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Preliminary Geochemical, Microbiological, and Epidemiological Investigations into Possible Linkages between Lignite Aquifers, Pathogenic Microbes, and Kidney Disease in Northwestern Louisiana

by

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ABSTRACT

In May 2002, 15 wells and four surface water sites were sampled, and in September 2002, those same wells and sites plus four additional surface sites were sampled in five parishes of northwestern Louisiana. A geographic information system (GIS) was used to select residential water wells for sampling. Well water samples were analyzed for pH, conductivity, organic compounds, and nutrient and anion concentrations. All samples were further tested for presence of fungi (maintained for up to 28 days and colonies counted and identified microscopically), and metal and trace element concentration by inductively-coupled plasma mass spectrometry and atomic emission spectrometry. Surface water samples were tested for dissolved oxygen and evidence of leptospiral bacterial presence. A polymerase chain reaction protocol was optimized for detection of pathogenic leptospires, and the sensitivity of the assay was determined. The Spearman correlation method was used to assess the association between the endpoints for these field/laboratory analyses and the incidence of cancer of the renal pelvis obtained from the Louisiana Tumor Registry. Significant associations were revealed between the cancer rate and the overall number of organic compounds, the fungi Zygomycetes, the nutrients PO_4 and NH_3, and thirteen chemical elements (As, B, Br, Cl, Cr, F, Li, Na, P, Rb, Se, Sr, W) from the well water as compared to the controls. Among the species of fungi from the total of 136 isolates were 12 *Penicillium* spp., at least two *Aspergillus* spp., a number of other genera (*Alternaria* sp., *Eupenicillium lapidosum*, *Cladosporium* sp., *Epicoccum* sp., *Trichoderma* sp., *Paecilomyces* sp., *Chrysosporium* sp., *Chloridium* sp.), and Zygomycetes, and Coelmycetes -- some of which are known mycotoxin producers. The two control wells yielded a mean of 6.5 (SD = 3.5355) individual isolates, while the mean number of isolates from all other sites was 7.6 (SD = 4.4866). Presence of human pathogenic leptospires was detected in 4/8 (50 percent) of the surface water sites sampled. These initial results suggest that additional investigation into these relationships is warranted.

INTRODUCTION

The state of Louisiana has one of the highest rates of kidney cancer in the United States of America (USA). Rural residents in the northwestern part of the state rely on well water for drinking, cooking, and other domestic uses. This region contains sizeable lignite (low-rank coal) deposits that could serve as sources for naturally occurring, potentially nephrotoxic compounds and contaminate ground water used as residential water supplies. Risk of kidney cancer could be elevated due to long-term exposure to low levels of such contaminants, especially in conjunction with infection by a nephrotropic pathogen such as *Leptospira* spp., the bacterial etiologic agent of leptospirosis, or Weil's disease. Conversely, history of long-term exposure to low levels of nephrotoxic organic compounds could predispose a leptospirosis patient to a more severe disease course. Certain fungal toxins have also been implicated as a possible contributing cause of Balkan endemic nephropathy, a potentially fatal kidney disease often associated with these same kidney cancers. Balkan endemic nephropathy (BEN) occurs in restricted geographic areas characterized by lignite deposits, stable rural populations, and untreated, non-municipally supplied water sources. The goals of this study are to: (1) gather and analyze preliminary data to determine if conducting a rigorous epidemiological study is warranted, (2) develop novel methods for detecting pathogenic leptospires from surface water using molecular biological markers, (3) adapt standard microbiological culturing techniques to determine the occurrence of nephrotoxin-producing fungi.

This Open-File Report (OFR) presents the results of the preliminary phase of an investigation designed to address the extent, if any, to which exposure to specific factors in the natural environment are in part, or wholly, responsible for kidney cancer, specifically cancers of the renal pelvis (RPC), in certain geographic areas of the USA. The state of Louisiana has the sixth highest kidney cancer mortality rate in the United States (Ries and others, 2001). Rates of cancer of the renal pelvis and kidney for the parishes in the study area are shown in figure 1. Wyoming ranks first in the USA for kidney cancer mortality, and North and South Dakota rank third and fourth, respectively (Ries and others, 2001). Large low-rank lignite deposits occur in several parishes of northwestern Louisiana (figures 2, 3 and 4). Lignite deposits are also present in the state of Wyoming and in the Dakotas, but not in many other states in the

USA. These low-rank coals are relatively unmetamorphosed and chemically reactive. All of the states mentioned have large rural populations that obtain drinking water from wells. The possibility of a link between lignite deposits, drinking water derived from rural wells, and RPC in these states is suggested by investigations undertaken by the United States Geological Survey (USGS) researchers into Balkan endemic nephropathy (BEN) and urothelial cancers (a type of RPC) in Eastern Europe (Tatu and others, 1998). Toxic organic compounds such as nitrogen-containing aromatic hydrocarbons have been identified in lignite and well water in Romania, Serbia, and Bulgaria. Microbial pathogens have also been implicated in the causation of BEN (Stoev, 1998; Uzelac-Keserovic and others, 1999). This OFR releases the data from initial feasibility studies; more interpretive work will follow.

We hypothesize that rural well water in northwestern Louisiana is contaminated with microbial pathogens associated with kidney disorders and/or toxic organic compounds at low concentrations that, when consumed over a long period of time, cause or contribute to RPC. Testing this hypothesis integrates geologic, geochemical, microbiological, and epidemiological approaches. This hypothesis involves the presence of aquifers containing low-rank coal (lignite) deposits, specific hydrologic conditions favorable to the leaching and transport of toxic organic compounds from the coal into the groundwater, possible microbial contamination, and stable rural populations. This hypothesis also accounts for the geographical restriction as well as the epidemiological and clinical features of BEN and American RPC. Preliminary investigations have suggested markedly different suites of compounds in BEN water as compared to control samples (Tatu and others, 2000). Fungal ochratoxin-A has also been implicated as a potential etiologic co-factor (Fillastre, 1997; Stoev, 1998; Abouzied and others, 2002; Pfohl-Leszkowicz and others, 2002). “Two-hit hypotheses,” such as the interactions between toxic organic compounds and microbial pathogens whereby both are necessary co-factors in RPC, are well documented in the cancer literature and have been noted with kidney disease (e.g., Knudson, 1996; Pei, 2001). Moreover, previous work on BEN has established the possibility of a multifactorial etiology (Toncheva and others, 1998).

Numerous sites containing low-rank lignite deposits occur in several parishes of northern Louisiana (figures 2, 3 and 4) with active lignite mining currently occurring in DeSoto and Red River Parishes (Chawner, 1936; Meagher and Aycock, 1942; Roland and others, 1976; Snider, 1982). Most of the lignite occurs in Lower Tertiary strata and is restricted to the northern part of Louisiana (Meagher and

Aycock, 1942; Roland and others, 1976). Similar lignite deposits extend from Texas through parts of Arkansas, Louisiana, and Mississippi, terminating in Alabama (Meagher and Aycock, 1942) and occur chiefly in the Wilcox Group and to a lesser extent in the overlying stratigraphic units of the Claiborne and Jackson Groups (figure 2) (Roland and others, 1976). A complete list of lignite localities was compiled in 1921 (Glenk), which included every location where lignite was known to outcrop in Louisiana, but many of these locations are no longer exposed due to weathering, erosion, and cultural alteration. However, Glenk (1921) also compiled a list of localities where lignite occurred in both water wells and oil wells. At present, lignite outcrops have been reported in13 parishes in northern Louisiana and are probably present in all or parts of 9 others. However, the issue is not one of mere presence as a surface outcrop, but whether lignite deposits are present in freshwater aquifers currently used by the general population.

There are four Tertiary-age freshwater aquifers in north Louisiana: Carizzo-Wilcox, Sparta, Cockfield, and Catahoula (figure 5). The Sparta Aquifer serves 15 parishes and serves the greatest number of people in Louisiana, about 650,000, with groundwater withdrawals amounting to about 70 million gallons per day (MGD). The other major aquifers in terms of areal extent and groundwater use are the Cockfield Aquifer, which serves all or parts of 16 parishes with ground water use of about 7 MGD, and the Carizzo-Wilcox Aquifer serving 8 parishes at a ground water use rate of about 15 MGD (Sargent, 2002). The Catahoula Aquifer serves parts of 8 parishes and provides about 3 MGD of ground water (Sargent, 2002). It will be the most southern aquifer examined for lignite deposits in subsequent phases of this project. If earlier work by Roland and others (1976) is correct, lignite should be absent from the Catahoula Aquifer because it lies stratigraphically above the Jackson Group (figure 5).

A collaborative team has been assembled consisting of researchers from the USGS (Eastern and Central Regions, Geology and Water Resources Disciplines, Louisiana and Ohio Districts), the Louisiana Geological Survey, and Louisiana State University School of Medicine. This team began preliminary work to test the above hypothesis in 2002.

MATERIALS AND METHODS

A. SITE SELECTION

In May, 2002, 12 private drinking water wells (figure 6) and four surface water sites (figure 7) were sampled, and in September 2002, those same wells and sites plus four additional surface water sites were sampled in four parishes (DeSoto, Natchitoches, Rapides, and Red River) of northwestern Louisiana (figure 8). Additionally, water and lignite samples were collected from an active coal mine in Red River Parish. In both May and September, two private wells in Vernon Parish served as "control wells," as they were located in a different aquifer than all of the sample wells (coastal lowlands as opposed to all others being located in the Mississippi embayment) and coincided with neither the Wilcox Group nor any known lignite (or coal) beds. A geographic information system (GIS) was used to select 12 residential water wells based on the following themes: well depth, lignite thickness, amount of overburden, depth below surface of lignite bed, mining lease areas, and geology. Additionally, computer software (Stratifact®, version 4.57, Stratifact Software, Wheat Ridge, CO) was used to compare known depths and thicknesses of lignite deposits based on drill well logs with drinking water well locations and depths (data on file at the Louisiana District Office of USGS, Ruston, LA) (figure 9).

B. WATER SAMPLES

B.1. Geochemical Analyses

B.1.a. *Inorganic*

For inorganic geochemical analyses, sample collection bottles (Nalgene® plastic) were cleaned with a 10 percent hydrochloric acid (HCl) solution and rinsed several times with Milli-Q (Millipore, Billerica, MA) water. Collection bottles were rinsed thrice with and then filled with untreated well water directly from homeowner's hose or spigot—as close to the well housing as possible. Well water was then sub-sampled by syringe for individual laboratory analyses. All well samples were analyzed for the following: pH, conductivity, anions (fluoride F^-, chloride Cl^-, bromide Br^-, nitrate NO_3^-, sulfate SO_4^{3-}), and nutrients (phosphate, PO_4^{3-} and ammonium, NH_4^+). All surface water samples were analyzed for pH, conductivity, and dissolved oxygen. A mobile laboratory facilitated on-site analyses, while the majority of the testing was done after water samples were shipped to USGS laboratories (figure 10).

B.1.a.*i*. Acidity (pH)

The pH meter was calibrated with two standard buffer solutions of pH 7 and pH 4, at least once every fourth sample. After subsamples were drawn, the pH of the sample was read (at the field location) directly from the collection bottle, using a Thermo Orion (Beverly, MA) semi-micro pH electrode and a temperature compensation probe. Readings were made at ambient temperature (mean temperature of 25.4 degrees C, with standard deviation of 1.802) shortly after sampling.

B.1.a.*ii*. Conductivity

Conductivity, salinity, total dissolved solids, and temperature were read simultaneously from the collection bottle using a Thermo Orion® (Beverly, MA) conductivity cell, after standardizing the instrument with a standard conductivity solution.

B.1.a.*iii*. Dissolved Oxygen (DO)

Dissolved oxygen and temperature readings were taken at surface water sites only, using a YSI (Yellow Springs, OH) Model 55 Handheld DO meter.

B.1.a.*iv*. Anions

For each sample, water was drawn from the original collection bottle into a prerinsed plastic syringe and then filtered through a 0.45 μm syringe filter disc (IC Acrodisc®, Supor® PES membrane: Pall Corp., Ann Arbor, MI). Samples were filtered into glass vials (pre-cleaned with 10 percent HCl), tightly capped, and stored at room temperature. Sample water was analyzed for fluoride (F^-), chloride (Cl^-), bromide (Br^-), nitrate (NO_3^-), and sulfate (SO_4^{3-}) by ion chromatography, using a Waters™ (Milford, MA) 2795 Separations Module. The sample was separated with an eluent of 1.7 millimolar (mM) bicarbonate/1.8mM carbonate (Alltech [Deerfield, IL] EZ-LUTE) through an Alltech Allsep™ Anion Column. F^-, Cl^-, and SO_4^{3-} were detected by a Waters™ 432 conductivity detector; Br^- and NO_3^- were detected by a Waters™ 486 Tunable Absorbance Detector at 210nm. Chromatogram peaks were identified and areas calculated with Waters™ Millenium^{32} software, version 4.00.

B.1.a.*v*. Nutrients (phosphate, PO_4^{3-} and ammonium, NH_4^+)

Sample water was drawn from the original collection bottle into a prerinsed plastic syringe, filtered through a 0.45 μm syringe filter disc (IC Acrodisc®, Supor® PES membrane: Pall Corp., Ann Arbor, MI), and dispensed into plastic storage bottles (pre-cleaned with 10 percent HCl). Samples were immediately

frozen on dry ice until they could be placed into freezer. Samples remained at -20ºC until they were removed from freezer approximately 15 hours before analysis and allowed to equilibrate at room temperature. Samples were analyzed for PO_4^{3-} and NH_4^+as outlined below:

a) Dissolved Inorganic Phosphate (PO_4^{3-})

Phosphate standards were prepared by dilution of potassium phosphate (KH_2PO_4) in distilled water at various concentrations. Plastic test tubes were pre-cleaned in 10 percent HCl, and 4.0 mL of each standard and sample were added to each. (Tubes of 4.0 mL distilled water were also prepared to act as "blanks.") A mixed reagent was then prepared as described in table 1—each pure reagent was diluted as specified, and the four resulting solutions were mixed in the quantities described in the last column. Next, 0.4 mL of mixed reagent was added to each test tube; tubes were then capped, shaken, and allowed to react for 10 min. for complete color development. Samples, standards, and blanks were then analyzed for absorbance using a colorimeter (Brinkman PC 900) with an 885 nm filter insert. Samples were analyzed within 60 min. after mixed reagent was added. Concentrations (micromoles/liter) of dissolved inorganic phosphate in samples were calculated by subtraction of blank values and linear regression on averaged standard values.

b) Ammonium (NH_4^+)

Procedure for ammonium analysis was parallel to that of phosphate. Ammonium standards were prepared by dilution of ammonium sulfate. As above, 4.0 mL of each sample, standard, and blank were added to test tubes. To each test tube, three separate reagents (a, b, and c in table 2) were added in the amounts shown in column 5 of table 2. The three reagents we re prepared in the proportions shown in columns 2 and 3, table 2. After addition of reagents, tubes were capped, shaken, and allowed to react a minimum of four hours for color development. The color complex [indophenol blue] remained stable for up to 30 hours. Samples were read through a 640 nm filter. Concentrations were calculated through linear regression of standard values as above.

B.1.a.*vi*. Metals

Metal and trace element concentrations were measured by inductively-coupled plasma mass spectrometry (ICP-MS) and atomic emission spectrometry (IC-AES). Approximately 2-3 mL of sample was needed for ICP-MS analysis using an ELAN 6000 (Perkin-Elmer/Sciex, Toronto, Ontario, Canada)

instrument. The instrument was calibrated using 2 calibration samples (diluted 100 : 1) supplied by Perkin-Elmer. A third calibration standard, consisting of multiple single element standards supplied by EM Science (VWR), was also employed. Samples were acidified prior to ICP-AES (model 3300DV, Perkin-Elmer). Six calibration standards, made up of 51 single, pure element standards, controlled for both analyzed elements as well as for potential elemental interferences.

B.1.b. *Organic: Hydrocarbons*

B.1.b.*i*. Sample collection

For collection of samples for hydrocarbon analysis, 1.0 liter amber glass bottles with Teflon-lined caps were selected. Bottles were cleaned using a pesticide-grade (99.99 percent pure) dichloromethane (DCM; chemical formula CH_2Cl_2) rinse and then dried completely. The sample was collected by filling two 1.0 liter bottles with untreated well water directly from home owner's hose or spigot—as close to well housing as possible. Immediately, at the field location, 10.0 mL DCM was added to each bottle to preserve the sample, and as the first step in the extraction process.

B.1.b.*ii*. Extraction

In the organic geochemistry laboratory facility, all glassware used in the extraction process was precleaned each day by washing with soap and water, rinsing with deionized water, and baking in a muffle furnace at 450°C for a minimum of four hours or rinsing with DCM. Upon returning from the field, an additional 50.0 mL pesticide-grade DCM was added to each bottle, for a total volume of 60.0 mL. To obtain the extracted organic compounds from the water, the sample (with added DCM) was poured into a furnace-fired (450°C) and DCM -rinsed separatory funnel. The sample was then hand-shaken ten times—shaking for 30 seconds each time and allowing separation into aqueous and organic phases between each shaking. After the last shaking, the sample was allowed to separate completely; then the layer of DCM and extracted compounds was drained through the stopcock (DCM -rinsed) into a furnace-fired flask. This shaking and draining process was performed three more times—adding 60.0 mL fresh pesticide-grade DCM, shaking, separation of phases, and draining. The entire process was repeated for the second bottle of sample. The drained DCM was continuously added to the same flask for a final total of 480.0 mL of DCM. The flask of extracted sample was then reduced from 480.0 mL to approximately 2.0 mL by a rotary evaporator (R-200 Rotavapor®, Büchi, Flawil, Switzerland). The remaining 2.0 mL

was transferred to a furnace-fired glass vial and then further evaporated under a stream of nitrogen gas. The vial was then stored at –20º C until morning of analysis.

B.1.b.*iii*. Gas Chromatography/Mass Spectrometry Analysis

On the day of analysis, the dried material in the vial was reconstituted with DCM to a volume of 50.0 µl—25.0 µl of this volume was then transferred to an autosampler vial to be analyzed by the gas chromatograph (GC) (Hewlett Packard 6890, Agilent Technologies, Rockville, MD); the remaining 25.0 µl was stored at –20º C as archive. The GC used a 5973 mass selective detector (Hewlett Packard/Agilent) set in the SCAN mode for a range of 50-650 m/z (atomic mass to charge ratio). The instrument was run in the auto sampler mode. A 2.0 µl injection was made, and the sample was separated on an HP-5MS (5 percent phenyl methyl siloxane) column, with dimensions of 30 meter x 0.25 mm x 0.25 µm. The GC was programmed with an injector temperature of 280°C and a constant helium flow of 1.3 mL/min. The temperature program ran from 60-300°C at 4°/min and was held at 300°C for 5 min. The solvent delay was 7.00 minutes. See table 3 for analysis parameters.

B.1.b.*iv*. Data interpretation

The peak identifications were made using the Willey 7.1 and NIST 2002 libraries as references. Future quantification of peaks (if necessary) will be made by comparison to external reference standards with similar retention times.

B.2. Microbiological Analyses

B.2.a. *Fungi*

B.2.a.*i*. Sample Collection:

Surface water. Surface water samples were collected by use of the hand-dip method (Myers and Sylvester, 1997). Sterile, 1.0 L polypropylene bottles were grasped near the base of the bottle with hand and arm on the downstream side of bottle. Without rinsing, the bottles were plunged below the water surface with the opening downward. The bottles were allowed to fill with the opening pointed slightly upward. The bottles were removed from the water with the opening pointed upward, leaving approximately 2.5 to 5 cm of headspace.

