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Determination of saccharides in biological materials by high-performance anion-exchange chromatography with pulsed amperometric detection

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ABSTRACT

High-performance anion-exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) under alkaline conditions (pH 9–13) separates aminosaccharides, neutral saccharides and glycuronic acids based upon their molecular size, saccharide composition and glycosidic linkages. Carbohydrates were extracted by utilizing 0.5 M H₂SO₄ (neutral monosaccharides), 0.25 M H₂SO₄ coupled with enzyme catalysis (glycuronic acids) and 3 M H₂SO₄ (aminosaccharides). Solid-phase extraction with strong cation and strong anion resins was used to partition the cationic aminosaccharides and anionic glycuronic acids and to deionize acid extracts for neutral saccharides. Separation was conducted on a medium-capacity anion-exchange column (36 mequiv.) utilizing sodium hydroxide (5–200 mM) and sodium acetate (0–250 mM) as the mobile phase. The saccharides were detected by oxidation at a gold working electrode with triple-pulsed amperometry. HPAEC–PAD was found superior to high-performance liquid chromatography with refractive index (RI) detection for neutral monosaccharides and aminosaccharides and to low-wavelength UV detection for glycuronic acids in terms of resolution and sensitivity. HPAEC–PAD was not subject to interferences as was the case for low UV detection (210 nm) or RI analyses and was highly selective for mono- and aminosaccharides and glycuronic acids. The use of HPAEC–PAD was applied for the determination of the saccharide composition of organic materials (plant residues, animal wastes and sewage sludge), microbial polymers and soil.

INTRODUCTION

Saccharides play a major role as structural (*e.g.*, cellulose and hemicellulose) components of plants and provide a major source of energy for microbial processes when cycled in soil. Aminosaccharides have been reported in plants [1], microorganisms [2], crustaceae and insects [3] and an important source of organic nitrogen in soil [4]. Uronic acids occur in animal tissue [5], microbes [6] and plant [7] structures. In addition to energy and structural roles in animals, insects, microbes and plants, some of the more important microbial ecological functions of saccharides include conferring virulence of pathogenic bacteria, protecting organisms from desiccation, regulating ionic traffic at the cell surface, concentrating nutrients, protecting microbes from toxic heavy metals or antibacterial agents and attachment to solid surfaces [8].

Detection of a wide diversity of monosaccharides, aminosaccharides and glycuronic acids in nature has led to the development of many analytical techniques.

Current methods for quantification of polysaccharides in biological materials include colorimetric assays [9–13], planar chromatography [14], gas chromatography (GC) [1,15,16] and high-performance liquid chromatography (HPLC) [18,19]. Colorimetric assays do not provide information on the composition of saccharides in biological materials and are often subject to many interferences. Planar chromatography has limitations in separation of similar saccharides. GC analyses involve the necessity of making derivatives. The most common method for quantification of saccharides with HPLC analysis is refractive index (RI) detection, which has several drawbacks. RI detection monitors the bulk property of the LC eluent, thus is non-selective, and cannot easily be used with gradient elution. It also lacks high sensitivity. Uronic acids can be analyzed by UV detection, although the use of low UV wavelengths (210 nm) is subject to interference from other low UV absorbing compounds. The use of high-performance anion-exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) has several advantages over RI and low-UV detection. PAD equipped with a gold electrode is selective only to compounds containing oxidizable functional groups including hydroxyl, amine and sulfide groups. Carboxylic acids and inorganic species do not interfere with detection by PAD. Glycosidic monomers derived from polysaccharides are weak acids with pK_a values in the range 9–13.5 and thus can be separated as anions by controlling the pH of the mobile phase.

Analysis of glycosidic units comprising polysaccharides presents many problems. The susceptibility of the glycosidic linkage to acid hydrolysis varies greatly along with the stability of the resulting monosaccharide unit in the acid solution. Pyranose forms of neutral monosaccharides exhibit the greatest stability to acid conditions, while the furanoses are more susceptible [20]. Polysaccharides containing aminosaccharides or uronic acid units are resistant to mild acid hydrolysis because of the amino and carboxylic moieties.

