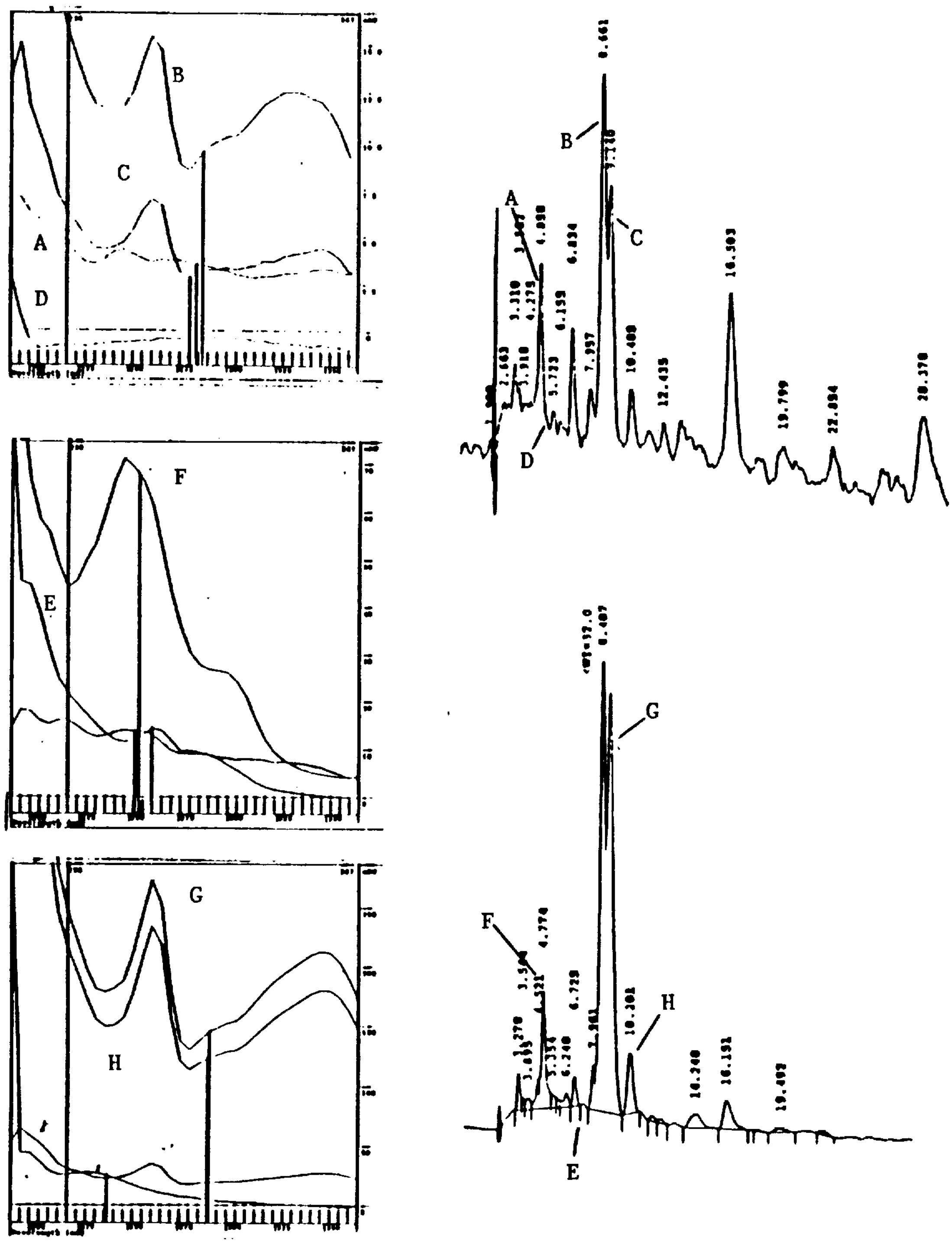


Figure 10. Ultraviolet Absorption Spectra from Selected Chromatogram Peaks Obtained with a Diode Array Detector. Column B; mobile phase MP1; sample LS-2, top chromatogram (f3b4 from 8 ml sample fractionation on -CH bed consisting of the contents from 7 cartridges), bottom chromatogram (f3b1 from 1.6 ml sample fractioned on single -CH bed). Spectra numbering coincides with chromatogram peak numbering. X-axis 190-375 nm.

nm range are similar to those of quercetin and morin(see Figure 7). This spectral evidence suggests that some of the components contained in SPE f3 may by flavonoid compounds. Further study in this direction would seem to be appropriate.

IV. SUMMARY COMMENTS

The use of solid phase extraction and high performance liquid chromatography for characterization of general similarities and differences in the chemical composition of aqueous leachates from crop residues in the Controlled Ecological Life Support System program has been accomplished. The composition is complex and potato, soybean and wheat leachates exhibit general similarities in composition due to the presence of highly colored anionic materials, as well as other colored compounds that can be fractioned from the samples by solid phase extraction. High performance liquid chromatography using ultraviolet absorption as the detection tool was used to further characterize composition. It is apparent that a modest number of individual compounds of unique structure and other groups of compounds with similar structure constitute the organic composition of the leachates. No specific compounds were identified, however, it is possible to determine the presence of tannin-like and phenolic-like classes of compounds in untreated leachate and in microbially treated leachate. It has been demonstrated that several specific phenolic-like compounds that are produced by many crop plants are not present in measurable quantities in the leachate samples that were available. However, it is possible that related compounds are responsible for the chromatographic behavior observed. An extension of this work should include the identification of some of these compounds. Once identified controlled experiments that relate to persistence and accumulation of these compounds in the treated leachate enriched hydroponic solutions used in the growth chambers will be possible. An exhaustive characterization is not possible because of the complexity associated with the leachate composition. In fact it is probably not necessary to fully characterize the composition as long as a general understanding of the type of compounds that are present is documented and studies undertaken to understand the role played by these compounds in the hydroponic solutions.

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