Ground water. Tap water was flushed for several minutes before collecting the sample. Sterile 1.0 L polypropylene bottles were filled, taking care not to touch the tap with the bottle. The 1.0 L samples were stored at 4°C and shipped overnight to the Ohio District Microbiology Laboratory (Columbus, OH).

B.2.a.*ii*. Sample Filtration and Culture Preparation:

For ground water samples, two aliquots of 500.0 mL each were filtered. For surface water samples, 1.0 mL of the undiluted sample was plated along with ten-fold dilutions (10^{-2}, 10^{-3}, and 10^{-4} dilutions) in duplicate. Samples were stored at 4°C until analyzed. To prepare for culturing, the samples were shaken vigorously at least 25 times and an appropriate amount of water was measured into a sterile filter funnel. Using a membrane filtration technique, the filter samples were aliquotted through a presterilized 0.45-μm membrane filter, 47-mm diameter mixed cellulose ester filter (Advantec MFS, Inc., Pleasanton, CA) (American Public Health Association and others, 1998). Sides of the filter funnel were rinsed with phosphate buffered dilution water. The filter membrane was removed from funnel and placed on Sabouraud Dextrose Agar (SDA) Emmons Modification, supplemented with chloramphenicol. This selective medium was prepared in the following manner: 20.0 g dextrose, 10.0 g peptone, and 17.0 g agar were added to 1.0 L deionized water. While boiling, 0.05 g of chloramphenicol in 10.0 mL 95 percent ethanol was added. Medium was removed from heat and mixed well, then autoclaved for 15 minutes and stored at 4°C. The filter membrane was incubated on agar at 22°C for a minimum of 7 days.

B.2.a.*iii*. Quality Control:

Field blank. A blind field blank was processed by pouring sterile deionized water into a sterile 1.0 L polypropylene bottle in the field. Bottle was shipped and analyzed with the environmental samples.

Laboratory controls.

Positive control: A loopful of an *Aspergillis* or *Penicillium* species was resuspended in 10.0 mL phosphate buffered dilution water. A 10^{-2} dilution was made and plated using 1.0 mL of that dilution using the same membrane filtration technique used for the environmental samples described above. A positive control was plated each day that environmental samples were filtered.

Negative control: Using the membrane filtration technique, 500 mL of sterile phosphate buffered dilution water was plated. A negative control was plated each day that environmental samples were filtered.

B.2.a.*iv*. Macroscopic Examination:

Plates were examined daily for a minimum of 7 days for growth of fungus. Each day, the number and color/characteristic of each colony type was recorded. Each colony type was subcultured immediately onto a new SDA with chloramphenicol plate to ensure viability and isolation of the organism. Colony identification (ID) number was recorded, along with the date transferred for isolation, and morphological characteristics including color, shape/size, texture, reverse color, and pigmentation in agar of the colony on the membrane filter. When mature growth developed on the isolation plate, the colony ID number, date examined, color, shape/size, texture, reverse color, and pigmentation in agar of the colony on the isolation plate were recorded. Isolation plates were kept for microscopic examination, and fungi were subcultured onto a potato dextrose agar (Sigma, St. Louis, MO) slant for long-term storage at -70°C (Larone, 2002) (see Maintenance of Fungal Cultures below).

B.2.a.*v*. Microscopic Examination:

A given sample fungus was examined microscopically using a tease mount when the culture first began to grow and form conidia. A drop of lactophenol cotton blue (LPCB) was placed on a clean glass slide. LPCB reagent was prepared as follows: 20 g phenol crystals were dissolved in 20.0 mL lactic acid, 40.0 mL glycerol, and 20.0 mL deionized water by gently heating. 0.05 g methyl blue was added and mixed well, then stored at room temperature. A small portion of the colony was removed with a dissecting needle and placed in the drop of LPCB. The mycelial mass was gently teased apart using two dissecting needles. Mass was covered with a coverslip and observed under the microscope. The colony ID number, date examined, morphology, drawing, and identification were recorded. If the colony was not actively producing conidia after 7 days of incubation, the colony was streaked on a sterile membrane filter saturated with sterile deionized water, placed in an empty petri dish, and incubated for 7 more days or until the colony sporulated. Colonies were then reexamined microscopically.

B.2.a.*vi*. Maintenance of Fungal Cultures:

The fungus was cultured on a slant of 15 mL potato dextrose agar (Sigma, St. Louis, MO) in a screw-cap glass test tube. Slants were incubated at 22°C until the organism actively produced conidia. Caps were sealed tightly and tubes were stored at -70°C. To subculture, the tube was removed from the freezer and a small portion of the colony was chipped from the agar slant. The chipped section was

placed on a fresh agar slant, plated and incubated. The frozen slant was immediately returned to the freezer, not having been allowed to thaw. If accidentally thawed, the fungus was transferred to a fresh slant, incubated to maturity as above, and then frozen.

B.2.b. *Leptospira spp*.

B.2.b.*i*. Sample Collection:

Surface water samples were collected by use of the hand-dip method (Myers and Sylvester, 1997), as described in section B.2.a.*i*, with the following modification: Sterile, 500 mL polypropylene bottles were used. The samples were shipped to USGS Ohio District Microbiology Laboratory (ODML) at ambient temperature within 48 hours of field collection.

B.2.b.*ii*. Sample Filtration And Concentration:

The following methods for sample filtration and concentration are modifications of methods found in American Public Health Association and others (1998) and Rossella and others (1997). The sample was vigorously shaken at least 25 times before water was decanted into a graduated cylinder. Using the membrane filtration technique, 100.0 mL of sample was filtered through a 0.65 μm pore size, 47-mm-diameter, mixed cellulose ester filter (Advantec MFS, Inc., Pleasanton, CA) and the filtrate was collected into a sterile side-arm flask. A second 100.0 mL aliquot was then measured and filtered through the same apparatus. Using a new sterile filter funnel, the combined filtrate from above was filtered through a 0.45 μm pore mixed cellulose ester filter. The filtrate was collected into a sterile side-arm flask. Using a new sterile filter funnel, the filtrate from above was filtered through a 0.20 μm pore filter. The filtrate was collected into a sterile side-arm flask. The final filtrate, consisting of 200 mL sample plus rinse water, was poured into a sterile 250 mL centrifuge tube.

The 0.65 μm and the 0.45 μm filters were discarded in a biohazard bag and autoclaved. Using sterile technique, the 0.20 μm filters were placed into a sterile 50 mL centrifuge tube. In most cases, use of two filters was required for effective filtration. Forty mL of sterile PBS was added, and the tube with filters was stored in the -70°C freezer, in case future recovery efforts were needed.

The 250 mL centrifuge tube was centrifuged at 3,370 x g for 7 hours, and the supernatant was aspirated to leave approximately 0.5 mL. The pellet was resuspended and transferred to a sterile 1.5 mL microcentrifuge tube. The 250 mL centrifuge tube was rinsed with 500 μL of PBS into the microcentrifuge

tube. The final volume in the microcentrifuge tube was approximately 1 mL. Cells were harvested by centrifugation at 15,000 x g for 15 minutes, and the supernatant was discarded. The pellet was resuspended in 100.0 μL of PBS and stored at -70°C for further processing.

B.2.b.*iii*. Quality Control:

Positive controls. Starting with live *Leptospira* culture of known concentration, nucleic acids were extracted by use of the same protocol as for all test samples. This served as a control for the extraction procedure and was used in determining the efficiency of recovery. When complete, 10-fold serial dilutions were made to test for optimum concentration to use in a polymerase chain reaction (PCR). Additionally, purified *Leptospira* DNA obtained from a CDC reference laboratory was resuspended and used as the PCR positive control. Control was diluted as above and optimum concentration was determined when using a standard volume of 1.0 µl DNA solution per reaction.

Blanks. Because PCR is so sensitive to minute contamination, both laboratory and field blanks were utilized. Two lab blanks were collected by pouring sterile deionized water into a sterile 500 mL polypropylene bottle in the lab. The blank samples were processed in the same manner as the environmental samples. One blind field blank was collected in September by pouring sterile deionized water into a sterile 500 mL polypropylene bottle in the field.

Split Replicate. One split replicate sample was analyzed in September. This was done by analyzing two 200 mL aliquots of sample from the same sample bottle.

B.2.b.*iv*. DNA Extraction:

The 1.5 mL microcentrifuge tubes containing concentrated cells were removed from -70°C storage and allowed to equilibrate to room temperature. Each tube was centrifuged for 10 minutes at 5,000 x g, and then the supernatant was discarded. DNA extraction was done by the protocol and products in the DNeasy Tissue Kit Handbook (Qiagen, Valencia, CA) for the isolation of genomic DNA from Gram-negative bacteria. After the extraction, 200 μL of undiluted DNA was stored in the -70°C freezer. Sample DNA was further concentrated by ethanol precipitation. The method for ethanol precipitation is a modification of the method found in Moore (1999), in which sodium chloride was used instead of sodium acetate. Following DNA extraction, the 200 μL of undiluted DNA was ethanol precipitated in the presence of sodium chloride. DNA was precipitated overnight at -20°C. The tubes

equilibrated to room temperature the following day and DNA was harvested by centrifugation at 15,000 x g for 15 minutes at 4°C. The supernatant was decanted, and the DNA was washed with 1 mL of 70 percent ethanol. Washed DNA was harvested by centrifugation at 15,000 x g for 5 minutes at 4°C, and the supernatant was decanted. The tubes were inverted and allowed to air dry. The DNA was resuspended in 25.0 μL of sterile molecular biology grade water, and stored at -70°C for later analysis.

B.2.b.*v*. Polymerase Chain Reaction (PCR):

The PCR analyses were performed using an Eppendorf Mastercycler. The sequences for the L and G primers are given in table 4. Primer stock concentrations were measured using absorbance at 260 nm. A sterile micropipetter dedicated exclusively to PCR was used in the procedure. Sterile disposable tips were always used; gloves and a lab coat with long sleeves were worn at all times. The PCR products (amplicons) were never brought into the area where reactions were prepared.

Oligonucleotide primers:

The forward oligonucleotide primers L737 and G1, and reverse primers L1218 and G2 were purchased from The Great American Gene Company (Ramona, CA). Sequences are shown in table 4. Primer stock concentrations were measured using absorbance at 260 nm. Primers were resuspended in sterile molecular grade water at a concentration such that 1.0 μl contained 50.0 pmol of primer. Fifty microliters were aliquotted into 0.5 mL sterile microcentrifuge tubes and stored at -20°C.

PCR mix preparation: For reactions of less than 20 samples, a Master Mix was made that contained enough for all of the reactions plus one-half of a reaction. In other words, if 12 samples were to be tested, plus positive and negative controls, a 14.5X Master Mix was made. For PCR runs with more than 20 samples, enough extra reagents were added to allow for one full additional reaction; *e.g.*, for 25 samples (+2 controls, as always), a 28X Master Mix was made. Doing this did not result in excessive waste of expensive reagents, but rather compensated for pipette error, and inevitable losses incurred in manipulating the reagents, even with the best technique. The smallest Master Mix ever prepared was 3.5X: one test sample, plus positive and negative controls.

Magnesium chloride concentrations [$MgCl_2$] were optimized by testing 1.0 mM, 2.0 mM, 3.0 mM, 4.0 mM with only positive and negative controls. Originally, the G1/G2 PCR used 1.0 μl of each primer (50 pmol). By testing with controls only, it was determined that 0.1 μl worked equally as well. Primers

were then titrated with 1.0 µl, 0.5 µl, and 0.1 µl [$MgCl_2$] for optimization. Similarly, originally 1.0 U of polymerase was used. However, 0.5 U actually produced cleaner bands. Further optimization included assessing the sensitivity of the PCR by titrating known concentrations of positive DNA. Good sensitivity detected less than one organism per reaction. One apparently negative test sample was taken, and it was spiked with the smallest concentration of known positive DNA. PCR was run on it as is, and when diluted with 25, 50, and 100 times volume water added. Such a dilution series tested for inhibitory effects in concentrated field samples.

PCR cycling parameters for *Leptospira* spp. using 23S rRNA primers L737 and L1218:

DNA was extracted as above. Included in each reaction were one positive control, and one water blank negative control. The semi-hot start technique was applied on the thermal cycler: *i.e.*, the sample block was allowed to equilibrate to 95 degrees C prior to loading of the sample tubes. The basic cycling parameters for the L primers were as follows: 95 degrees C for 3 minutes; then 35 cycles of 95 degrees C for 30 seconds, 50 degrees C for 30 seconds, and 72 degrees C for 90 seconds; then a final hold at 4 degrees C (modified/optimized from Woo and others, 1997). The basic cycling parameters for the G primers were as follows: 32 cycles of 94˚C for 90 seconds, 55˚C for 60 seconds, and 72˚C for 120 seconds; then a final hold at 4˚C (Gravekamp and others, 1993). Included in each reaction were one positive control, and one water blank negative control.

B.2.b.*vi*. Optimization of PCR

The following steps were performed for the optimization of PCR:

1. To determine the detection limit for the assay, the original, positive control DNA sample (kindly provided by Paul Levett, Centers for Disease Control) was diluted to extinction of PCR product generation. The lowest detected concentration was defined to be 1 detectable unit (DU). The original concentration of DNA was not known, and a fluorometer suitable for DNA quantification was not available; therefore the concentration of DNA in one DU was not quantified. For all experiments, 1 DU was 10 µL of a 10^{-5} dilution of original DNA, which previously had been diluted up to a working stock of 1 mL. No products of the expected size for *Leptospira* (or any other size) were detected in the 14 reaction blanks using L primers and 4 reaction blanks using G primers.

2. Triplicate experiments were run with various combinations of magnesium, primer, enzyme, and annealing temperature in an effort to improve consistency and sensitivity (tables 5 and 6). Several combinations of reaction parameters resulted in consistent generation of product from 5 DU template DNA using the L primers. The highlighted reaction parameters in table 5 were chosen as the optimized protocol. Consistent product generation using G primers was achieved only using 50 DU template DNA in initial screenings. Further optimization of G primer protocol was discontinued to meet the workplan schedule.

3. Sensitivity was confirmed using the optimized protocol for the L primers only. In several confirmation runs, consistency between 60-70 percent of product generation using 5 DU template DNA with the optimized protocol for the L primers was achieved.

B.2.b.*vii*. Gel Electrophoresis:

Gel electrophoresis was performed using 2 percent agarose with 1X TAE buffer. The gels were run at 4 V/cm (80 V for short gels and 200 V for long gels). Ten μL of product plus loading buffer was added to each lane. A 100-1000 base pair ladder was used for reference. The gels were stained for 1 hour with ethidium bromide (10 mg/mL) and then destained for 30 minutes. A 481-base-pair product was expected using the L primers, and a 285-base-pair product was expected using the G primers. 1X TAE buffer was used. 1.5 percent agarose gels were made by adding 7.5 g agar to 500.0 mL TAE buffer. 1.6 μL 6X loading buffer was used with 10 μL PCR product. Thirty microliters ethidium bromide was used in 1000 mL (10 mg/mL) TAE buffer. At least one lane with a DNA ladder (size marker) was electrophoresed per 10 lanes of sample. A ladder in the 100 - 1000 bp product size range was used. Gels were kept cool by not running higher than 100 volts (e.g., 80 V for 2 hours).

C. COAL SAMPLES

C.1. Geochemical analysis

C.1.a. Organics (hydrocarbons)

C.1.a.*i. Sample Collection and Preparation*

The lignite sample (LA-02-B) was collected from the Dolet Hills Lignite Mine (surface), De Soto Parish, Louisiana in early spring/summer 2002 (figure 11). A channel sample of coal was collected from a face that had been exposed for about 10 days. The sampled coal bed is the Chemard Lake Lignite of

the Naborton Formation, Wilcox Group, Paleocene (channel sample location: Lat. - 32.00847^{o} N, Long. – 93.60043^{o} W, approximately 328 ft elevation). The section dips about 25 degrees NW due to a small local fault, but otherwise the coal bed is generally flat lying with a gentle, regional dip to the south. See table 7 for stratigraphic details.

The raw coal sample was sent to a commercial lab (Geochemical Testing; Somerset, PA) for grinding, sieving and bulk chemical analysis. At the USGS, a 400 g split from the ground sample (8 mesh, < 2.38 mm) was transferred to a clean glass tray and dried under air in a gravity convection oven at 40°C for 48 h to remove ambient moisture. After drying, the sample was ground with a ceramic mortar and pestle and sieved to obtain a fraction between 60 and 120 mesh (250-125 μm). The sieved fraction was then rinsed several times with organic-free, deioinized water and dried again under air at 40°C. The dried sample was transferred to a precleaned glass jar and stored at room temperature until extraction.

C.1.a.*ii. Hydrocarbon Extraction*

Prior to extraction, the sample was dried overnight at 40°C in a gravity convection oven to remove any absorbed moisture. A 5.00 g aliquot of sample was loaded into a glass extraction thimble containing 5 g precleaned quartz sand. The sample was extracted for 24 h using 150 mL of pesticide-grade dichloromethane (DCM) in a Soxhlet extraction apparatus. Following extraction, the sample was evaporated under nitrogen to 10mL and transferred to a glass graduated cylinder. A 100 μL portion of the extract was removed to determine the total extractable organic matter (EOM) content.

C.1.a.*iii. GC-MS Analyses*

Total extracts from the water and coal samples were analyzed using an Agilent 6890 Gas Chromatograph (GC) interfaced with an Agilent 5973 Mass Selective Detector (MSD). The MSD was operated in the total ion current (TIC) mode to scan masses from 50 to 650. See table 3 for analysis parameters. Both the water and the lignite sample extracts were analyzed using the same column and GC/MS parameters.

D. STATISTICAL ANALYSIS

The incidence of cancer of the renal pelvis (RPC) was derived from total number of the RPC cases divided by total number of population in that parish at the same period. The average value of

variables was calculated from the several measurements of point and/or time period in that parish. Statistical software SAS 8.2 (SAS Institute Inc., Cary, NC) was used to analyze the data. The Spearman correlation method was used to assess the association between the endpoints for these field, laboratory analyses and cancer incidence of the renal pelvis obtained from the Louisiana Tumor Registry. Significance level for each variable was set at 0.05.

RESULTS AND DISCUSSION

Results are presented in table 8. Explanation of the terminology and abbreviations used in table 8 are shown in table 9. Locations of sampling sites may be found in figure 8 using the Map Code common identifier. Figures 12 - 17 show representative total ion current chromatograms (TIC) from the mass spectrometry. A comparison between TICs of two representative wells in the Wilcox Group (potentially intersecting a lignite aquifer) and the two control wells in the coastal lowlands aquifer system not coincident with coal deposits is presented in figure 18. Seasonal variation in organic content of the wells was also observed (*e.g.*, figure 19).

Endpoints for these field and laboratory analyses were tested by Spearman correlation for association with cancer of the renal pelvis mortality data, obtained from the Louisiana Tumor Registry. Significant associations were revealed between the cancer rate and the overall number of organic compounds, the fungi Zygomycetes, the nutrients PO_4 and NH_3, and thirteen chemical elements (As, B, Br, Cl, Cr, F, Li, Na, P, Rb, Se, Sr, W) from the well water as compared to the controls (table 10). Among the species of fungi from the total of 136 isolates were 12 *Penicillium* spp., at least two *Aspergillus* spp., a number of other genera (*Alternaria* sp., *Eupenicillium lapidosum*, *Cladosporium* sp., *Epicoccum* sp., *Trichoderma* sp., *Paecilomyces* sp., *Chrysosporium* sp., *Chloridium* sp.), and Zygomycetes, and Coelmycetes -- some of which are known mycotoxin producers. The two control wells yielded a mean of 6.5 (SD = 3.5355) individual isolates, while the mean number of isolates from all other sites was 7.6 (SD = 4.4866). These initial results suggest that additional investigation is warranted.