The objective of this study was to evaluate saccharide extraction, purification and detection of aminosaccharides, neutral saccharides and glycuronic acids present in various biological materials. HPAEC–PAD was used to detect the saccharide composition of various organic materials including plant residues, animal wastes, sewage sludge and soil extracts.

EXPERIMENTAL

Reagents

All neutral monosaccharide and aminosaccharide standards were obtained from Supelco (Bellefonte, PA, U.S.A.). D-Glucuronic acid, D-galacturonic acid and the enzymes, pectolyase from *Aspergillus japonicus* [reported to contain both endopolygalacturonase (EC 3.2.1.15) and endopectin lyase (EC 4.2.2.10)] and β -D-glucuronidase (EC 3.2.1.31) were obtained from Sigma (St. Louis, MO, U.S.A.).

Chromatographic instrumentation

HPAEC–PAD. The HPAEC–PAD analysis was performed on a Dionex (Sunnyvale, CA, U.S.A.) LC gradient pump module and a Model PAD2 detector. Sample injection was via a Dionex autosampler equipped with a 200- μ l sample loop. Neutral monosaccharides, aminosaccharides and glycuronic acids were separated on a CarboPac PA1 pellicular anion-exchange resin (250 \times 4 mm I.D.) and a CarboPac PA

guard column (25 × 3 mm I.D.) at a flow-rate of 0.8 ml min⁻¹ at ambient temperature. Neutral monosaccharides were separated with the following gradient [21]:

Eluent A: 18 MΩ 0.22-μm filtered water; Eluent B: 50 mM sodium hydroxide containing 1.5 mM sodium acetate

| Time (min) | Eluent | |
|------------|--------|-------|
| | A (%) | B (%) |
| 0 | 93 | 7 |
| 15 | 93 | 7 |
| 25 | 0 | 100 |
| 30 | 93 | 7 |

Aminosaccharides were separated with the following gradient [22]:

Eluent A: 18 MΩ 0.22-μm filtered water; Eluent B: 100 mM sodium hydroxide; Eluent C: 200 mM sodium hydroxide

| Time (min) | Eluent | | |
|------------|--------|-------|-------|
| | A (%) | B (%) | C (%) |
| 0 | 95 | 5 | 0 |
| 15 | 95 | 5 | 0 |
| 20 | 0 | 0 | 100 |
| 25 | 0 | 0 | 100 |
| 30 | 95 | 5 | 0 |

Sodium hydroxide (200 mM) was used to elute interfering species that may act as displacing ions and shorten the retention times of subsequent runs. Uronic acids were separated with the following gradient [23]:

Eluent A: 18 MΩ 0.22-μm filtered water; Eluent B: 200 mM sodium hydroxide; Eluent C: 100 mM sodium hydroxide containing 250 mM sodium acetate

| Time (min) | Eluent | | |
|------------|--------|-------|-------|
| | A (%) | B (%) | C (%) |
| 0 | 25 | 25 | 50 |
| 10 | 25 | 25 | 50 |
| 25 | 8 | 8 | 84 |
| 30 | 25 | 25 | 50 |

All mobile phases were degassed to prevent absorption of carbon dioxide producing carbonate. Carbonate will act as a displacing ion and shorten retention times. Detection was by triple-pulsed amperometry with a gold working electrode [24]. The following working pulse potentials (E) and durations (t) were used for the detection of saccharides: $E_1 = 0.10$ V ($t_1 = 300$ ms); $E_2 = 0.60$ V ($t_2 = 120$ ms); $E_3 = -0.60$ V ($t_3 = 60$ ms). The CHOH groups are oxidized at E_1 , E_2 removes the reaction prod-

ucts, while E_3 cleans the electrode at a negative potential. Cyclic voltammetry was used to choose the three potentials. The PAD response time was set to 1 s. Chromatographic data were collected and plotted using the Dionex AutoIon 300 software. HPLC-RI and UV determinations were described in previous studies for aminosaccharides [22], neutral monosaccharides [21] and glycuronic acids [23].

Saccharide extraction

Neutral monosaccharides and glycuronic acids were extracted by the methods described by Martens and Frankenberg [21,23]. Briefly, the samples were pretreated with 0.4 ml 6 M H_2SO_4 for 2 h at room temperature and then refluxed with 0.5 M H_2SO_4 for 16 h at 80°C. Neutral saccharides in poultry manure and sewage sludge were extracted as described above and, in addition, refluxed with 3 M H_2SO_4 for 5 h at 80°C. Aminosaccharides were extracted by the method of Martens and Frankenberg [22] with a 6 M H_2SO_4 pretreatment and a 3 M H_2SO_4 reflux for 16 h at 90°C. The neutral monosaccharides, aminosaccharides and glycuronic acid samples were then treated with 1 ml 0.1 M EDTA, titrated to pH 4 with 5 M KOH and centrifuged at 10 000 rpm (RCF = 12 062).

Purification of acidic extracts

Neutral monosaccharides. The colored materials present in the refluxed organic materials (poultry manure, sewage sludge, straw and alfalfa) and soil extracts were removed by filtration through a Supelco solid-phase extraction column system composed of a 3-ml strong cation (SCX, 3-propylsulfonic acid, H^+) and a 3-ml strong anion (SAX, 3-quaternary propylammonium, Cl^-) column [21].

Aminosaccharides. A 1-ml aliquot of the refluxed organic amendments, microbial polymers, or soil extracts was diluted to 5 ml with water and passed through a Supelco 3-ml SCX solid-phase extraction column. The SCX column was then rinsed with 3 ml of water to elute all non-retained compounds. The aminosaccharides were eluted with 5 ml of 0.3 M HCl [22].

Glycuronic acids. A 1-ml aliquot of the acid-hydrolyzed sample was diluted to 5 ml and passed through a SCX column (Supelco). Pectolyase (3 units ml^{-1} ; pH 5.5) or β -D-glucuronidase (30 units ml^{-1} ; pH 6.8) were added to the SCX eluent and the mixture was incubated at ambient temperatures overnight (16 h). The enzyme-extract mixture was passed through a SAX column (Supelco) and rinsed with several ml of water. The glycuronic acids were then eluted with 0.1 M NaCl (pH 8.0) [23].

All samples were filtered before analysis through a Millipore type GS 0.22- μm filters (Bedford, MA, U.S.A.).

RESULTS AND DISCUSSION

Extraction

The following solvents have been reported for extraction of saccharides in biological materials: water, aqueous buffers, complexing reagents, mineral acids (0.25–12 M), organic reagents, alkali and combinations of the above [25]. The association between saccharides and soil constituents is believed to involve hydrogen and covalent bonds [26]. Sulfuric acid is often used as the extracting reagent to break these bonds.

Several reports have shown that aminosaccharides can be extracted from biological materials by treatment with hot mineral acids [1,27,28]. Isolation of uronic acids from polysaccharides, however, is very difficult because their glycosidic linkages are more stable than that of the neutral saccharides. Higher concentrations of mineral acids (3–6 $M H^+$) have been employed to release glycuronic acids [16,29,30], but these concentrations can also decarboxylate the free glycuronic residues [31] and convert them into lactones which are difficult to separate from the neutral saccharides [17].

Various extraction procedures have been used for quantifying saccharides in soils and organic amendments with different degrees of effectiveness. Seven monosaccharides in soil were detected by GC [15]. Angers *et al.* [19], using a lead-loaded cation-exchange column with HPLC-RI, detected five monosaccharides in soil. They recognized problems with coelution of certain monosaccharides with their technique. Coelution of saccharides with HPLC-RI is a common problem as illustrated in Fig. 1. A sample of barley straw was separated on a calcium-based resin column [21]. The solutes were collected with a fraction collector and analyzed with HPAEC-PAD. This work clearly shows that glucose and galactose coelute during the analysis. Retention times of standards analyzed by HPLC-RI indicated that complex materials may be prone to coelution of xylose, mannose and rhamnose with galactose and fucose with arabinose [21]. These saccharides must be resolved because they are important constituents of soils. HPAEC-PAD analyses indicated that in addition to these saccharides, inositol, mannitol and/or ribitol are also present in soils, plant residues, animal wastes and sewage sludge. A HPAEC-PAD chromatogram of ten saccharide standards shows the potential of ion-exchange chromatography for the analysis of saccharides with high resolution (Fig. 2).