A polymerase chain reaction protocol was optimized for detection of pathogenic leptospires, and the sensitivity of the assay was ascertained. Using the optimized DNA extraction techniques and PCR conditions, three parallel sets of samples were run using the whole cell suspension received from CDC. Each set consisted of the same nominal number of cells from the 10^{-1} through 10^{-5} dilutions and a blank.

The first set of samples (F series, filtered) was diluted to 200 mL, filtered, and centrifuged according to the protocol described in the methods section. The second set of samples (U series, unfiltered) was diluted to 200 mL and centrifuged according to the protocol. The third set of samples (C series, cells) was simply harvested by microcentrifugation. All three series were subjected to the same lysis and purification steps. DNA extracts from the 18 controlled experiment samples were tested by the optimized protocol for amplification using the L primers. There was an apparent 2-log reduction in *Leptospira* cells recovered through each step (filtration and centrifugation). *Leptospira* were detected in the C dilution series 10^{-5}; in the U dilution series 10^{-3}; and in the F dilution series 10^{-1}. No product was detected in any of the three blanks. See figure 20 for the results of the controlled experiment.

Environmental samples were filtered and centrifuged then held for analyses. Five μL of 1:10 dilution and undiluted DNA extracts from each sample were tested in replicate against the optimized protocol for L primers. See table 11 and figure 21 for results of environmental samples. Matrix spikes were run in the same set with the replicate runs. Matrix spike positive controls failed to amplify (1 out of 5 produced products); therefore, the potential for inhibitory compounds in the sample matrix may not have been adequately tested. Half of the surface water sites tested positive for the presence of *Leptospira* spp. pathogenic to humans.

The average number of organic compounds detected in the sample well water was 104.9, while a mean of 60.0 different such chemicals was found in the control wells. Integrated areas (in absorbance units x 10E8) under the mass spectrometry peaks (indicative of the relative amounts or concentrations of all organic compounds combined), 84.0 for sample well water versus 6.40 for control well water, were calculated. It is important to note, however, that many of the organic compounds identified were phthalates -- ubiquitous man-made chemicals used in the manufacture of plastics. These phthalates accounted for some of the largest areas under the peaks, and results will be adjusted for this potential confounder in future work.

A TIC similar to those resulting from the well water samples was observed from the lignite extract sample, both in relative abundance and concentration of organic compounds. The lignite was sampled from an exposed mine cut at the surface mining operation, Dolet Hills. While the lignite was exposed to the elements for not likely more than a week, oxidization may have occurred. In future analyses, we will

compare the chromatograms from the well water with that from the lignite, and search for compounds present in both, especially those that have been implicated as possible causal factors in kidney disease and cancer.

Together, these results strongly suggest that the water coming from the lignite aquifers in this region is significantly more contaminated by naturally occurring organic compounds that may cause greater exposure to potentially nephrotoxic/carcinogenic nitroaromatics and other similar aromatic compounds. The detection of pathogenic leptospires and ochratoxin-producing fungi in surface and well water samples raise the possibility that the geochemical milieu in drinking water combined with microbial exposure in northwestern Louisiana where lignite acts as aquifers may contribute to incidence of kidney disease and RPC.

SUMMARY

Overall, the presence of chemicals and microbes with known or suspected links to kidney disease including cancer of the renal pelvis appears to be greater in ground water sources derived from lignite aquifers of the Wilcox group in northwestern Louisiana than in water from nearby sources not coincident with known lignite or coal deposits (chemicals are greater, but fungi do not appear to be). Some of the organic compounds found in the water samples appear to be similar to those found in the lignite sample collected from the coal mine in the area, suggesting that the lignite beds may in fact be the source of some of the water contaminants. The infection of humans by pathogenic leptospires that were found in 50 percent of the surface water sites in the studied region provides an additional potential means by which kidney diseases including cancer may result. Preliminary epidemiological evidence suggests that there are significant associations between some of the variables measured and kidney cancer rates. Future work is planned to investigate biological and toxicological mechanisms to test for causation in the observed statistical correlation.

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Table 1. Specifications of reagents used in phosphate analysis.

REAGENT NAME	AMOUNT OF PURE REAGENT	AMOUNT OF DISTILLED WATER FOR DILUTION	AMOUNT OF SOLUTION IN "MIXED REAGENT"
ammonium molybdate	3 g	100 mL	10 mL
sulfuric acid (concentrated)	28 mL	180 mL	25 mL
ascorbic acid (powder)	2.7g	50 mL (prepared day of analysis)	10 mL
potassium antimonyl tartrate	0.17g	125 mL	5 mL

Table 2. Specifications of reagents used in ammonium analysis.

REAGENT NAME	REAGENT AMOUNT	DILUTION	INSTRUCTIONS	AMOUNT ADDED TO EACH TEST TUBE
Phenol	10 g (reagent grade)	100 mL 95% ethanol	Refrigerate; remove night before analysis	0.16 mL
sodium nitroprusside	0.5 g	100 mL ammonium-free distilled water	Store in dark bottle Refrigerate; remove night before analysis	0.16 mL
“oxidizing regent”	4 parts “alkaline reagent” (see proportions in next row)	1 part Clorox bleach	Prepare just before use	0.4 mL
“alkaline reagent”:	50 g sodium citrate -and- 2.5 g sodium hydroxide	250 mL ammonia-free distilled water		

Table 3. Gas chromatography-mass spectrometry conditions for organic compound analyses of water and coal samples.

Parameter	Setting
Injector Temp	280°C; splitless mode
Injection Volume	2 μL
Carrier Gas / Flow Rate	He; 1.3 mL min^{-1}
Initial Oven Temp	60°C
Oven Program	4°C min^{-1} to 300°C, hold 5 min
Column	HP5-MS; 30m x 0.25mm x 0.25μm

Table 4. Nucleic acid sequences for the oligonucleotide primers used to amplify DNA from human pathogenic *Leptospira* spp.

Name	Sequence (5' to 3')
L737	5′ GAC CCG AAG CCT GTC GAG 3′
L1218	5′ GCC ATG CTT AGT CCC GAT TAC 3′
G1	5′ CTG AAT CGC TGT ATA AAA GT 3′
G2	5′ GGA AAA CAA ATG GTC GGA AG 3′

Table 5. Success rate of different reaction mixes for L primer set (169 total reactions)

Enzyme (μL)	Mg (mM)	L Primer (μM)	Temp (°C)	DNA (DU)	# hits	# runs	Success rate
0.5	1.5	1	50	10, 50	0	6	0%
1	1	1	50	10, 50	0	6	0%
1	1.25	1	50	10, 50	2	6	33%
1	1.5	1	47.5	10	1	2	50%
1	1.5	0.1	50	0.1 - 10	0	5 @ 1 each	0%
1	1.5	0.2	50	10	0	3	0%
1	1.5	0.2	50	50	1	3	33%
1	1.5	0.5	50	0.1 - 5	0	4	0%
1	1.5	0.5	50	10	3	4	75%
1	1.5	0.5	50	50	1	3	33%
1	1.5	0.75	50	0.1 - 10	0	5 @ 1 each	0%
1	1.5	1	50	10	1	1	100%
1	1.5	1	50	0.05 - 0.5	0	6	0%
1	1.5	1	50	1	1	2	50%
1	1.5	1	50	5	2	5	40%
1	1.5	1	50	10	1	5	20%
1	1.5	1	50	50	3	5	60%
1	1.5	1	50	100	6	11	55%
1	1.5	1	50	500	2	2	100%
1	1.5	1	50	1000	1	2	50%
1	1.5	1	50	5000	1	1	100%
1	1.5	1	50	10000	1	1	100%
1	1.5	1	50	10	0	1	0%
1	1.5	1.5	50	0.1 - 10	0	5 @ 1 each	0%
1	1.5	1	52.5	10	1	2	50%
1	1.75	1	50	10	0	3	0%
1	1.75	1	50	50	2	3	67%
1	2	1	50	10	0	3	0%
1	2	1	50	50	1	3	33%
2	1.5	0.5	47.5	5	1	2	50%
2	1.5	1	47.5	1	0	2	0%
2	1.5	1	47.5	5	1	2	50%
2	1.5	1	47.5	10	1	2	50%
2	1.5	0.5	50	5	1	2	50%
2	1.5	1	50	1	1	2	50%
2	1.5	1	50	5	1	2	50%
2	1.5	1	50	10	4	5	80%
2	1.5	1	50	50	2	3	67%
2	1.5	0.5	52.5	5	2	2	100%
2	1.5	1	52.5	1	1	2	50%
2	1.5	1	52.5	5	1	2	50%
2	1.5	1	52.5	10	2	2	100%
2	1.75	0.5	47.5	1	1	2	50%
2	1.75	0.5	47.5	5	1	2	50%
2	1.75	1	47.5	5	2	2	100%
2	1.75	0.5	50	1	1	2	50%
2	1.75	0.5	50	5	0	2	0%
2	1.75	1	50	5	4	5	80%
2	1.75	1	50	1	1	3	33%
2	1.75	1	50	10	0	2	0%
2	**1.75**	**1**	**52**	**5**	**3**	**5**	**60%**
2	1.75	0.5	52.5	1	2	2	100%
2	1.75	0.5	52.5	5	1	2	50%
2	1.75	1	52.5	5	2	2	100%

Table 6. Success rate of different reaction mixes for G primer set (60 total reactions)

Enzyme (μL)	Mg (mM)	G Primer (μM)	Temp (°C)	DNA (DU)	# hits	# runs	Success rate
1	1.5	0.1	55	0.1 - 100	0	7	0%
1	1.5	1	55	0.05 - 1	0	7	0%
1	1.5	1	55	5	3	7	43%
1	1.5	1	55	10	4	7	57%
1	1.5	1	55	50	5	7	71%
1	1.5	1	55	100	3	7	43%
1	1.5	1	55	500	1	1	100%
1	1.5	1	55	1000	0	1	0%
2	1.5	1	55	1	0	4	0%
2	1.5	1	55	5	2	4	50%
2	1.5	1	55	10	1	4	25%
2	1.5	1	55	50	0	4	0%

Table 7. Lithologic description of strata at lignite sample location in Dolet Hills Lignite Mine area.

Top of section (ft)	Lithologic description
30–40	Sandstone, light gray, medium to fine grained, oxidized red, sharp basal contact with coal
0.7	Coal, black, dull with bright stringers, may be slightly weathered?, mostly massive, SAMPLE LA-02-A
0.25	Claystone, medium to dark gray, irregular and uneven thickness, with ripples and vitrain stringers, Tonstein?, SAMPLE LA-02-T
7.25	Coal, black, hard, banded, bright and dull bands medium to finely banded (2 in – 0.25 in), woody lenses with pyrite crystals on cleat faces, SAMPLE LA-02-B
1.60	Coal, black, massive, woody?, no apparent bedding, irregular faces, cleat is well developed in lower half of coal, cleat orientations: face cleat – N 42^{o} E, Butt cleat – N 45^{o} W, SAMPLE LA-02-C
1.00+	Claystone, dark gray, rooted, not measured

Table 8. Complete set of raw data and means for all of the geochemical and microbiological data collected from northwestern Louisiana well and mine water samples in May and September 2002.

site	map code	id #	LAT	LONG	well depth (m)	dissolved oxygen (mg/L	diss ox temp (deg C)	pH	salinity (ppt)	TDS (mg/l)	conductivity (uS)	temp (deg C)	phosphate (umoles/l)
W1a	1	02050701	31.9454	-93.1855	64.01	nd	nd	7.71	0.2	176	367.0	26.6	6.891
W1b	1	02090501	31.9454	-93.1855	64.01	nd	nd	7.81	0.2	168	354.0	26.4	6.524
W1ab	1	na	31.9454	-93.1855	na	#DIV/0!	#DIV/0!	7.76	0.2	172	360.5	26.5	6.708
W2a	2	02050702	31.9444	-93.1876	9.75	nd	nd	5.98	0.1	80	169.4	26.2	0.414
W2b	2	02090502	31.9445	-93.1876	9.75	nd	nd	5.46	0.1	82	173.5	27.9	0.491
W2ab	2	na	31.9446	-93.1876	na	#DIV/0!	#DIV/0!	5.72	0.1	81	171.5	27.1	0.453
W3a	4	02050704	32.0826	-93.3522	100.58	nd	nd	5.62	0.0	26	55.5	26.2	0.515
W3b	4	02090512	32.0826	-93.3522	100.58	nd	nd	5.29	0.0	14	29.8	24.6	0.502
W3ab	4	na	32.0826	-93.3522	na	#DIV/0!	#DIV/0!	5.46	0.0	20	42.7	25.4	0.509
W4a	6 (also 24)	02050706	32.0276	-93.2143	62.48	nd	nd	6.28	0.1	59	124.9	28.5	6.944
W4b	6 (also 24)	02090511	32.0276	-93.2143	62.48	nd	nd	6.38	0.1	63	133.7	30.1	4.426
W4ab	6 (also 24)	na	32.0276	-93.2143	na	#DIV/0!	#DIV/0!	6.33	0.1	61	129.3	29.3	5.685
W5a	7	02050707	31.9713	-93.9408	91.44	nd	nd	8.26	2.0	1970	3850.0	24.2	7.042
W5b	7	02090408	31.9713	-93.9408	91.44	nd	nd	8.22	2.1	2050	3990.0	23.4	7.847
W5ab	7	na	31.9713	-93.9408	na	#DIV/0!	#DIV/0!	8.24	2.1	2010	3920.0	23.8	7.445
W6a	9	02050709	32.0722	-94.0023	nd	nd	nd	8.59	0.5	472	972.0	26.0	24.823
W6b	9	02090409	32.0722	-94.0023	nd	nd	nd	6.54	0.3	277	582.0	29.8	0.994
W6ab	9	na	32.0722	-94.0023	na	#DIV/0!	#DIV/0!	7.57	0.4	375	777.0	27.9	12.909
W7a	10	02050710	32.0483	-94.0331	nd	nd	nd	8.70	1.0	991	1997.0	22.7	25.556
W7b	10	02090410	32.0483	-94.0331	nd	nd	nd	8.66	1.0	972	1970.0	23.6	26.928
W7ab	10	na	32.0483	-94.0331	na	#DIV/0!	#DIV/0!	8.68	1.0	982	1983.5	23.2	26.242
W8a	11	02050801	31.9946	-93.2845	nd	nd	nd	7.67	0.2	153	318.0	23.8	4.763
W8b	11	02090507	31.9946	-93.2845	nd	nd	nd	7.88	0.2	156	328.0	25.4	4.950
W8ab	11	na	31.9946	-93.2845	na	#DIV/0!	#DIV/0!	7.78	0.2	155	323.0	24.6	4.857
W9a	13	02050804	32.0801	-93.2189	nd	nd	nd	7.31	0.1	71	149.9	25.9	0.032
W9b	13	02090504	32.0801	-93.2189	nd	nd	nd	6.11	0.0	47	100.3	27.3	1.169
W9ab	13	na	32.0801	-93.2189	na	#DIV/0!	#DIV/0!	6.71	0.1	59	125.1	26.6	0.601
W10a	14	02050805	31.9948	-93.6973	57.91	nd	nd	7.89	0.3	306	636.0	27.3	0.387
W10b	14	02090406	31.9948	-93.6973	57.91	nd	nd	8.87	0.4	358	744.0	24.8	13.245
W10ab	14	na	31.9948	-93.6973	na	#DIV/0!	#DIV/0!	8.38	0.4	332	690.0	26.1	6.816
W11a	15	02050806	32.0989	-93.3110	70.10	nd	nd	8.45	0.9	880	1784.0	24.3	1.550
W11b	15	02090510	32.0989	-93.3110	70.10	nd	nd	8.52	0.9	893	1816.0	26.9	10.841
W11ab	15	na	32.0989	-93.3110	na	#DIV/0!	#DIV/0!	8.49	0.9	887	1800.0	25.6	6.196
W12a	16	02050807	31.9948	-93.2831	60.96	nd	nd	7.99	0.2	154	323.0	24.4	3.949
W12b	16	02090506	31.9948	-93.2831	60.96	nd	nd	7.77	0.2	160	337.0	24.4	5.158
W12ab	16	na	31.9948	-93.2831	na	#DIV/0!	#DIV/0!	7.88	0.2	157	330.0	24.4	4.554
MW1a	25	02050903	30.9641	-93.2819	nd	nd	nd	nd	nd	nd	nd	nd	1.169
MW2a	nd	03041002	31.9943	-93.5939	39.62	nd	nd	4.95	0.0	45	94.3	19.4	0.291
MW1a2a	25	na	31.9943	-93.4379	39.624	#DIV/0!	#DIV/0!	4.95	0	45	94.3	19.4	0.730
CW1a	18	02050901	32.0055	-93.6023	nd	nd	nd	6.08	0.0	16	34.3	26.3	5.839
CW1b	18	02090514	32.0055	-93.6023	nd	nd	nd	6.51	0.0	15	32.8	28.8	0.458
CW1ab	18	na	32.0055	-93.6023	na	#DIV/0!	#DIV/0!	6.30	0.0	16	33.6	27.6	3.149
CW2a	19	02050902	30.9911	-93.2811	nd	nd	nd	5.65	0.0	14	30.7	29.0	0.090
CW2b	19	02090513	30.9911	-93.2811	nd	nd	nd	5.59	0.0	13	28.3	26.7	0.382
CW2ab	19	na	30.9911	-93.2811	na	#DIV/0!	#DIV/0!	5.62	0.0	14	29.5	27.9	0.236
S1a	3	02050703	32.0189	-93.2635	na	6.30	27.5	5.79	0.0	28	58.5	28.5	nd
S1b	3	02090505	32.0189	-93.2635	na	7.00	nd	7.45	0.0	34	72.0	32.0	nd
S1ab	3	na	32.0189	-93.2635	na	6.65	27.5	6.62	0.0	31	65.3	30.3	#DIV/0!
S2a	8	02050708	31.9652	-93.9406	na	0.73	25.6	6.53	0.1	87	185.1	27.5	nd
S2b	8	02090407	31.9652	-93.9406	na	4.30	nd	6.68	0.1	75	159.9	28.9	nd
S2ab	8	na	31.9652	-93.9406	na	2.52	25.6	6.61	0.1	81	172.5	28.2	#DIV/0!
S3a	12	02050803	32.0028	-93.1993	na	0.63	25.0	6.56	0.2	159	333.0	26.0	nd
S3b	12	02090503	32.0028	-93.1993	na	0.25	nd	6.37	0.2	217	456.0	24.8	nd
S3ab	12	na	32.0028	-93.1993	na	0.44	25.0	6.47	0.2	188	394.5	25.4	#DIV/0!
S4a	17	02050808	31.9607	-93.2899	na	3.60	26.5	7.59	0.1	150	315.0	27.1	nd
S4b	17	02090509	31.9607	-93.2899	na	3.60	nd	7.38	0.1	118	248.0	28.8	nd
S4ab	17	na	31.9607	-93.2899	na	3.60	26.5	7.49	0.1	134	281.5	28.0	#DIV/0!
S5b	20	02090401	30.2500	-92.6050	na	nd	nd	nd	nd	nd	nd	nd	nd
S6b	21	02090402	31.1050	-93.3633	na	nd	nd	nd	nd	nd	nd	nd	nd
S7b	22	02090403	31.2216	-93.3375	na	nd	nd	nd	nd	nd	nd	nd	nd
S8b	23	02090405	31.7500	-93.1167	na	nd	nd	nd	nd	nd	nd	nd	nd