Recent reports have suggested that saccharides determined in hot water extracts of soil samples by the anthrone-sulfuric acid analysis were significantly correlated

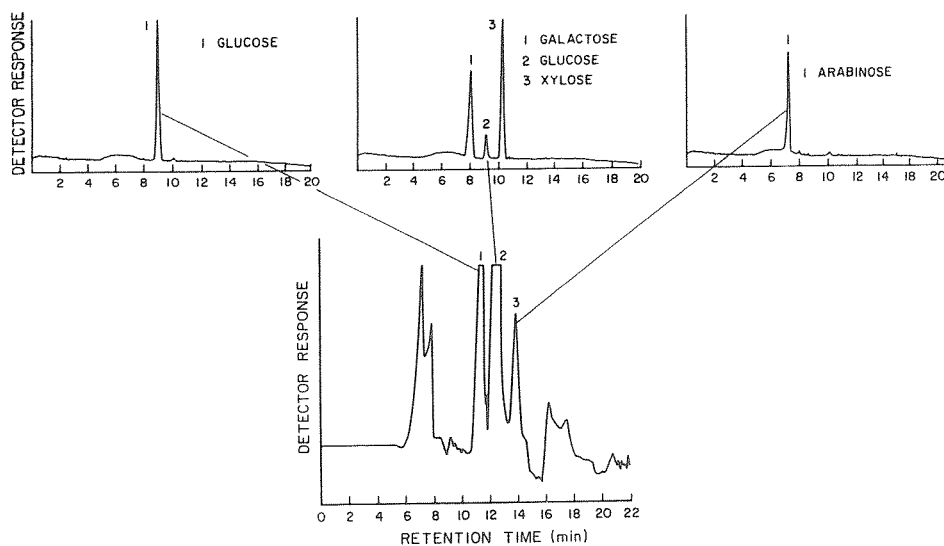


Fig. 1. Chromatogram of an acidic extract of barley straw detected by HPLC-RI. Insets are HPLC-RI fractions collected and then analyzed by HPAEC-PAD.

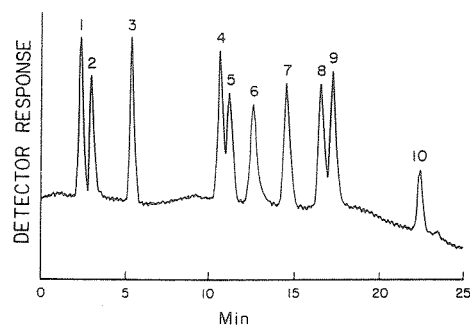


Fig. 2. Chromatogram of saccharides detected by HPAEC-PAD. Peaks: 1 = inositol; 2 = ribitol; 3 = fucose; 4 = arabinose; 5 = rhamnose; 6 = galactose; 7 = glucose; 8 = xylose; 9 = mannose; 10 = lactose (internal standard).

with the structural improvement in soil [32–33]. Martens and Frankenberg [34] found that little or no saccharides were extracted from organic-amended soil with hot (80°C) or cold (25°C) water and that the anthrone–sulfuric acid analysis of these extracts was prone to many interferences. However, the combination of heat (80°C) with H_2SO_4 concentrations as low as 0.13 M was effective in extracting saccharides (Fig. 3). The optimum concentration of H_2SO_4 for extraction of saccharides in soils and plant materials was determined to be 0.5 M (Table I). However, this $[H^+]$ is not

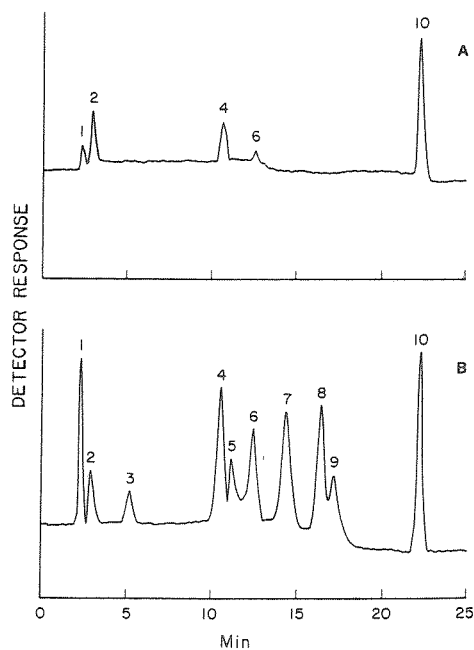


Fig. 3. HPAEC-PAD chromatograms of the acidic extracts of (A) straw-amended soil extracted with 2.5 M H_2SO_4 (25°C) and (B) straw-amended soil extracted with 0.13 M H_2SO_4 (80°C). Peaks as in Fig. 2.

TABLE I
COMPARISON OF H₂SO₄ CONCENTRATIONS AND HEAT ON EXTRACTION OF SACCHARIDES FROM BIOLOGICAL SAMPLES DETECTED BY HPAEC-PAD

| Treatment | Saccharides extracted (mg kg ⁻¹) | | | | | | | | | | |
|--|--|---------|-----------------|-----------|----------|-----------|---------|--------|---------|-------|--|
| | Inositol | Ribitol | Fucose | Arabinose | Rhamnose | Galactose | Glucose | Xylose | Mannose | Total | |
| <i>Soil</i> | | | | | | | | | | | |
| 0.13 M H ₂ SO ₄ (80°C) | 35 | 53 | 20 | 279 | 48 | 265 | 192 | 242 | 62 | 1196 | |
| 0.5 M H ₂ SO ₄ (80°C) | 38 | 46 | 30 | 303 | 45 | 365 | 423 | 208 | 157 | 1616 | |
| 2.5 M H ₂ SO ₄ (25°C) | 13 | 25 | ND ^a | 133 | ND | Trace | ND | ND | ND | 172 | |
| <i>Poultry manure</i> | | | | | | | | | | | |
| 0.5 M H ₂ SO ₄ (80°C) | 213 | 232 | 160 | 2313 | ND | ND | 172 | ND | ND | 3090 | |
| 3.0 M H ₂ SO ₄ (80°C) | 300 | 104 | 231 | 2889 | 349 | 2055 | 2906 | 6425 | 488 | 15644 | |
| <i>Earthworm casts</i> | | | | | | | | | | | |
| 0.5 M H ₂ SO ₄ (80°C) | 38 | 39 | 288 | 2210 | 396 | 1736 | 1099 | 2951 | 658 | 9518 | |
| 3.0 M H ₂ SO ₄ (80°C) | 59 | 32 | ND | 318 | ND | 189 | 1196 | 320 | 462 | 2547 | |

^a ND = not determined.

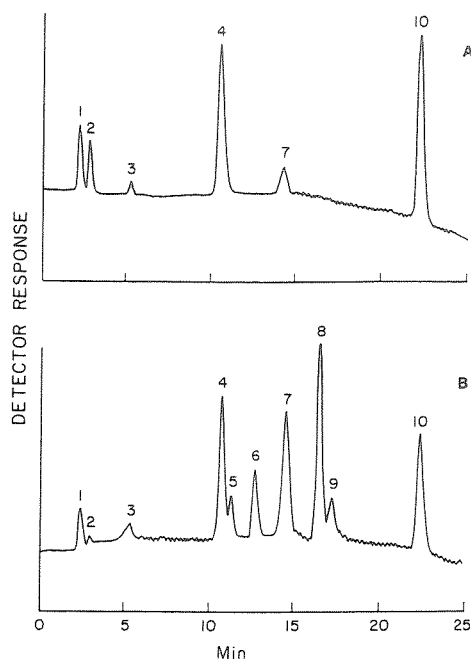


Fig. 4. HPAEC-PAD chromatograms of the acidic extracts of (A) poultry manure extracted with 0.5 M H₂SO₄ (80°C) and (B) poultry manure extracted with 3.0 M H₂SO₄ (80°C). Peaks as in Fig. 2.

effective for extracting saccharides in sewage sludge or poultry manure. Fig. 4 indicates that low levels of saccharides were extracted by 0.5 M H₂SO₄ from poultry manure and that much higher H₂SO₄ concentrations were required. The optimum H₂SO₄ concentration for saccharide determination in animal waste and sludge was 3.0 M H₂SO₄.