Table 8. Continued.

site	ammonium (umoles/l)	F (ppm)	Cl (ppm)	Br (ppm)	NO3 (ppm)	SO4 (ppm)	Ag ppb	Al ppb	As ppb	B ppm	Ba ppm	Be ppb
W1a	12.611	0.456	8.281	*	*	0.520	<2	<3	<1	1.260	0.066	<0.1
W1b	9.324	0.659	8.241	*	*	0.637	<2	< 3	<1	1.270	0.017	<0.1
W1ab	10.967	0.558	8.261	#DIV/0!	#DIV/0!	0.579	#DIV/0!	#DIV/0!	#DIV/0!	1.265	0.042	#DIV/0!
W2a	0.241	*	9.978	*	47.930	8.614	<2	31.400	<1	0.044	0.178	0.720
W2b	0.226	*	9.650	*	52.517	8.285	<2	4.900	<1	0.050	0.198	0.330
W2ab	0.234	#DIV/0!	9.814	#DIV/0!	50.224	8.450	#DIV/0!	18.150	#DIV/0!	0.047	0.188	0.525
W3a	0.413	*	3.384	*	9.758	0.660	<2	12.800	<1	<0.02	0.051	0.190
W3b	*	*	2.231	*	5.806	0.710	<2	3.700	<1	<0.02	0.029	<0.1
W3ab	0.413	#DIV/0!	2.808	#DIV/0!	7.782	0.685	#DIV/0!	8.250	#DIV/0!	#DIV/0!	0.040	0.190
W4a	1.088	*	6.906	*	*	2.717	<2	<3	<1	0.027	0.070	<0.1
W4b	1.250	*	6.281	*	*	2.315	<2	< 3	<1	0.052	0.059	<0.1
W4ab	1.169	#DIV/0!	6.594	#DIV/0!	#DIV/0!	2.516	#DIV/0!	#DIV/0!	#DIV/0!	0.040	0.065	#DIV/0!
W5a	39.078	nd	925.160	*	0.648	*	<2	38.700	3.500	3.000	0.095	<0.1
W5b	42.756	4.030	921.951	*	*	0.570	<20	< 30	<10	2.980	0.027	<1
W5ab	40.917	4.030	923.556	#DIV/0!	0.648	0.570	#DIV/0!	38.700	3.500	2.990	0.061	#DIV/0!
W6a	24.204	1.389	50.670	0.510	*	9.010	<2	21.700	<1	1.710	0.027	<0.1
W6b	2.021	*	77.677	*	*	88.232	<2	4.600	1.200	0.219	0.076	<0.1
W6ab	13.113	1.389	64.174	0.510	#DIV/0!	48.621	#DIV/0!	13.150	1.200	0.965	0.052	#DIV/0!
W7a	28.704	0.823	297.637	*	0.244	*	<2	3.600	1.400	1.820	0.026	<0.1
W7b	25.666	1.476	286.811	1.173	*	*	<20	< 30	<10	1.860	<0.005	<1
W7ab	27.185	1.150	292.224	1.173	0.244	#DIV/0!	#DIV/0!	3.600	1.400	1.840	0.026	#DIV/0!
W8a	24.579	0.153	6.032	*	*	2.258	<2	<3	<1	0.337	0.109	<0.1
W8b	27.802	0.326	5.668	*	*	2.344	<2	< 3	<1	0.289	0.041	<0.1
W8ab	26.191	0.240	5.850	#DIV/0!	#DIV/0!	2.301	#DIV/0!	#DIV/0!	#DIV/0!	0.313	0.075	#DIV/0!
W9a	17.330	0.107	8.146	*	*	0.540	<2	21.800	<1	0.040	0.010	<0.1
W9b	7.184	*	5.025	*	*	1.023	<2	< 3	<1	<0.02	0.022	<0.1
W9ab	12.257	0.107	6.586	#DIV/0!	#DIV/0!	0.782	#DIV/0!	21.800	#DIV/0!	0.040	0.016	#DIV/0!
W10a	0.323	0.300	27.034	*	*	39.422	<2	31.400	<1	0.873	0.019	<0.1
W10b	17.239	0.722	26.122	0.224	0.246	0.880	<20	< 30	<10	1.110	<0.005	<1
W10ab	8.781	0.511	26.578	0.224	0.246	20.151	#DIV/0!	31.400	#DIV/0!	0.992	0.019	#DIV/0!
W11a	31.079	2.603	243.863	*	0.188	0.455	<2	<3	1.100	3.370	0.035	<0.1
W11b	29.107	3.092	239.031	0.795	*	*	<20	< 30	<10	3.350	<0.005	<1
W11ab	30.093	2.848	241.447	0.795	0.188	0.455	#DIV/0!	#DIV/0!	1.100	3.360	0.035	#DIV/0!
W12a	18.955	0.144	6.705	*	*	3.173	<2	<3	<1	0.290	0.131	<0.1
W12b	24.004	0.306	6.438	*	*	4.161	<2	< 3	<1	0.307	0.076	<0.1
W12ab	21.480	0.225	6.572	#DIV/0!	#DIV/0!	3.667	#DIV/0!	#DIV/0!	#DIV/0!	0.299	0.104	#DIV/0!
MW1a	0.849	*	3.179	*	*	1.080	<2	21.900	<1	0.042	0.017	0.510
MW2a	9.144	*	11.706	*	*	nd	nd	nd	nd	nd	nd	nd
MW1a2a	4.997	#DIV/0!	7.4425	#DIV/0!	#DIV/0!	1.08	#DIV/0!	21.9	#DIV/0!	0.042	0.017	0.51
CW1a	5.682	0.084	13.276	*	*	7.459	<2	<3	<1	0.037	0.027	<0.1
CW1b	1.977	*	3.148	*	*	1.122	<2	< 3	<1	<0.02	0.025	<0.1
CW1ab	3.830	0.084	8.212	#DIV/0!	#DIV/0!	4.291	#DIV/0!	#DIV/0!	#DIV/0!	0.037	0.026	#DIV/0!
CW2a	0.222	*	3.754	*	0.320	0.907	<2	11.400	<1	0.028	0.024	<0.1
CW2b	0.256	*	3.640	*	0.502	1.007	<2	< 3	<1	<0.02	0.020	<0.1
CW2ab	0.239	#DIV/0!	3.697	#DIV/0!	0.411	0.957	#DIV/0!	11.400	#DIV/0!	0.028	0.022	#DIV/0!
S1a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S1b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S1ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
S2a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S2b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S2ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
S3a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S3b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S3ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
S4a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S4b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S4ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
S5b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S6b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S7b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S8b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 8. Continued.

site	Bi ppb	Ca ppm	Cd ppb	Ce ppm	Co ppb	Cr ppb	Cs ppb	Cu ppb	Fe ppm	K ppm	La ppm	Li ppm	Mg ppm	Mn ppb
W1a	<0.4	2.430	<0.1	<0.05	<0.1	<2	<0.1	<1	<0.02	0.751	<0.01	<0.02	1.090	3.400
W1b	< 0.4	2.390	<0.1	<0.05	<0.1	22.100	<0.1	<1	<0.02	0.760	<0.01	<0.02	1.240	<0.3
W1ab	#DIV/0!	2.410	#DIV/0!	#DIV/0!	#DIV/0!	22.100	#DIV/0!	#DIV/0!	#DIV/0!	0.756	#DIV/0!	#DIV/0!	1.165	3.400
W2a	<0.4	2.730	<0.1	<0.05	9.800	<2	<0.1	29.600	<0.02	4.950	<0.01	<0.02	4.150	98.800
W2b	< 0.4	2.780	<0.1	<0.05	8.600	<2	<0.1	28.000	<0.02	5.780	<0.01	<0.02	5.010	108.000
W2ab	#DIV/0!	2.755	#DIV/0!	#DIV/0!	9.200	#DIV/0!	#DIV/0!	28.800	#DIV/0!	5.365	#DIV/0!	#DIV/0!	4.580	103.400
W3a	<0.4	0.669	<0.1	<0.05	1.200	<2	<0.1	5760.000	<0.02	0.923	<0.01	<0.02	0.757	2.200
W3b	< 0.4	0.528	<0.1	<0.05	0.680	<2	<0.1	68.500	<0.02	0.691	<0.01	<0.02	0.604	1.700
W3ab	#DIV/0!	0.599	#DIV/0!	#DIV/0!	0.940	#DIV/0!	#DIV/0!	2914.250	#DIV/0!	0.807	#DIV/0!	#DIV/0!	0.681	1.950
W4a	<0.4	6.790	<0.1	<0.05	0.150	<2	<0.1	3.000	1.890	1.890	<0.01	<0.02	2.820	43.300
W4b	< 0.4	8.220	<0.1	<0.05	<0.1	6.100	<0.1	<1	1.160	2.160	<0.01	<0.02	3.620	25.900
W4ab	#DIV/0!	7.505	#DIV/0!	#DIV/0!	0.150	6.100	#DIV/0!	3.000	1.525	2.025	#DIV/0!	#DIV/0!	3.220	34.600
W5a	<0.4	2.670	<0.1	<0.05	<0.1	<2	0.130	3.800	0.099	2.590	<0.01	0.058	1.120	2.100
W5b	< 4	2.320	<1	<0.05	<1	39.000	<1	<10	<0.02	2.760	<0.01	0.071	1.120	<3
W5ab	#DIV/0!	2.495	#DIV/0!	#DIV/0!	#DIV/0!	39.000	0.130	3.800	0.099	2.675	#DIV/0!	0.065	1.120	2.100
W6a	<0.4	1.190	<0.1	<0.05	<0.1	<2	<0.1	<1	0.024	1.210	<0.01	<0.02	0.388	3.200
W6b	< 0.4	29.300	<0.1	<0.05	0.240	6.100	<0.1	5.100	<0.02	5.330	<0.01	0.031	6.910	<0.3
W6ab	#DIV/0!	15.245	#DIV/0!	#DIV/0!	0.240	6.100	#DIV/0!	5.100	0.024	3.270	#DIV/0!	0.031	3.649	3.200
W7a	<0.4	0.948	<0.1	<0.05	<0.1	<2	<0.1	<1	<0.02	1.280	<0.01	0.031	0.330	3.100
W7b	< 4	0.638	<1	<0.05	<1	20.000	<1	<10	<0.02	1.300	<0.01	0.036	0.287	<3
W7ab	#DIV/0!	0.793	#DIV/0!	#DIV/0!	#DIV/0!	20.000	#DIV/0!	#DIV/0!	#DIV/0!	1.290	#DIV/0!	0.034	0.309	3.100
W8a	<0.4	5.330	<0.1	<0.05	<0.1	<2	<0.1	<1	0.044	1.570	<0.01	<0.02	1.640	12.200
W8b	< 0.4	5.340	<0.1	<0.05	<0.1	21.500	<0.1	1.300	<0.02	1.540	<0.01	0.021	1.850	<0.3
W8ab	#DIV/0!	5.335	#DIV/0!	#DIV/0!	#DIV/0!	21.500	#DIV/0!	1.300	0.044	1.555	#DIV/0!	0.021	1.745	12.200
W9a	<0.4	16.600	<0.1	<0.05	0.500	<2	<0.1	19.700	1.990	1.980	<0.01	<0.02	1.980	95.400
W9b	< 0.4	5.310	<0.1	<0.05	0.890	<2	<0.1	<1	<0.02	0.467	<0.01	<0.02	0.608	142.000
W9ab	#DIV/0!	10.955	#DIV/0!	#DIV/0!	0.695	#DIV/0!	#DIV/0!	19.700	1.990	1.224	#DIV/0!	#DIV/0!	1.294	118.700
W10a	<0.4	4.860	<0.1	<0.05	0.150	<2	<0.1	167.000	0.231	1.650	<0.01	<0.02	0.755	31.600
W10b	< 4	0.752	<1	<0.05	<1	20.000	<1	<10	<0.02	0.957	<0.01	<0.02	0.119	4.200
W10ab	#DIV/0!	2.806	#DIV/0!	#DIV/0!	0.150	20.000	#DIV/0!	167.000	0.231	1.304	#DIV/0!	#DIV/0!	0.437	17.900
W11a	<0.4	1.440	<0.1	<0.05	<0.1	<2	<0.1	<1	0.024	1.530	<0.01	0.030	0.537	0.990
W11b	< 4	0.732	<1	<0.05	<1	21.000	<1	<10	<0.02	1.600	<0.01	0.036	0.436	<3
W11ab	#DIV/0!	1.086	#DIV/0!	#DIV/0!	#DIV/0!	21.000	#DIV/0!	#DIV/0!	0.024	1.565	#DIV/0!	0.033	0.487	0.990
W12a	<0.4	6.180	<0.1	<0.05	<0.1	<2	<0.1	1.900	0.142	1.580	<0.01	<0.02	1.790	10.200
W12b	< 0.4	8.480	<0.1	<0.05	<0.1	21.400	<0.1	<1	<0.02	1.770	<0.01	0.022	2.560	<0.3
W12ab	#DIV/0!	7.330	#DIV/0!	#DIV/0!	#DIV/0!	21.400	#DIV/0!	1.900	0.142	1.675	#DIV/0!	0.022	2.175	10.200
MW1a	<0.4	0.816	<0.1	<0.05	6.800	<2	<0.1	<1	0.194	1.440	<0.01	<0.02	0.908	45.500
MW2a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MW1a2a	#DIV/0!	0.816	#DIV/0!	#DIV/0!	6.8	#DIV/0!	#DIV/0!	#DIV/0!	0.194	1.44	#DIV/0!	#DIV/0!	0.908	45.5
CW1a	<0.4	1.970	<0.1	<0.05	0.280	<2	<0.1	20.400	<0.02	1.340	<0.01	<0.02	0.435	0.600
CW1b	< 0.4	2.260	<0.1	<0.05	0.210	<2	<0.1	8.700	<0.02	1.630	<0.01	<0.02	0.514	0.880
CW1ab	#DIV/0!	2.115	#DIV/0!	#DIV/0!	0.245	#DIV/0!	#DIV/0!	14.550	#DIV/0!	1.485	#DIV/0!	#DIV/0!	0.475	0.740
CW2a	<0.4	0.983	<0.1	<0.05	0.700	<2	<0.1	766.000	0.071	0.708	<0.01	<0.02	0.386	7.400
CW2b	< 0.4	1.060	<0.1	<0.05	0.320	<2	<0.1	106.000	<0.02	0.735	<0.01	<0.02	0.441	4.500
CW2ab	#DIV/0!	1.022	#DIV/0!	#DIV/0!	0.510	#DIV/0!	#DIV/0!	436.000	0.071	0.722	#DIV/0!	#DIV/0!	0.414	5.950
S1a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S1b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S1ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
S2a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S2b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S2ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
S3a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S3b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S3ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
S4a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S4b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S4ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
S5b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S6b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S7b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S8b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 8. Continued.

site	Mo ppb	Na ppm	Ni ppb	P ppm	Pb ppb	Rb ppb	Sb ppb	Sc ppb	Se ppb	Si ppm	Sn ppb	Sr ppb	Th ppm	Ti ppm
W1a	<3	77.200	<0.5	0.298	<0.1	0.700	0.800	2.700	<1	5.970	<1	190.000	<0.02	<0.01
W1b	<3	79.300	<0.5	0.216	<0.1	0.630	0.500	2.000	<1	6.600	<1	148.000	<0.02	<0.01
W1ab	#DIV/0!	78.250	#DIV/0!	0.257	#DIV/0!	0.665	0.650	2.350	#DIV/0!	6.285	#DIV/0!	169.000	#DIV/0!	#DIV/0!
W2a	<3	16.300	3.200	<0.2	2.800	6.300	<0.5	3.100	<1	8.070	<1	36.800	<0.02	<0.01
W2b	<3	18.200	4.000	<0.2	0.750	6.600	<0.5	2.300	<1	9.080	<1	37.000	<0.02	<0.01
W2ab	#DIV/0!	17.250	3.600	#DIV/0!	1.775	6.450	#DIV/0!	2.700	#DIV/0!	8.575	#DIV/0!	36.900	#DIV/0!	#DIV/0!
W3a	<3	3.700	2.100	<0.2	5.900	3.000	<0.5	3.800	<1	10.800	<1	14.700	<0.02	<0.01
W3b	<3	3.270	0.560	<0.2	0.170	2.200	<0.5	2.300	<1	10.300	<1	9.300	<0.02	<0.01
W3ab	#DIV/0!	3.485	1.330	#DIV/0!	3.035	2.600	#DIV/0!	3.050	#DIV/0!	10.550	#DIV/0!	12.000	#DIV/0!	#DIV/0!
W4a	<3	13.200	1.300	0.213	0.120	3.200	<0.5	8.500	<1	23.900	<1	87.600	<0.02	<0.01
W4b	<3	13.800	0.560	<0.2	<0.1	3.000	<0.5	6.000	<1	25.500	<1	75.300	<0.02	<0.01
W4ab	#DIV/0!	13.500	0.930	0.213	0.120	3.100	#DIV/0!	7.250	#DIV/0!	24.700	#DIV/0!	81.450	#DIV/0!	#DIV/0!
W5a	<3	910.000	<0.5	0.431	1.100	2.300	<0.5	2.600	12.500	4.870	<1	432.000	<0.02	<0.01
W5b	<30	896.000	<5	0.269	<1	2.000	<5	<10	<10	4.760	<10	401.000	<0.02	<0.01
W5ab	#DIV/0!	903.000	#DIV/0!	0.350	1.100	2.150	#DIV/0!	2.600	12.500	4.815	#DIV/0!	416.500	#DIV/0!	#DIV/0!
W6a	<3	227.000	<0.5	0.674	0.100	1.000	<0.5	1.800	1.100	4.490	<1	126.000	<0.02	<0.01
W6b	9.900	73.900	2.900	<0.2	<0.1	7.200	2.100	<1	1.800	1.780	<1	299.000	<0.02	<0.01
W6ab	9.900	150.450	2.900	0.674	0.100	4.100	2.100	1.800	1.450	3.135	#DIV/0!	212.500	#DIV/0!	#DIV/0!
W7a	<3	446.000	<0.5	1.050	<0.1	1.000	<0.5	2.200	4.600	5.060	<1	126.000	<0.02	<0.01
W7b	<30	469.000	<5	1.110	<1	1.000	<5	<10	<10	5.320	<10	113.000	<0.02	<0.01
W7ab	#DIV/0!	457.500	#DIV/0!	1.080	#DIV/0!	1.000	#DIV/0!	2.200	4.600	5.190	#DIV/0!	119.500	#DIV/0!	#DIV/0!
W8a	<3	66.000	<0.5	0.222	0.240	1.200	<0.5	3.000	<1	8.240	<1	376.000	<0.02	<0.01
W8b	<3	69.200	<0.5	<0.2	<0.1	1.100	<0.5	2.400	<1	8.830	<1	333.000	<0.02	<0.01
W8ab	#DIV/0!	67.600	#DIV/0!	0.222	0.240	1.150	#DIV/0!	2.700	#DIV/0!	8.535	#DIV/0!	354.500	#DIV/0!	#DIV/0!
W9a	<3	12.000	0.690	<0.2	9.100	5.100	<0.5	1.200	<1	3.090	<1	111.000	<0.02	<0.01
W9b	<3	3.530	<0.5	<0.2	<0.1	1.600	<0.5	1.400	<1	5.600	<1	18.900	<0.02	<0.01
W9ab	#DIV/0!	7.765	0.690	#DIV/0!	9.100	3.350	#DIV/0!	1.300	#DIV/0!	4.345	#DIV/0!	64.950	#DIV/0!	#DIV/0!
W10a	<3	139.000	1.500	<0.2	4.800	1.800	0.600	1.300	<1	3.020	<1	121.000	<0.02	<0.01
W10b	<30	179.000	<5	0.384	6.200	<1	<5	<10	<10	2.110	<10	35.000	<0.02	<0.01
W10ab	#DIV/0!	159.000	1.500	0.384	5.500	1.800	0.600	1.300	#DIV/0!	2.565	#DIV/0!	78.000	#DIV/0!	#DIV/0!
W11a	<3	435.000	<0.5	0.670	<0.1	1.400	<0.5	2.000	3.800	4.330	<1	173.000	<0.02	<0.01
W11b	<30	447.000	<5	0.439	1.700	1.300	<5	<10	<10	4.460	<10	159.000	<0.02	<0.01
W11ab	#DIV/0!	441.000	#DIV/0!	0.555	1.700	1.350	#DIV/0!	2.000	3.800	4.395	#DIV/0!	166.000	#DIV/0!	#DIV/0!
W12a	<3	66.200	<0.5	0.366	<0.1	1.300	<0.5	3.000	<1	7.890	<1	458.000	<0.02	<0.01
W12b	<3	66.800	<0.5	<0.2	<0.1	1.200	<0.5	2.100	<1	7.610	<1	499.000	<0.02	<0.01
W12ab	#DIV/0!	66.500	#DIV/0!	0.366	#DIV/0!	1.250	#DIV/0!	2.550	#DIV/0!	7.750	#DIV/0!	478.500	#DIV/0!	#DIV/0!
MW1a	<3	9.220	9.800	<0.2	<0.1	4.500	<0.5	5.400	<1	14.900	<1	19.200	<0.02	<0.01
MW2a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MW1a2a	#DIV/0!	9.22	9.8	#DIV/0!	#DIV/0!	4.5	#DIV/0!	5.4	#DIV/0!	14.9	#DIV/0!	19.2	#DIV/0!	#DIV/0!
CW1a	<3	2.960	<0.5	<0.2	0.610	4.800	<0.5	3.000	<1	8.330	<1	13.200	<0.02	<0.01
CW1b	<3	2.850	<0.5	<0.2	1.400	4.300	<0.5	2.000	<1	9.040	<1	10.200	<0.02	<0.01
CW1ab	#DIV/0!	2.905	#DIV/0!	#DIV/0!	1.005	4.550	#DIV/0!	2.500	#DIV/0!	8.685	#DIV/0!	11.700	#DIV/0!	#DIV/0!
CW2a	<3	2.650	7.400	<0.2	29.300	2.700	<0.5	2.500	<1	6.910	<1	8.500	<0.02	<0.01
CW2b	<3	2.770	1.500	<0.2	6.300	2.500	<0.5	1.600	<1	7.160	<1	7.400	<0.02	<0.01
CW2ab	#DIV/0!	2.710	4.450	#DIV/0!	17.800	2.600	#DIV/0!	2.050	#DIV/0!	7.035	#DIV/0!	7.950	#DIV/0!	#DIV/0!
S1a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S1b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S1ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
S2a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S2b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S2ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
S3a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S3b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S3ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
S4a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S4b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S4ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
S5b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S6b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S7b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S8b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 8. Continued.

site	Tl ppb	U ppb	V ppb	W ppm	Y ppm	Zn ppb	Zr ppm	# of organic compounds	sum of integrated areas	Number of Isolates	Penicillium sp. Total	P. citrinum	P. canescens	P. waksmanii
W1a	<0.3	<0.3	<1	<0.1	<0.01	1.500	<0.02	60.0	6.78	5	0	0	0	0
W1b	<0.3	<0.3	6.300	<0.1	<0.01	<1	<0.02	99.0	247.59	nd	nd	nd	nd	nd
W1ab	#DIV/0!	#DIV/0!	6.300	#DIV/0!	#DIV/0!	1.500	#DIV/0!	79.5	127.19	5	0	0	0	0
W2a	<0.3	<0.3	<1	<0.1	<0.01	194.000	<0.02	80.0	12.61	2	0	0	0	0
W2b	<0.3	<0.3	<1	<0.1	<0.01	277.000	<0.02	75.0	7.70	nd	nd	nd	nd	nd
W2ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	235.500	#DIV/0!	77.5	10.16	2	0	0	0	0
W3a	<0.3	<0.3	<1	<0.1	<0.01	182.000	<0.02	39.0	13.08	9	21	20	0	0
W3b	<0.3	<0.3	<1	<0.1	<0.01	3.800	<0.02	218.0	229.16	nd	nd	nd	nd	nd
W3ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	92.900	#DIV/0!	128.5	121.12	9	21	20	0	0
W4a	<0.3	<0.3	<1	<0.1	<0.01	21.200	<0.02	71.0	50.70	5	23	0	0	2
W4b	<0.3	<0.3	1.700	0.110	<0.01	15.200	<0.02	99.0	28.55	nd	nd	nd	nd	nd
W4ab	#DIV/0!	#DIV/0!	1.700	0.110	#DIV/0!	18.200	#DIV/0!	85.0	39.62	5	23	0	0	2
W5a	<0.3	<0.3	<1	<0.1	<0.01	44.800	<0.02	76.0	57.30	6	0	0	0	0
W5b	<3	<3	11.000	<0.1	<0.01	31.000	<0.02	108.0	168.44	nd	nd	nd	nd	nd
W5ab	#DIV/0!	#DIV/0!	11.000	#DIV/0!	#DIV/0!	37.900	#DIV/0!	92.0	112.87	6	0	0	0	0
W6a	<0.3	<0.3	<1	<0.1	<0.01	1.600	<0.02	90.0	15.19	2	0	0	0	0
W6b	<0.3	<0.3	1.800	0.110	<0.01	239.000	<0.02	190.0	288.11	nd	nd	nd	nd	nd
W6ab	#DIV/0!	#DIV/0!	1.800	0.110	#DIV/0!	120.300	#DIV/0!	140.0	151.65	2	0	0	0	0
W7a	<0.3	<0.3	<1	<0.1	<0.01	1.400	<0.02	25.0	1.89	5	5	0	0	0
W7b	<3	<3	<10	0.100	<0.01	22.000	<0.02	51.0	3.78	nd	nd	nd	nd	nd
W7ab	#DIV/0!	#DIV/0!	#DIV/0!	0.100	#DIV/0!	11.700	#DIV/0!	38.0	2.84	5	5	0	0	0
W8a	<0.3	<0.3	<1	<0.1	<0.01	2.400	<0.02	67.0	10.74	6	0	0	0	0
W8b	<0.3	<0.3	6.000	<0.1	<0.01	<1	<0.02	80.0	14.49	nd	nd	nd	nd	nd
W8ab	#DIV/0!	#DIV/0!	6.000	#DIV/0!	#DIV/0!	2.400	#DIV/0!	73.5	12.61	6	0	0	0	0
W9a	<0.3	<0.3	<1	0.120	<0.01	143.000	<0.02	125.0	43.01	5	5	0	0	0
W9b	<0.3	<0.3	<1	<0.1	<0.01	9.800	<0.02	138.0	83.99	nd	nd	nd	nd	nd
W9ab	#DIV/0!	#DIV/0!	#DIV/0!	0.120	#DIV/0!	76.400	#DIV/0!	131.5	63.50	5	5	0	0	0
W10a	<0.3	<0.3	<1	<0.1	<0.01	182.000	<0.02	160.0	148.25	7	0	0	0	0
W10b	<3	<3	<10	<0.1	<0.01	501.000	<0.02	215.0	392.03	nd	nd	nd	nd	nd
W10ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	341.500	#DIV/0!	187.5	270.14	7	0	0	0	0
W11a	<0.3	<0.3	<1	<0.1	<0.01	1.700	<0.02	170.0	120.42	10	2	0	0	0
W11b	<3	<3	<10	<0.1	<0.01	39.000	<0.02	99.0	37.43	nd	nd	nd	nd	nd
W11ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	20.350	#DIV/0!	134.5	78.93	10	2	0	0	0
W12a	<0.3	<0.3	<1	<0.1	<0.01	<1	<0.02	62.0	12.52	9	0	0	0	0
W12b	<0.3	<0.3	6.000	<0.1	<0.01	<1	<0.02	120.0	23.00	nd	nd	nd	nd	nd
W12ab	#DIV/0!	#DIV/0!	6.000	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	91.0	17.76	9	0	0	0	0
MW1a	<0.3	<0.3	<1	<0.1	<0.01	65.400	<0.02	nd	nd	nd	nd	nd	nd	nd
MW2a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MW1a2a	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	65.4	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
CW1a	<0.3	<0.3	<1	<0.1	<0.01	19.800	<0.02	35.0	1.95	4	0	0	0	0
CW1b	<0.3	<0.3	<1	<0.1	<0.01	16.100	<0.02	101.0	13.06	nd	nd	nd	nd	nd
CW1ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	17.950	#DIV/0!	68.0	7.50	4	0	0	0	0
CW2a	<0.3	<0.3	<1	<0.1	<0.01	224.000	<0.02	21.0	4.46	9	19	0	0	0
CW2b	<0.3	<0.3	<1	<0.1	<0.01	81.400	<0.02	83.0	6.17	nd	nd	nd	nd	nd
CW2ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	152.700	#DIV/0!	52.0	5.31	9	19	0	0	0
S1a	nd	nd	nd	nd	nd	nd	nd	nd	nd	15	0	0	0	0
S1b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S1ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	nd	nd	15	0	0	0	0
S2a	nd	nd	nd	nd	nd	nd	nd	nd	nd	17	5	0	0	0
S2b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S2ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	nd	nd	17	5	0	0	0
S3a	nd	nd	nd	nd	nd	nd	nd	nd	nd	4	1	0	0	0
S3b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S3ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	nd	nd	4	1	0	0	0
S4a	nd	nd	nd	nd	nd	nd	nd	nd	nd	14	6	0	0	0
S4b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S4ab	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	nd	nd	14	6	0	0	0
S5b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S6b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S7b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S8b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 8. Continued.

site	P. decumbens	P. glabrum	P. chrysogenum	P. sclerotiorum	P. restrictum	P. miczynskii	P. raistrickii	P. simplicissimum	P. expansum	Eupenicillium lapidosum	Unkown Penicillium sp.	Aspergillus sp. Total	A. parasiticus	A. niger
W1a	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W1b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W1ab	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W2a	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W2b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W2ab	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W3a	0	0	0	0	0	0	0	0	0	1	0	0	0	0
W3b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W3ab	0	0	0	0	0	0	0	0	0	1	0	0	0	0
W4a	21	0	0	0	0	0	0	0	0	0	0	0	0	0
W4b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W4ab	21	0	0	0	0	0	0	0	0	0	0	0	0	0
W5a	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W5b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W5ab	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W6a	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W6b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W6ab	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W7a	0	5	0	0	0	0	0	0	0	0	0	0	0	0
W7b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W7ab	0	5	0	0	0	0	0	0	0	0	0	0	0	0
W8a	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W8b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W8ab	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W9a	0	0	0	5	0	0	0	0	0	0	0	0	0	0
W9b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W9ab	0	0	0	5	0	0	0	0	0	0	0	0	0	0
W10a	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W10b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W10ab	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W11a	0	0	0	0	2	0	0	0	0	0	0	0	0	0
W11b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W11ab	0	0	0	0	2	0	0	0	0	0	0	0	0	0
W12a	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W12b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W12ab	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MW1a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MW2a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MW1a2a	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
CW1a	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CW1b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
CW1ab	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CW2a	0	0	0	0	0	0	0	0	0	4	15	8	8	0
CW2b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
CW2ab	0	0	0	0	0	0	0	0	0	4	15	8	8	0
S1a	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S1b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S1ab	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S2a	0	1	0	1	0	1	0	0	0	0	2	0	0	0
S2b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S2ab	0	1	0	1	0	1	0	0	0	0	2	0	0	0
S3a	0	0	0	0	0	0	0	1	0	0	0	0	0	0
S3b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S3ab	0	0	0	0	0	0	0	1	0	0	0	0	0	0
S4a	0	0	0	0	0	0	2	1	3	0	0	1	0	1
S4b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S4ab	0	0	0	0	0	0	2	1	3	0	0	1	0	1
S5b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S6b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S7b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S8b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 8. Continued.

site	Alternaria sp.	Cladosporium Sp.	Epicoccum sp.	Trichoderma	Zygomycetes Class	Paecilomyces	Chrysosporium sp.	Coelmycete	Chloridium sp.	Unknowns	Unknown dermatophyte	Bacteria	Leptospira spp.
W1a	0	209	1	0	0	0	0	0	0	1	0	1	nd
W1b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W1ab	0	209	1	0	0	0	0	0	0	1	0	1	#DIV/0!
W2a	0	0	tntc	0	0	0	0	0	0	tntc	0	0	nd
W2b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W2ab	0	0	#DIV/0!	0	0	0	0	0	0	#DIV/0!	0	0	#DIV/0!
W3a	0	101	8	59	0	0	0	0	0	0	0	0	nd
W3b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W3ab	0	101	8	59	0	0	0	0	0	0	0	0	#DIV/0!
W4a	0	0	2	0	0	0	0	0	0	27	0	0	nd
W4b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W4ab	0	0	2	0	0	0	0	0	0	27	0	0	#DIV/0!
W5a	0	143	4	0	0	0	0	0	0	16	0	0	nd
W5b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W5ab	0	143	4	0	0	0	0	0	0	16	0	0	#DIV/0!
W6a	0	0	14	0	0	0	0	0	0	0	0	0	nd
W6b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W6ab	0	0	14	0	0	0	0	0	0	0	0	0	#DIV/0!
W7a	0	55	33	0	0	0	0	0	0	0	0	0	nd
W7b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W7ab	0	55	33	0	0	0	0	0	0	0	0	0	#DIV/0!
W8a	1	36	8	0	0	0	0	0	0	0	0	1	nd
W8b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W8ab	1	36	8	0	0	0	0	0	0	0	0	1	#DIV/0!
W9a	0	0	0	0	59	0	0	0	0	0	0	0	nd
W9b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W9ab	0	0	0	0	59	0	0	0	0	0	0	0	#DIV/0!
W10a	0	270	5	0	7	0	0	0	0	3	0	0	nd
W10b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W10ab	0	270	5	0	7	0	0	0	0	3	0	0	#DIV/0!
W11a	0	23	2	0	0	23	0	0	0	4	5	0	nd
W11b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W11ab	0	23	2	0	0	23	0	0	0	4	5	0	#DIV/0!
W12a	0	153	25	8	2	0	0	0	0	0	0	0	nd
W12b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
W12ab	0	153	25	8	2	0	0	0	0	0	0	0	#DIV/0!
MW1a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MW2a	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MW1a2a	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
CW1a	0	29	0	0	0	33	0	0	0	0	0	0	nd
CW1b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
CW1ab	0	29	0	0	0	33	0	0	0	0	0	0	#DIV/0!
CW2a	0	157	19	0	0	0	0	0	0	0	0	0	nd
CW2b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
CW2ab	0	157	19	0	0	0	0	0	0	0	0	0	#DIV/0!
S1a	0	tntc	5	0	0	0	2	14	0	2	0	1	0
S1b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1
S1ab	0	#DIV/0!	5	0	0	0	2	14	0	2	0	1	1
S2a	0	23	0	2	0	0	0	0	1	6	0	1	0
S2b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0
S2ab	0	23	0	2	0	0	0	0	1	6	0	1	0
S3a	0	0	0	0	10	0	0	0	0	0	0	0	0
S3b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0
S3ab	0	0	0	0	10	0	0	0	0	0	0	0	0
S4a	0	255	1	0	0	0	0	0	0	5	0	0	0
S4b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0
S4ab	0	255	1	0	0	0	0	0	0	5	0	0	0
S5b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1
S6b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1
S7b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0
S8b	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1

Table 9. Explanation of notation used in Table 8.

SAMPLE ID NOTATION

W= well
MW= mine well
CW= control well
S= surface water site
MC= mine coal
CC= control coal

a= May (2002) sample
b= September (2002) sample
ab= mean of May and September samples

DATA NOTATIONS

nd= no data available
0= actual zero value
***=** below detection limit (of instrument or analysis technique)
tntc= too numerous to count
na= not applicable (e.g.-no well depth for a surface site)
#DIV/0!= not applicable (cell is mathematical equation with illegal values)

DETECTION LIMITS

nutrients (ammonium & phosphate) = +/- 1.0 nanomole/liter
anions = +/- 10 ppb

Table 10. Results of the Spearman correlation under H0: Rho = 0. Only parameters with significance values < 0.1500 are presented.

covariate	coefficient (γ)	p-value
total number of organic compounds (TIC data)	1.0000	<0.0001
fungi: Class Zygomycetes	0.8944	0.1056
nutrient: NH_3	1.0000	<0.0001
nutrient: PO_4	1.0000	<0.0001
element: As	0.9487	0.0513
element: B	1.0000	<0.0001
element: Br	0.9487	0.0513
element: Cl	1.0000	<0.0001
element: Cr	1.0000	<0.0001
element: F	1.0000	<0.0001
element: Li	0.9487	0.0513
element: Na	1.0000	<0.0001
element: P	1.0000	<0.0001
element: Rb	-1.0000	<0.0001
element: Se	0.9487	0.0513
element: Sr	1.0000	<0.0001
element: W	0.9487	0.0513

Table 11. Results of environmental samples using L primers

Sample ID	Undiluted	1:10 dilution	Summary
02050703	0 of 2	0 of 2	Possible*
02050708	0 of 2	0 of 2	Possible
Lab blank	0 of 2	0 of 2	ND**
02050803	0 of 2	0 of 2	Possible
02050808	0 of 2	0 of 2	Possible
02090401	1 of 2	1 of 2	Present
02090402	1 of 2	0 of 2	Present
02090403	1 of 2	0 of 2	Possible
02090404	0 of 2	0 of 2	Possible
02090405	2 of 2	1 of 1	Present
Lab blank	0 of 2	0 of 1	ND
02090407	0 of 2	0 of 2	Possible
02090503	0 of 2	0 of 2	Possible
02090505A	2 of 2	2 of 2	Present
02090505B (replicate)	1 of 2	0 of 2	Present
02090508	0 of 2	0 of 1	Possible
02090509	0 of 2	0 of 1	Possible

* refers to the possible presence of pathogenic leptospires in sample as indicated by a PCR band too faint to be conclusively considered positive (present)

** ND, not detected

Figure Captions

Figure 1. Map showing distribution by Parish of kidney cancer and cancer of the renal pelvis cases, 1998-1999.

Figure 2. Map of Gulf Coast region showing distribution of coal bearing formations (such as the Wilcox Group). This geologic data comprised one of the geographic information systems themes that were used to help select sample wells.

Figure 3. Map of Louisiana parishes, showing the location of Sabine Uplift coinciding with lignite deposits of the Wilcox group, along the Red River valley. From Mineral Resources of Louisiana, Department of Conservation, Louisiana Geological Survey, Baton Rouge, LA, 1976.

Figure 4. Map showing parishes in Louisiana that contain lignite deposits. Locations of lignite outcrops are shown as points. "Control group" sites are recommendations for future work, and were not tested in the present study. The two control wells used in this pilot phase were located in Vernon Parish (sites not shown on this map; see Figure 8).

Figure 5. Map of Louisiana indicating the approximate boundaries for the major aquifers in the northern part of the state that are Tertiary in age. "Control group" sites are recommendations for future work, and were not tested in the present study. The two control wells used in this pilot phase were located in Vernon Parish.