Research has shown that earthworm (*Lumbricus terrestris*) activity can have a beneficial effect on soil. Part of this benefit may be due to the breakdown of organic residues and deposition of saccharide-rich worm casts on the soil surface (Fig. 5).

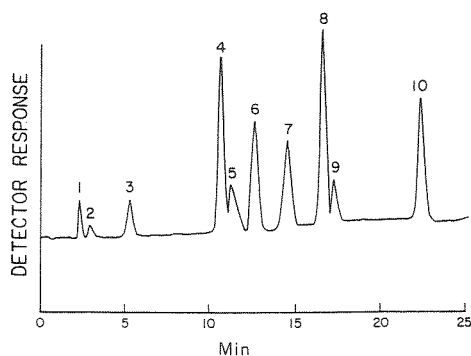


Fig. 5. HPAEC-PAD chromatogram of the acidic extract of worm casts (0.5 M H₂SO₄; 80°C). Peaks as in Fig. 2.

These casts were found to be $7.5 \times$ richer in saccharide content than the surrounding soil (Table I). The optimum H_2SO_4 concentration for saccharide extraction from earthworm casts was determined to be 1 *M*.

Saccharide analysis of acid extracts of various biological materials by HPAEC-PAD has revealed new information on the composition of these materials. The saccharide composition of plant residues appears to be composed mainly of arabinose, galactose, glucose and xylose (Table II). The composition of poultry manure, sewage sludge and soil, in addition to the above four listed saccharides, included fucose, rhamnose and mannose units (Tables II and III). The analysis of worm casts showed the same trend as soil and animal wastes but tended to be richer in the saccharides arabinose, galactose, glucose and xylose, reflecting the decomposition of plant residues (Table I).

TABLE II
COMPOSITION AND QUANTITY OF AMINO-, MONOSACCHARIDE AND URONIC ACIDS
EXTRACTED FROM ORGANIC AMENDMENTS

| Polysaccharide material | Organic amendment (mg kg ⁻¹) | | | |
|-------------------------|--|---------------|--------|---------|
| | Poultry manure | Sewage sludge | Straw | Alfalfa |
| <i>Monosaccharides</i> | | | | |
| Inositol | 514 | 556 | 1042 | 11842 |
| Ribitol | ND ^b | 196 | 271 | ND |
| Mannitol | 337 | ND | 490 | ND |
| Fucose | 391 | 1320 | ND | ND |
| Arabinose | 5203 | 10503 | 26407 | 40961 |
| Rhamnose | 350 | 1846 | ND | 46 |
| Galactose | 2296 | 4409 | 8754 | 20222 |
| Glucose | 3079 | 3338 | 12457 | 31337 |
| Xylose | 6575 | 2599 | 154035 | 58045 |
| Mannose | 734 | 2930 | ND | 373 |
| Total | 19479 | 27697 | 203456 | 162826 |
| <i>Aminosaccharides</i> | | | | |
| Galactosamine | 49 | 188 | 1405 | 1825 |
| Mannosamine | 39 | 90 | 230 | 685 |
| Glucosamine | 38 | 115 | 905 | 1212 |
| N,N'-Diacetylchitobiose | 187 | 84 | 13140 | 6040 |
| Acetylgalactosamine | 18 | 35 | 149 | 245 |
| Acetylmannosamine | 67 | 73 | 390 | 1565 |
| Acetylglucosamine | 60 | 59 | 968 | 1950 |
| Total | 450 | 643 | 17195 | 13533 |
| <i>Glycuronic acids</i> | | | | |
| Galacturonic acid | 212 | 740 | 39790 | 34900 |
| Glucuronic acid | 195 | 987 | 58890 | 43880 |
| Total | 407 | 1787 | 98680 | 78780 |
| Total ^a | 20336 | 30127 | 319331 | 255139 |

^a Sum of monosaccharides + aminosaccharides + glycuronic acids.