Figure 6. Representative household from which well water sample was collected in northwestern Louisiana. Note pump housing in foreground.

Figure 7. Representative field site (bayou) in northwestern Louisiana showing source of surface water collected for microbiological assays.

Figure 8. Detailed map of study area showing locations of water sample collection sites (green dots = wells, blue dots = surface sites), hydrologic units, and aquifers.

Figure 9. Example of cross section derived from the Stratifact ® software (version 4.57, Stratifact Software, Wheat Ridge, Colorado) used to help select sample wells based on their probable intersection with coal beds.

Figure 10. USGS researchers collecting well water samples that will be shipped to laboratories for geochemical and microbiological testing, and conducting on-site analysis of physical properties of well water in Red River Parish, Louisiana.

Figure 11. USGS researcher collecting a sample of lignite from the Dolet Hills coal mine that will be shipped to laboratories for geochemical and physical characterization.

Figure 12. Total ion current chromatogram showing identification of selected organic compounds from water collected at site W2a (map code 2).

Figure 13. Total ion current chromatogram showing identification of selected organic compounds from water collected at site W3a.

Figure 14. Total ion current chromatogram showing identification of selected organic compounds from water collected at site W10b.

Figure 15. Total ion current chromatogram showing identification of selected organic compounds from water collected at site W6b.

Figure 16. Total ion current chromatogram showing identification of selected organic compounds from water collected at site W2b.

Figure 17. Total ion current chromatogram showing identification of selected organic compounds from water collected at site W3b.

Figure 18. Total ion current chromatograms of Louisiana drinking well water samples collected from areas with high incidence of urinary tract cancer and underlying lignite deposits (W1 and W2) and control drinking well water samples from areas lacking lignite deposits (CW1 and CW 2).

Figure 19. Total ion current chromatograms demonstrating seasonal variation in the number of the organic components and abundances in a drinking well water sample collected from Louisiana from a parish with high incidence of urinary tract cancer and underlying low rank coal (lignite) deposits.

Figure 20. Two percent agarose gel visualized under UV with ethidium bromide staining showing PCR results of controlled experiment for pathogenic *Leptospira* spp. Each series is in dilution order (10^{-1} to 10^{-5}) followed by a blank. The first set is the filtered (F) series, followed by unfiltered (U) series and cells (C) series. Four wells were loaded and run with a 100 base pair ladder was used for reference (each product is just under 500 base pairs) – the two outside lanes and the two others evenly spaced across gel.

Figure 21. Composite image of individual lanes from two percent agarose gels visualized under UV with ethidium bromide staining showing PCR results of environmental samples in which *Leptospira* was present (expected band is 481 base pairs).

Figure 1.

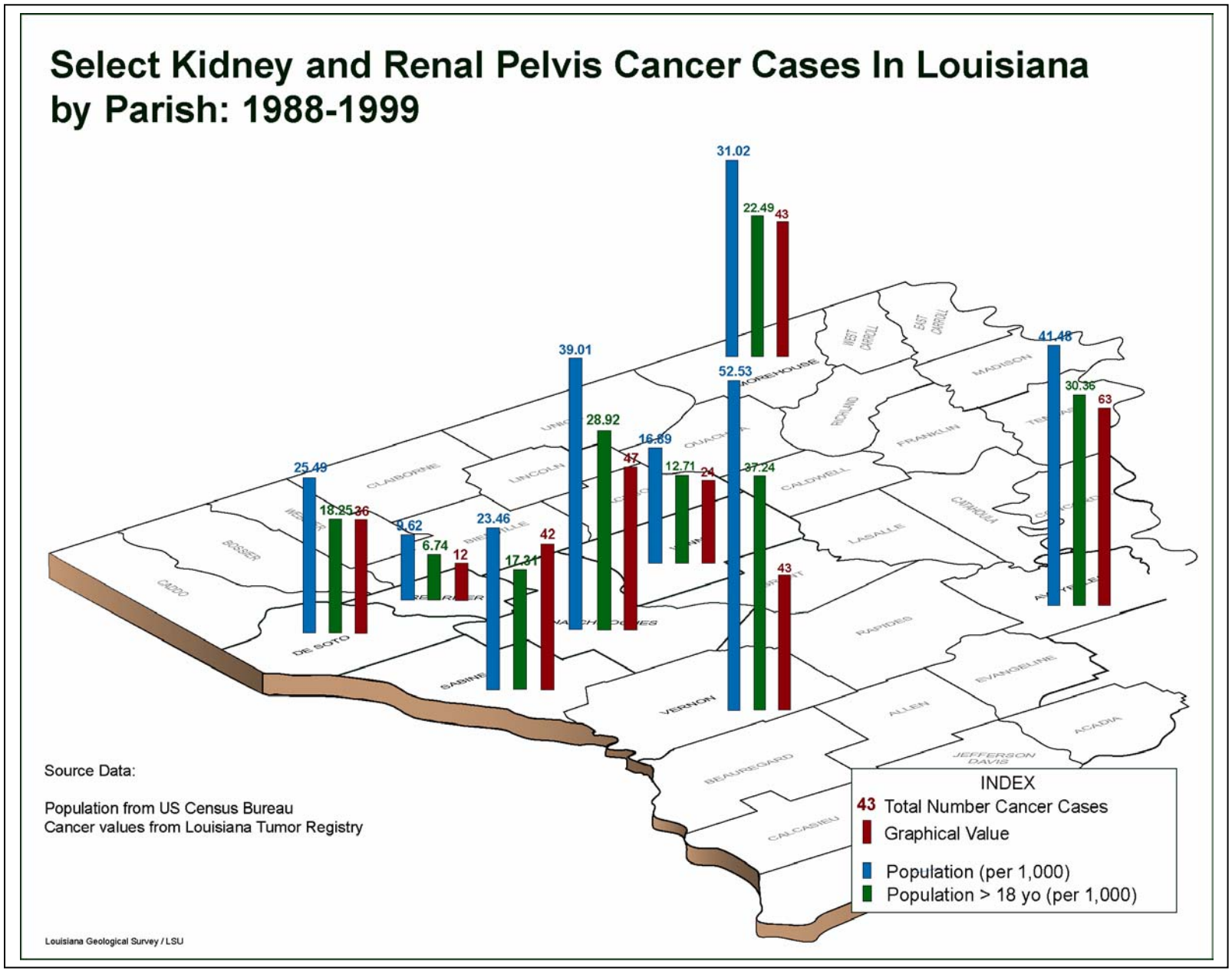

Select Kidney and Renal Pelvis Cancer Cases In Louisiana by Parish: 1988-1999
31.02
22.49
43
39.01
28.92
47
52.53
37.24
43
41.48
30.36
63
25.49
18.25
36
16.89
12.71
24
9.62
6.74
12
23.46
17.31
42
CLAIBORNE
LINCOLN
OUACHITA
RICHLAND
FRANKLIN
CALDWELL
LASALLE
CATAHOULA
BOSSIER
CADDO
DE SOTO
SABINE
VERNON
RAPIDES
EVANGELINE
ACADIA
ALLEN
BEAUREGARD
CALCASIEU
JEFFERSON DAVIS
MADISON
WEST CARROLL
EAST CARROLL
Source Data:
Population from US Census Bureau
Cancer values from Louisiana Tumor Registry
INDEX
43 Total Number Cancer Cases
Graphical Value
Population (per 1,000)
Population > 18 yo (per 1,000)
Louisiana Geological Survey / LSU

Figure 2.

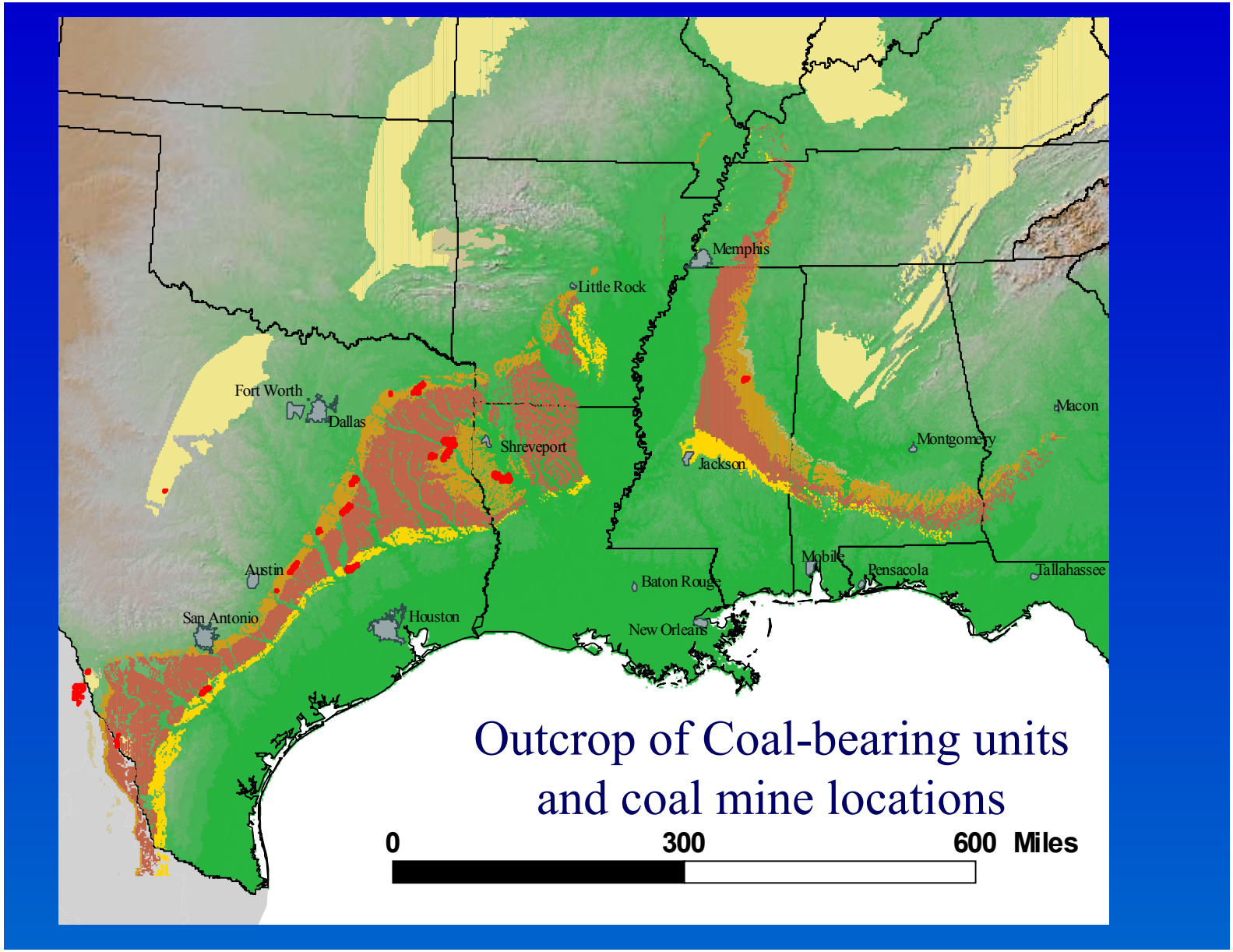

Memphis
Little Rock
Fort Worth
Dallas
Shreveport
Jackson
Macon
Montgomery
Austin
San Antonio
Houston
Baton Rouge
New Orleans
Mobile
Pensacola
Tallahassee
Outcrop of Coal-bearing units
and coal mine locations
0
300
600 Miles

Figure 3.

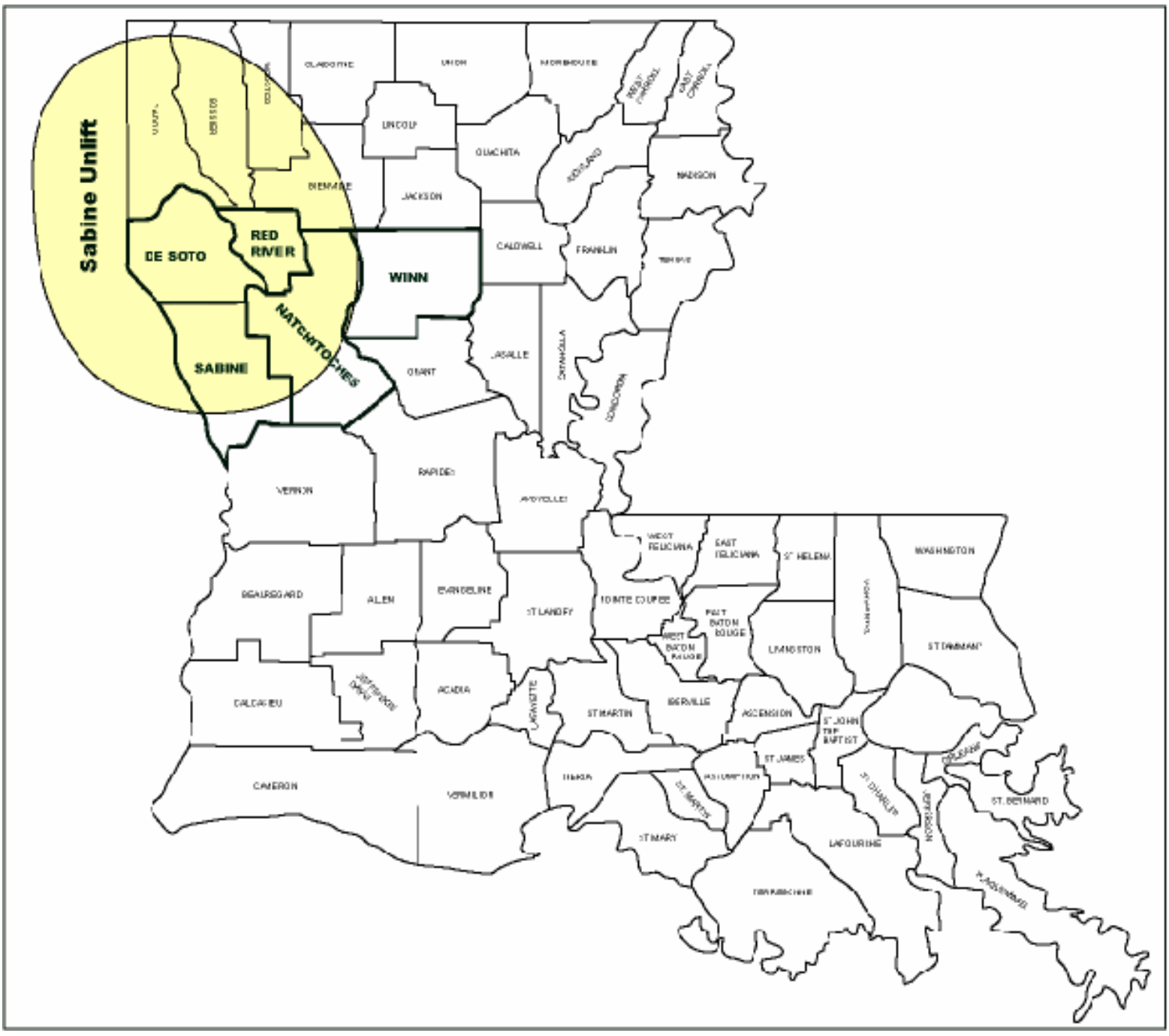

Sabine Uplift
DE SOTO
RED RIVER
SABINE
NATCHITOCHES
WINN
CLAIBORNE
UNION
MORNHOUSE
LINCOLN
OUACHITA
RICHLAND
MADISON
BIENVILLE
JACKSON
CALDWELL
FRANKLIN
GRANT
LASALLE
CATAHOULA
CONCORDIA
VERNON
RAPIDES
AVOYELLES
WEST FELICIANA
EAST FELICIANA
ST. HELENA
WASHINGTON
BEAUREGARD
ALLEN
EVANGELINE
ST LANDRY
POINTE COUPEE
EAST BATON ROUGE
WEST BATON ROUGE
LIVINGSTON
TANGIPAHOA
ST TAMMANY
CALCASIEU
JEFFERSON DAVIS
ACADIA
LAFAYETTE
ST MARTIN
IBERVILLE
ASCENSION
ST JOHN THE BAPTIST
ST JAMES
ST CHARLES
JEFFERSON
ST BERNARD
CAMERON
VERMILION
IBERIA
ST MARY
LAFOURCHE
TERREBONNE

Figure 4.

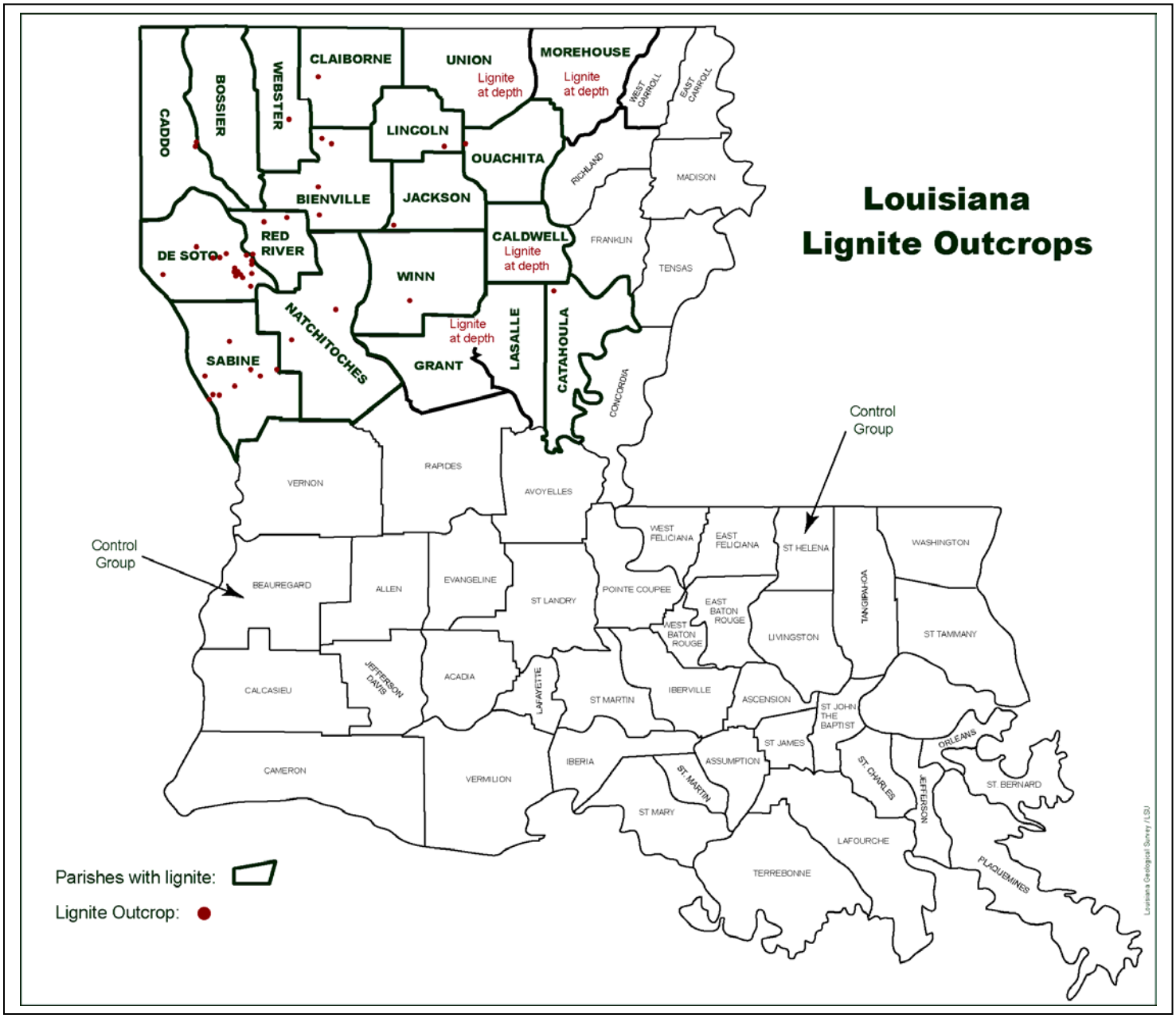
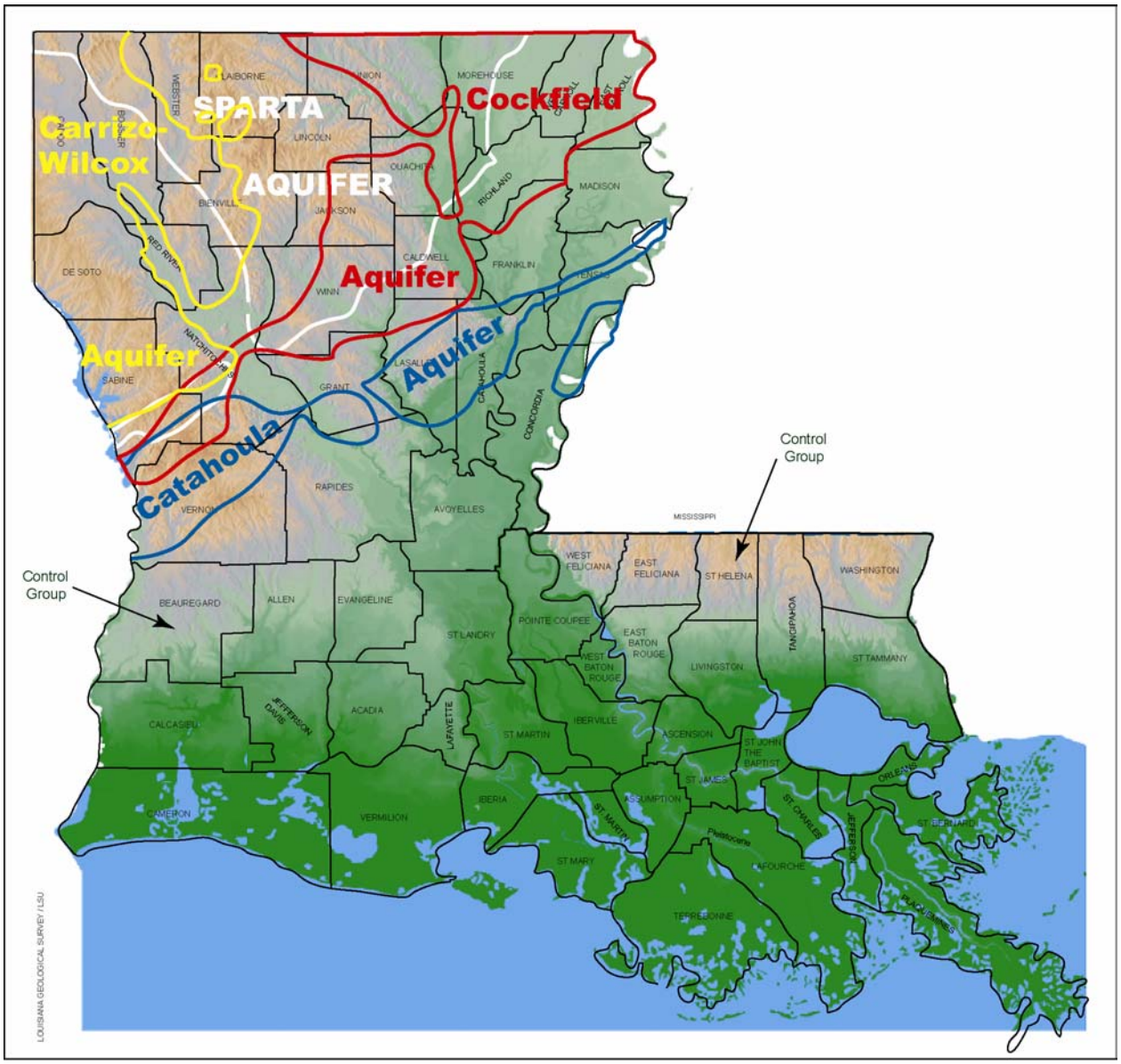

Louisiana
Lignite Outcrops
CLAIBORNE
UNION
Lignite at depth
MOREHOUSE
Lignite at depth
WEST CARROLL
EAST CARROLL
CADDO
BOSSIER
WEBSTER
LINCOLN
OUACHITA
RICHLAND
MADISON
BIENVILLE
JACKSON
RED RIVER
DE SOTO
CALDWELL
Lignite at depth
FRANKLIN
TENSAS
WINN
NATCHITOCHES
Lignite at depth
LASALLE
CATAHOULA
CONCORDIA
SABINE
GRANT
Control Group
VERNON
RAPIDES
AVOYELLES
Control Group
WEST FELICIANA
EAST FELICIANA
ST HELENA
WASHINGTON
BEAUREGARD
ALLEN
EVANGELINE
ST LANDRY
POINTE COUPEE
EAST BATON ROUGE
WEST BATON ROUGE
LIVINGSTON
TANGIPAHOA
ST TAMMANY
CALCASIEU
JEFFERSON DAVIS
ACADIA
LAFAYETTE
ST MARTIN
IBERVILLE
ASCENSION
ST JOHN THE BAPTIST
ST JAMES
ORLEANS
CAMERON
VERMILION
IBERIA
ASSUMPTION
ST. CHARLES
JEFFERSON
ST. BERNARD
ST MARY
LAFOURCHE
TERREBONNE
PLAQUEMINES
Parishes with lignite:
Lignite Outcrop:
Louisiana Geological Survey / LSU

Figure 5.



Approximate boundaries for major aquifers in north Louisiana that are Tertiary in age.

Figure 6.

Figure 7.

Figure 8.

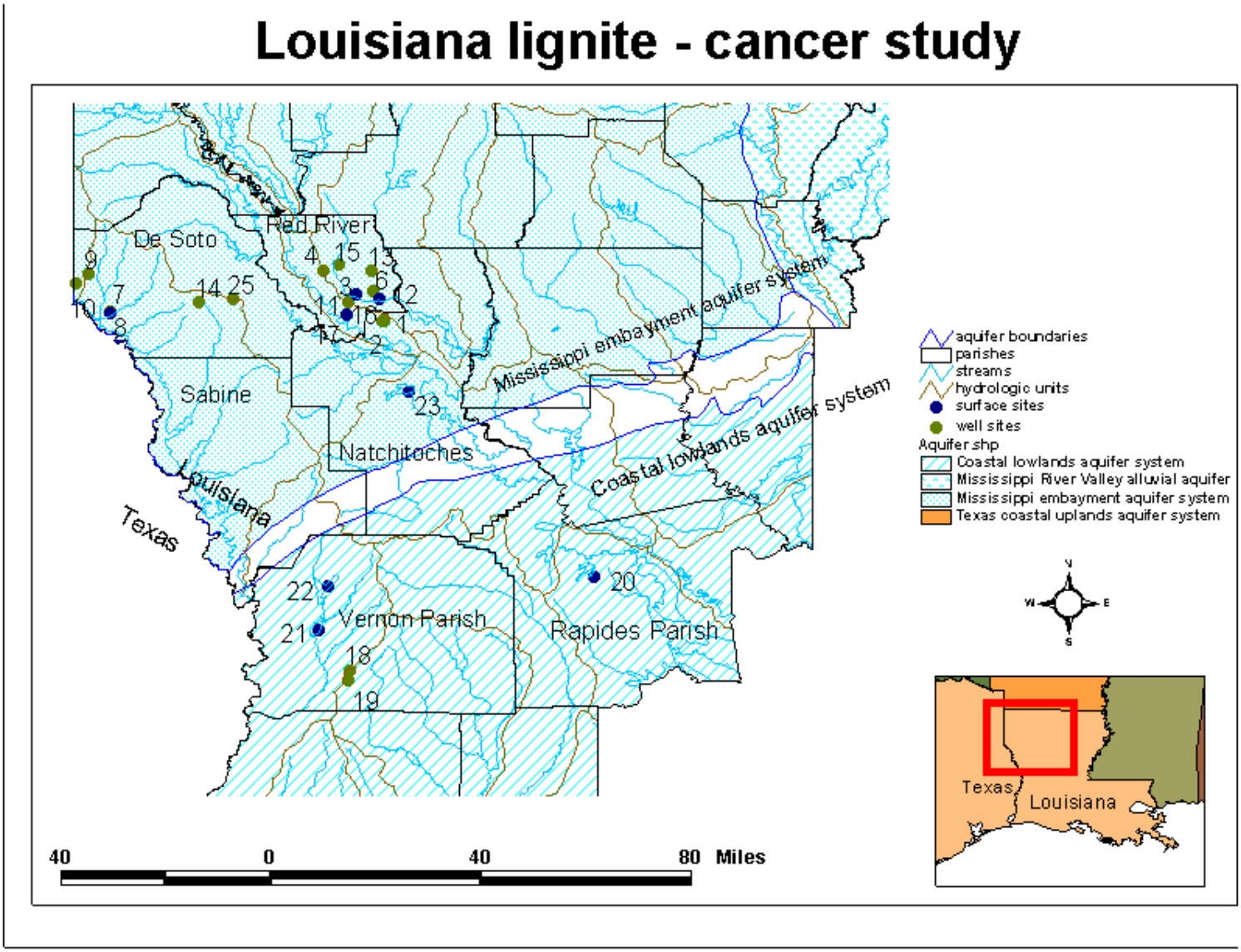

Louisiana lignite - cancer study
Red River
De Soto
Sabine
Natchitoches
Louisiana
Texas
Vernon Parish
Rapides Parish
Mississippi embayment aquifer system
Coastal lowlands aquifer system
aquifer boundaries
parishes
streams
hydrologic units
surface sites
well sites
Aquifer.shp
Coastal lowlands aquifer system
Mississippi River Valley alluvial aquifer
Mississippi embayment aquifer system
Texas coastal uplands aquifer system
40 0 40 80 Miles
Texas
Louisiana

Figure 9.

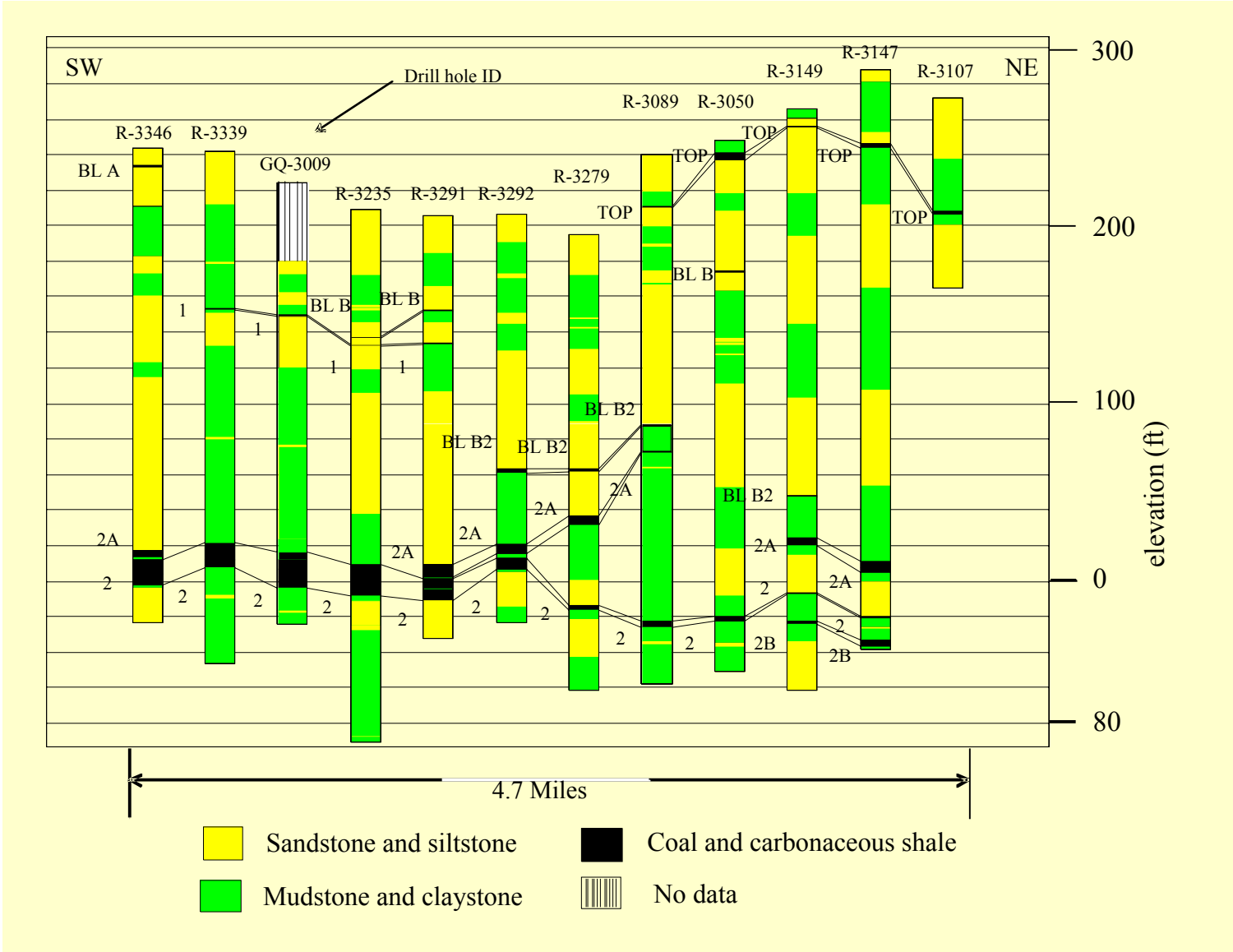

SW
NE
Drill hole ID
R-3346
R-3339
GQ-3009
R-3235
R-3291
R-3292
R-3279
R-3089
R-3050
R-3149
R-3147
R-3107
BL A
BL B
BL B2
2A
TOP
1
2
2B
300
200
100
0
80
elevation (ft)
4.7 Miles
Sandstone and siltstone
Coal and carbonaceous shale
Mudstone and claystone
No data

Figure 10.

Figure 11.

Figure 12.

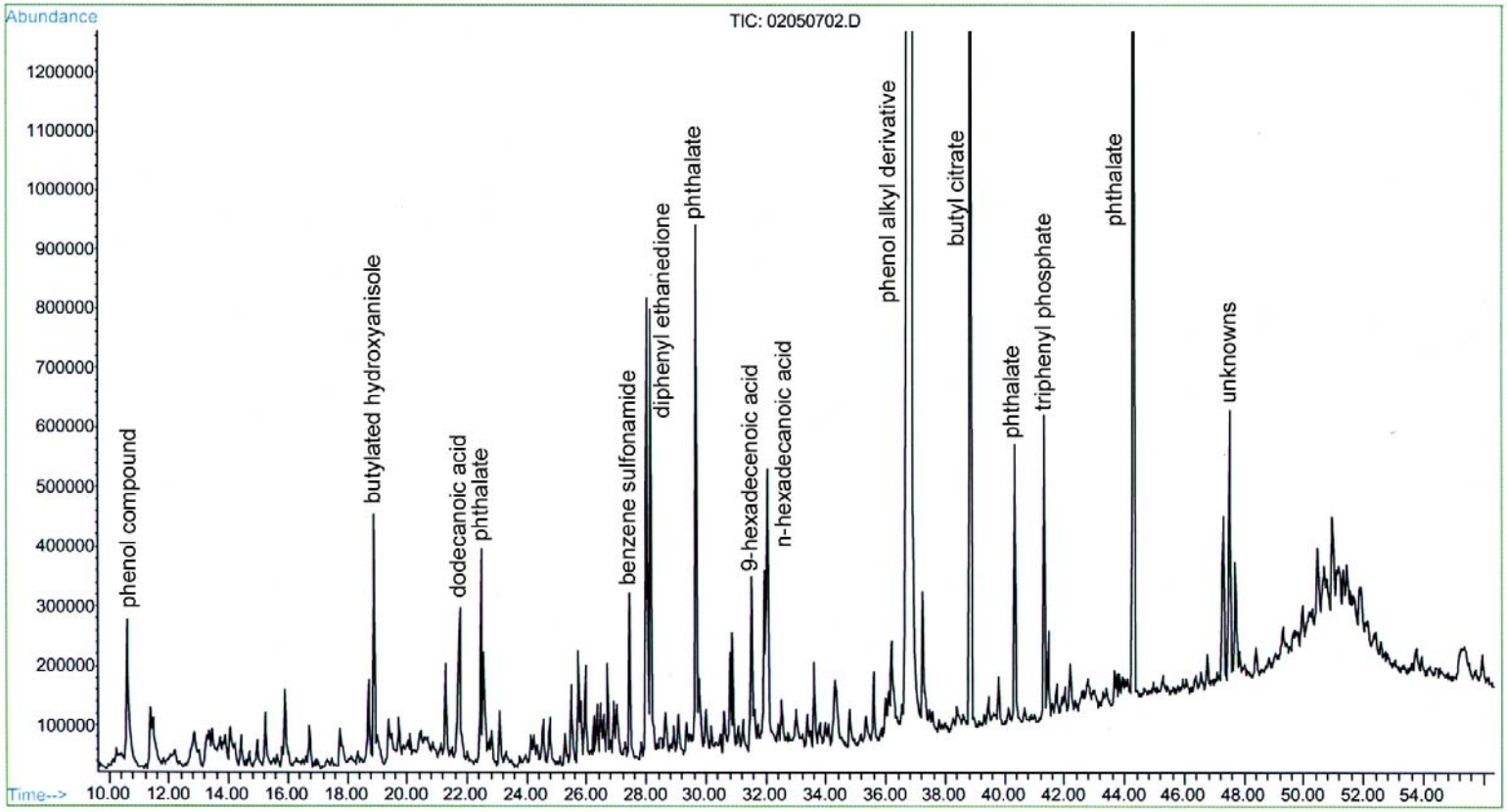

Abundance
TIC: 02050702.D
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
phenol compound
butylated hydroxyanisole
dodecanoic acid
phthalate
benzene sulfonamide
diphenyl ethanedione
phthalate
9-hexadecenoic acid
n-hexadecanoic acid
phenol alkyl derivative
butyl citrate
phthalate
triphenyl phosphate
phthalate
unknowns
Time-->
10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00 40.00 42.00 44.00 46.00 48.00 50.00 52.00 54.00

Figure 13.