^b ND = Not determined.

TABLE III
COMPOSITION AND QUANTITY OF AMINO-, MONOSACCHARIDE AND URONIC ACIDS
EXTRACTED FROM ORGANIC AMENDED AND UNAMENDED SOIL

| Polysaccharide material | Organic-amended soil (mg kg ⁻¹) | | | | |
|-------------------------|---|---------------|-------|---------|-------------------|
| | Poultry manure | Sewage sludge | Straw | Alfalfa | Check (unamended) |
| <i>Monosaccharides</i> | | | | | |
| Inositol | 189 | 212 | 238 | 232 | 187 |
| Ribitol | 64 | 65 | 97 | 61 | 49 |
| Fucose | 91 | 88 | 88 | 94 | 82 |
| Arabinose | 421 | 386 | 419 | 404 | 288 |
| Rhamnose | 120 | 169 | 109 | 128 | 89 |
| Galactose | 407 | 413 | 391 | 332 | 292 |
| Glucose | 282 | 307 | 308 | 237 | 201 |
| Xylose | 708 | 342 | 497 | 335 | 252 |
| Mannose | 62 | 67 | 74 | 78 | 55 |
| Total | 2344 | 2049 | 2221 | 1901 | 1495 |
| <i>Aminosaccharides</i> | | | | | |
| Galactosamine | 4 | 5 | 5 | 13 | 1 |
| Mannosamine | 3 | 3 | 3 | 2 | <1 |
| Glucosamine | 4 | 5 | 5 | 34 | 2 |
| N,N'-diacetylchitobiose | 6 | 3 | 4 | 5 | 1 |
| Acetylgalactosamine | <1 | 1 | 13 | 1 | 1 |
| Acetylmannosamine | 2 | 3 | 3 | 0 | 1 |
| Acetylglucosamine | 3 | 2 | 3 | 1 | <1 |
| Total | 24 | 23 | 36 | 56 | 7 |
| <i>Glycuronic acids</i> | | | | | |
| Galacturonic acid | 94 | 20 | 2 | 7 | 2 |
| Glucuronic acid | 28 | 132 | 16 | 42 | 11 |
| Total | 122 | 170 | 218 | 49 | 13 |
| Total ^a | 2490 | 2242 | 2275 | 2006 | 1515 |

^a Sum of monosaccharides + aminosaccharides + glycuronic acids.

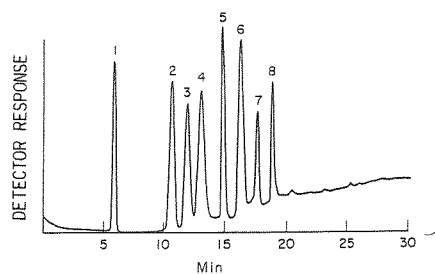


Fig. 6. Chromatogram of aminosaccharides detected by HPAEC-PAD. Peaks: 1 = 2-deoxyribose (internal standard); 2 = galactosamine; 3 = mannosamine; 4 = glucosamine; 5 = N,N'-diacetylchitobiose; 6 = acetylgalactosamine; 7 = acetylmannosamine; 8 = acetylglucosamine.

Many of the oligosaccharides found in plant, microbial and animal tissues consist of various combinations of neutral monosaccharides, aminosaccharides and uronic acids [20]. Fig. 6 shows the separation of seven aminosaccharides by HPAEC-PAD. Cation-exchange chromatography (SXC) [22] separates the aminosaccharides from the neutral monosaccharides and glycuronic acids released by the hydrolysis process. Aminosaccharides, principally chitin (acetyl-D-glucosamine repeating units), have been reported to be present in the cell walls of algae and fungi but have not been found as a component in higher plants [35]. Both straw (Fig. 7) and alfalfa were found to contain large amounts of aminosaccharides (Table II), with the majority of aminosaccharides present in straw as chitobiose (N,N'-diacetylchitobiose) [22]. In lower plants, chitin frequently replaces cellulose as the structural entity in cell walls and may have the same role in maintaining the structural integrity of straw and alfalfa. The aminosaccharide-N fraction in straw was found to account for about 20% of the total N content [22]. Aminosaccharides were also found to compose about 8% of a bacterial polymer produced by *Chromobacterium violaceum* [22] (Fig. 7). Incorporation of straw and alfalfa residues into soil and incubation for eight months resulted in decomposition of nearly all of the added aminosaccharides (Table III).

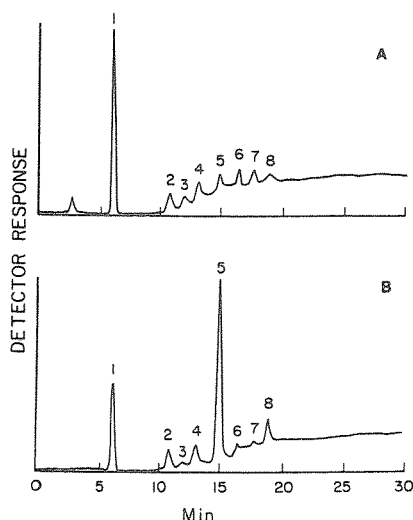


Fig. 7. HPAEC-PAD chromatograms of the acidic extracts (3.0 M H₂SO₄; 90°C) of (A) a bacterial polymer produced by *Chromobacterium violaceum* and (B) barley straw. Peaks as in Fig. 6.

The use of pectolyase and β -D-glucuronidase enzymes can be a powerful tool in the preparative separation and elucidation of the structure of polysaccharides. The use of mild acid hydrolysis (0.25 M H₂SO₄) to fragment the saccharide polymers coupled with enzyme catalysis released large amounts of uronic acids from the plant materials but released less from the animal waste or sewage sludge and only trace amounts from the organic-amended soil (Tables II and III). Fig. 8 shows a chromatogram of galacturonic acid and glucuronic acid as detected by HPAEC-PAD. Structural analysis of a hemicellulose fraction of barley straw indicated that uronic acids

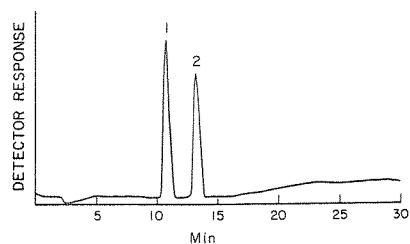


Fig. 8. Chromatogram of glycuronic acids detected by HPAEC-PAD. Peaks: 1 = galacturonic acid; 2 = glucuronic acid.

and L-arabinose monomers link the xylose chains to form the structural polymers [36]. Treatment of the barley straw extract with pectolyase resulted in the release of low levels of uronic acids but use of β -D-glucuronidase with the same extract resulted in a $10\times$ increase in the release of uronic acids (Fig. 9). Incorporation of the straw residue into soil resulted in a rapid decomposition of added uronic acids and after eight months, only trace amounts were present (Table III).

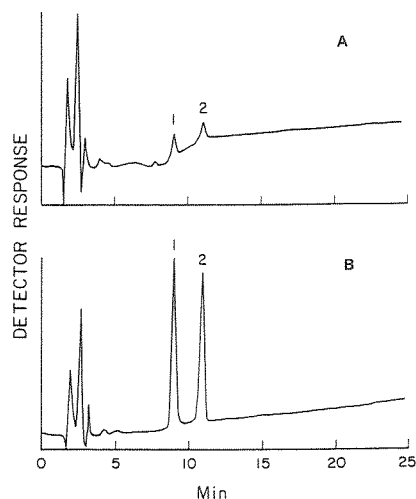


Fig. 9. HPAEC-PAD chromatograms of the acidic-enzyme extracts of (A) barley straw treated with pectolyase and (B) barley straw treated with β -D-glucuronidase. Peaks as in Fig. 8.

CONCLUSIONS

The work presented here showed that plant materials, animal waste, sewage sludge and soil contain neutral monosaccharides, aminosaccharides and glycuronic acids. The saccharide composition of each of these materials was quantified by optimizing the conditions for extraction, purification and chromatography. The use of HPAEC-PAD for separation of saccharides in these complex materials was superior to HPLC-RI and HPLC-UV in terms of sample preparation, resolution and sensitivity.

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