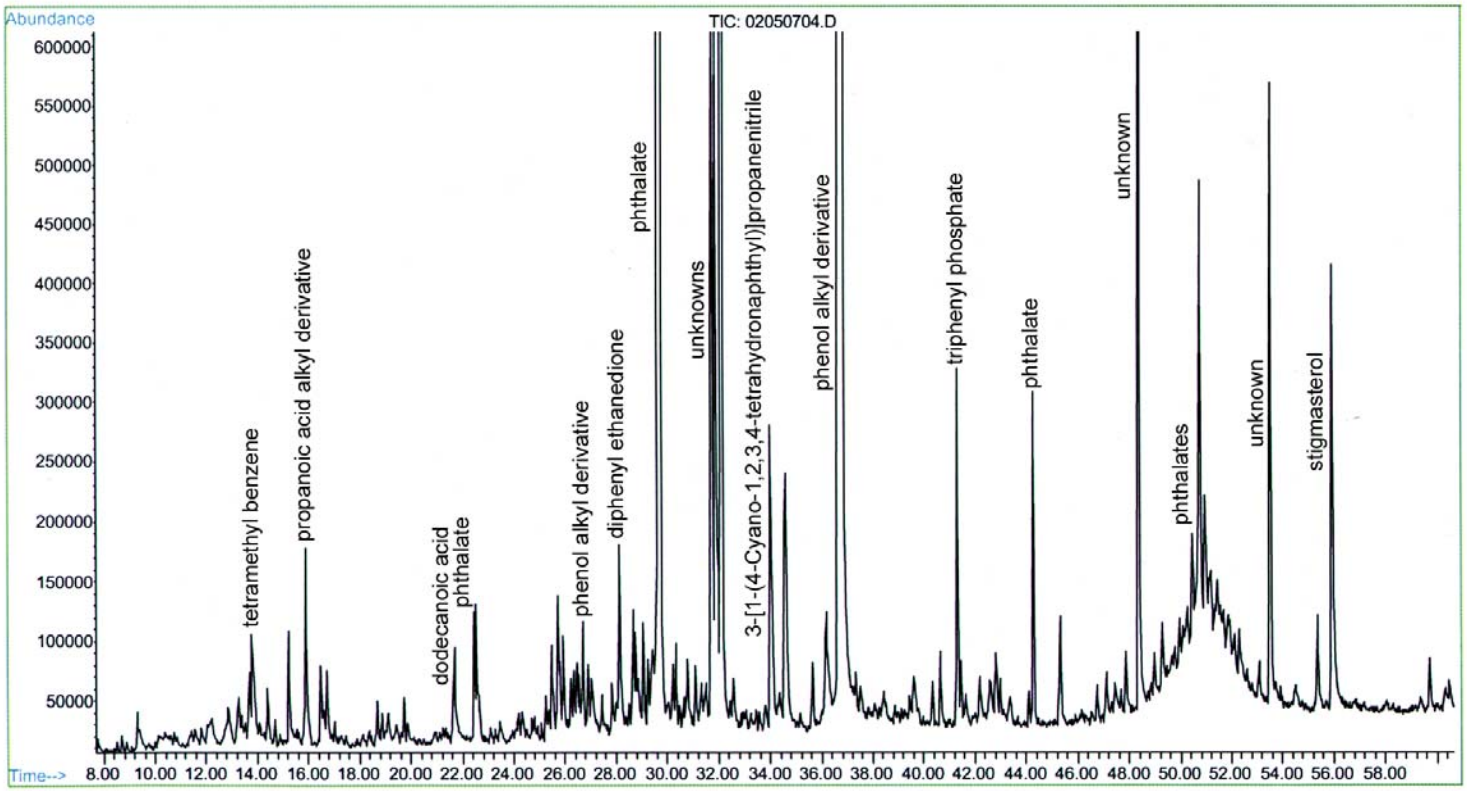
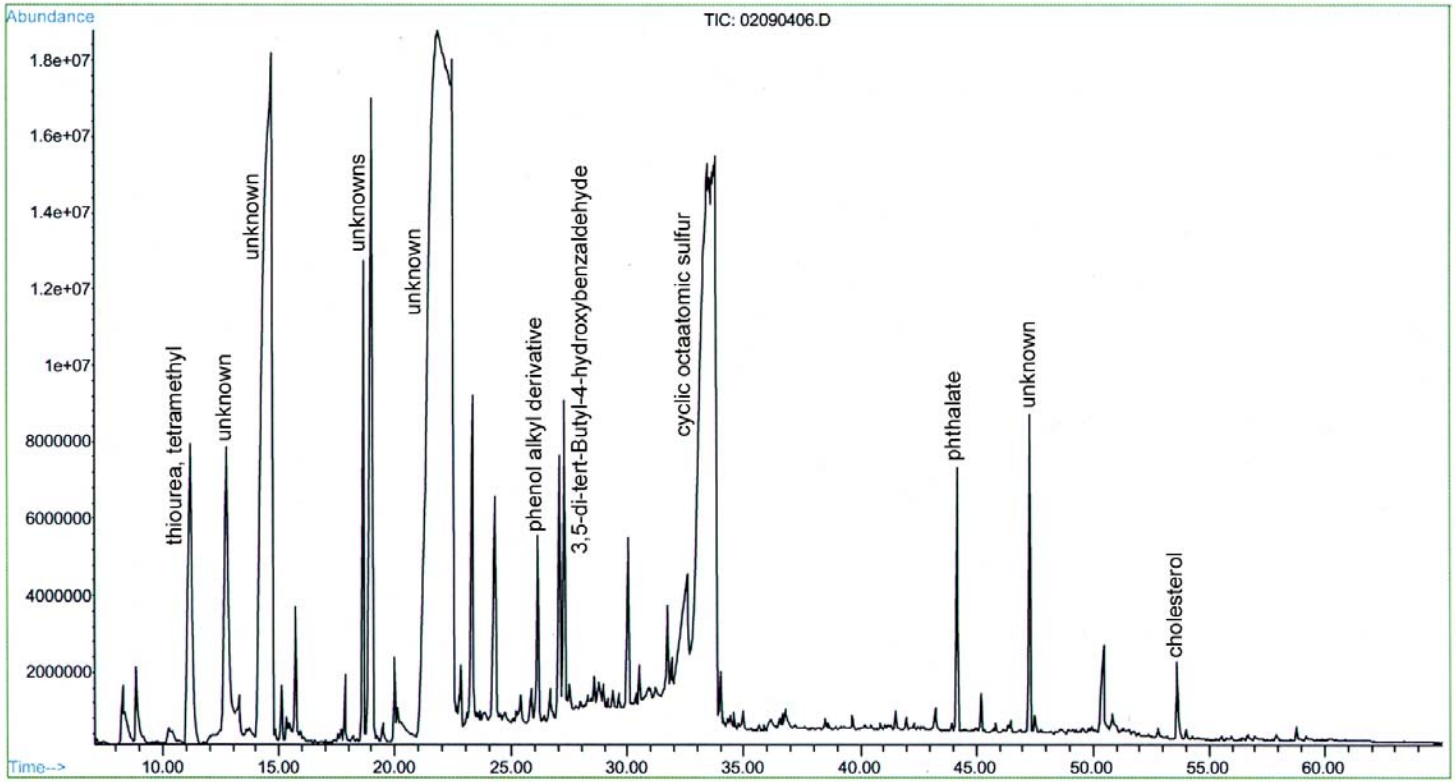

Abundance
TIC: 02050704.D
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
Time-->
8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00 40.00 42.00 44.00 46.00 48.00 50.00 52.00 54.00 56.00 58.00
tetramethyl benzene
propanoic acid alkyl derivative
dodecanoic acid
phthalate
phenol alkyl derivative
diphenyl ethanedione
phthalate
unknowns
3-[1-(4-Cyano-1,2,3,4-tetrahydronaphthyl)]propanenitrile
phenol alkyl derivative
triphenyl phosphate
phthalate
unknown
phthalates
unknown
stigmasterol

Figure 14.


Abundance
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1.8e+07
1.6e+07
1.4e+07
1.2e+07
1e+07
8000000
6000000
4000000
2000000
Time-->
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00
thiourea, tetramethyl
unknown
unknown
unknowns
unknown
phenol alkyl derivative
3,5-di-tert-Butyl-4-hydroxybenzaldehyde
cyclic octaatomic sulfur
phthalate
unknown
cholesterol

Figure 15.

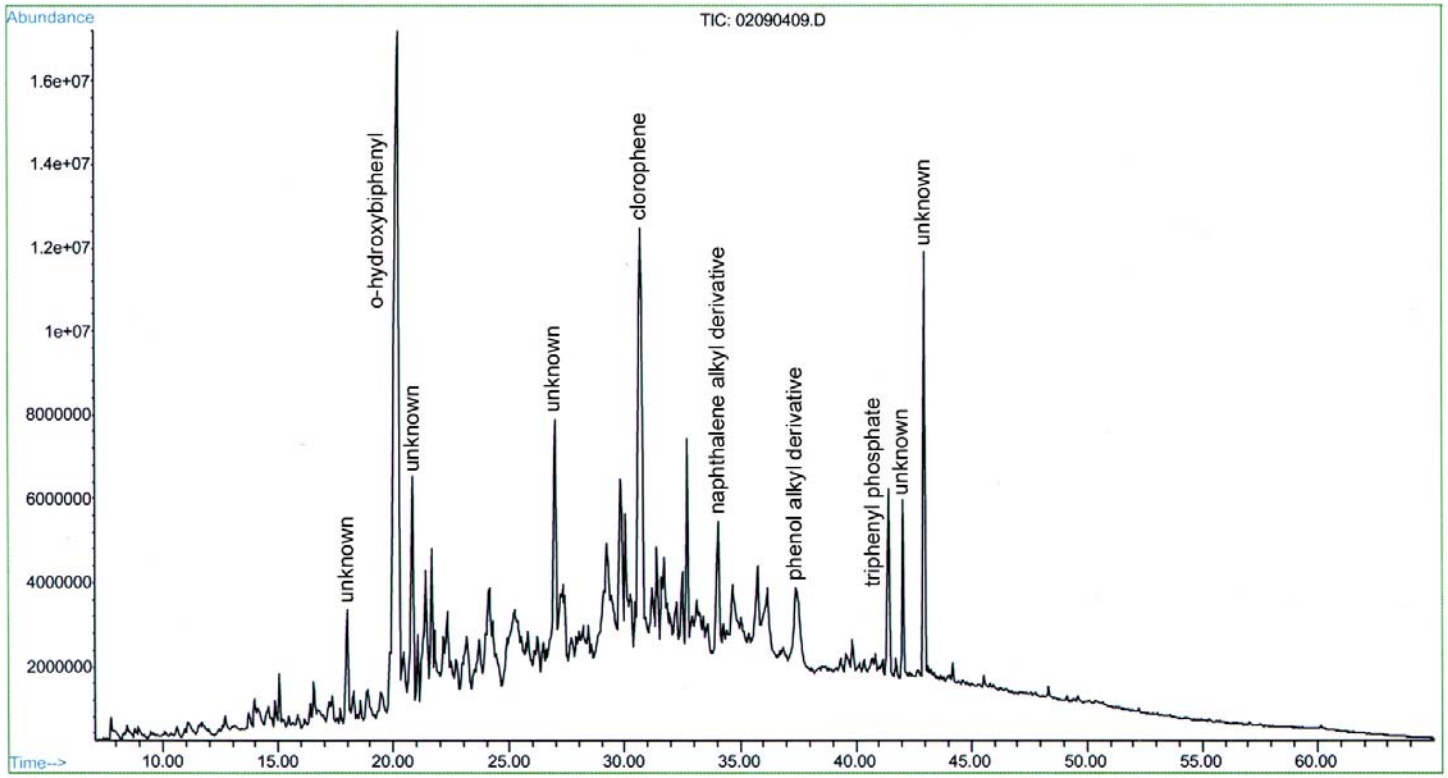
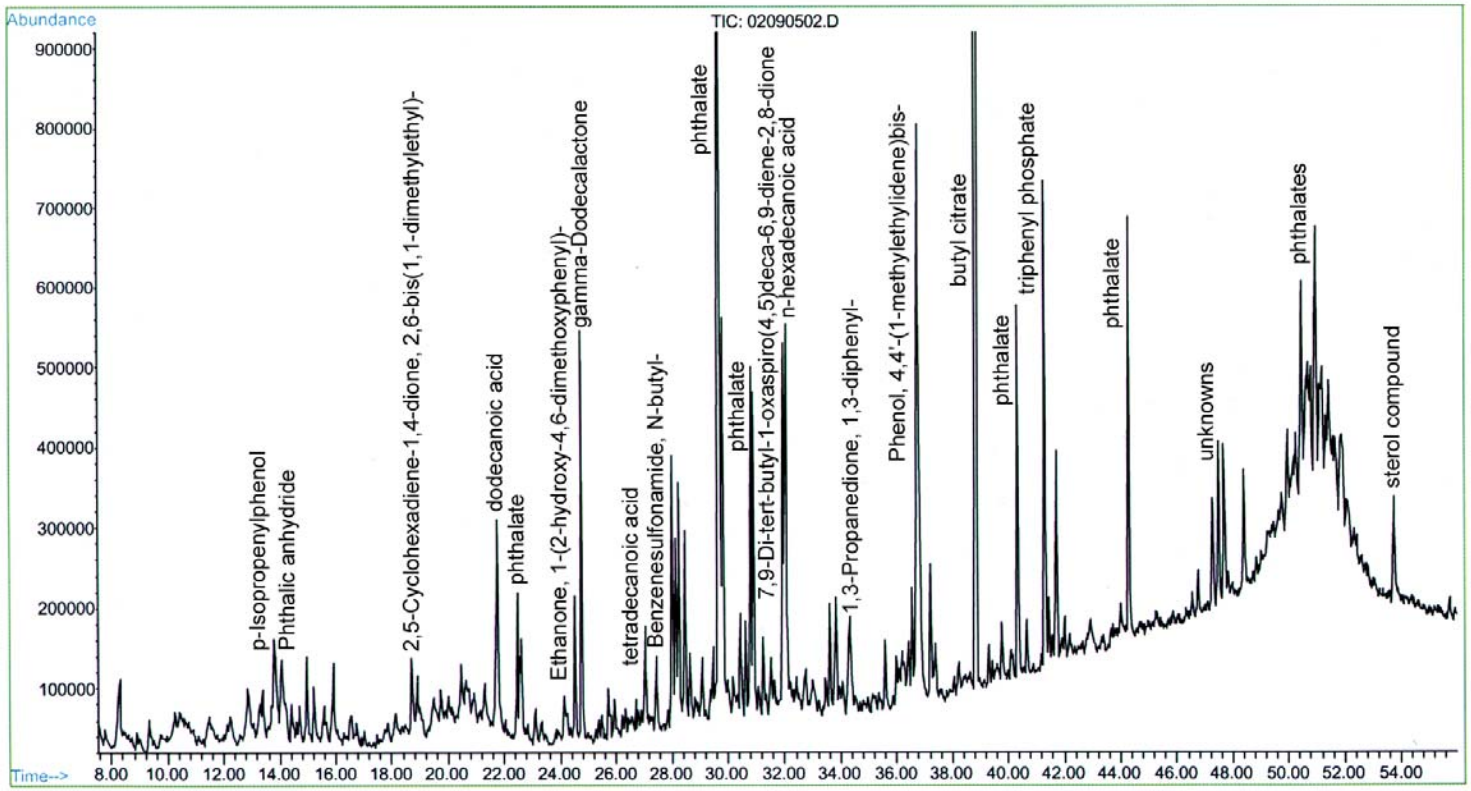

Abundance
TIC: 02090409.D
1.6e+07
1.4e+07
1.2e+07
1e+07
8000000
6000000
4000000
2000000
Time-->
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00
unknown
o-hydroxybiphenyl
unknown
unknown
clorophene
naphthalene alkyl derivative
phenol alkyl derivative
triphenyl phosphate
unknown
unknown

Figure 16.


Abundance
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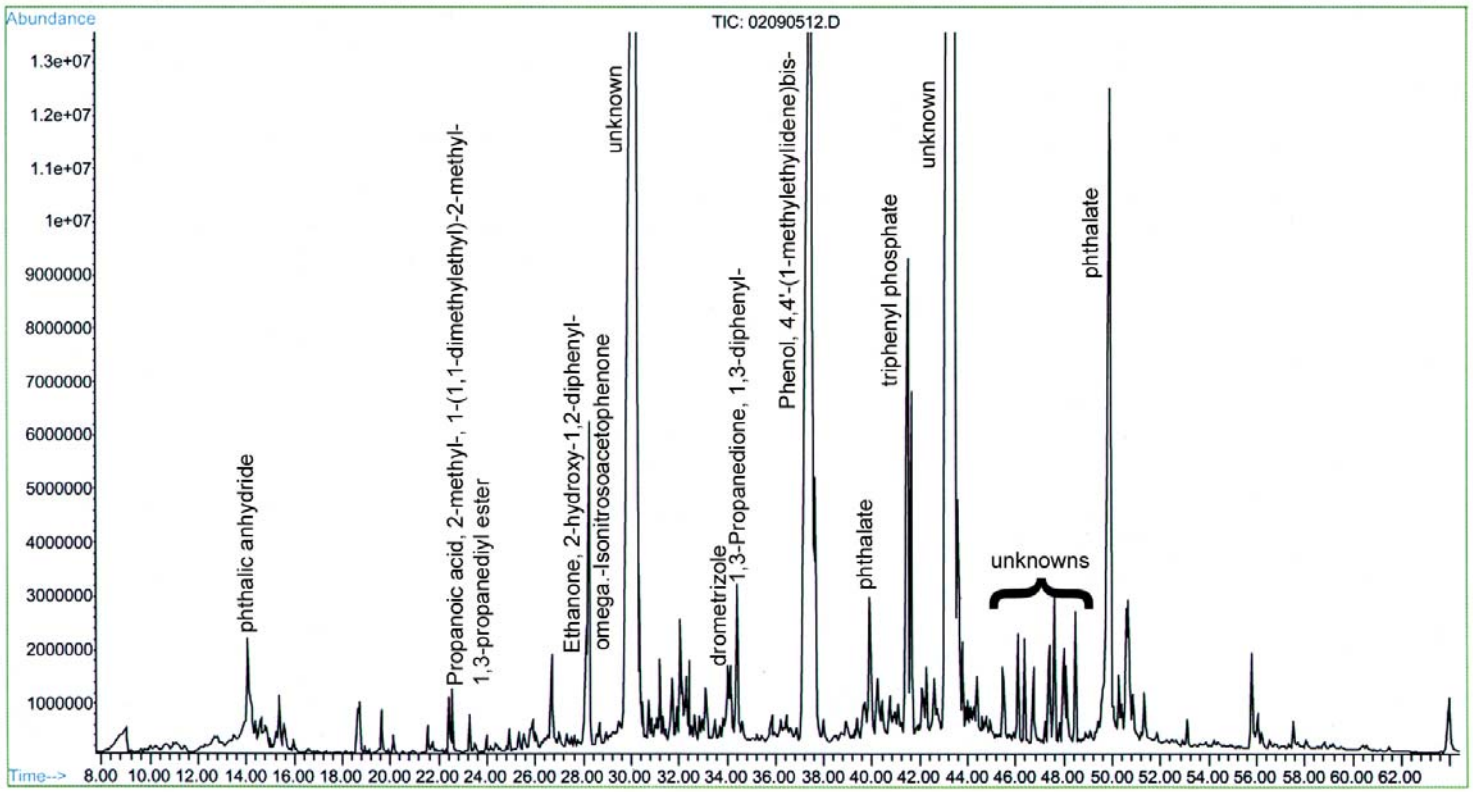
800000
700000
600000
500000
400000
300000
200000
100000
Time-->
8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00 40.00 42.00 44.00 46.00 48.00 50.00 52.00 54.00
p-Isopropenylphenol
Phthalic anhydride
2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-
dodecanoic acid
phthalate
Ethanone, 1-(2-hydroxy-4,6-dimethoxyphenyl)-
gamma-Dodecalactone
tetradecanoic acid
Benzenesulfonamide, N-butyl-
phthalate
phthalate
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
n-hexadecanoic acid
1,3-Propanedione, 1,3-diphenyl-
Phenol, 4,4'-(1-methylethylidene)bis-
butyl citrate
phthalate
triphenyl phosphate
phthalate
unknowns
phthalates
sterol compound

Figure 17.


Abundance
TIC: 02090512.D
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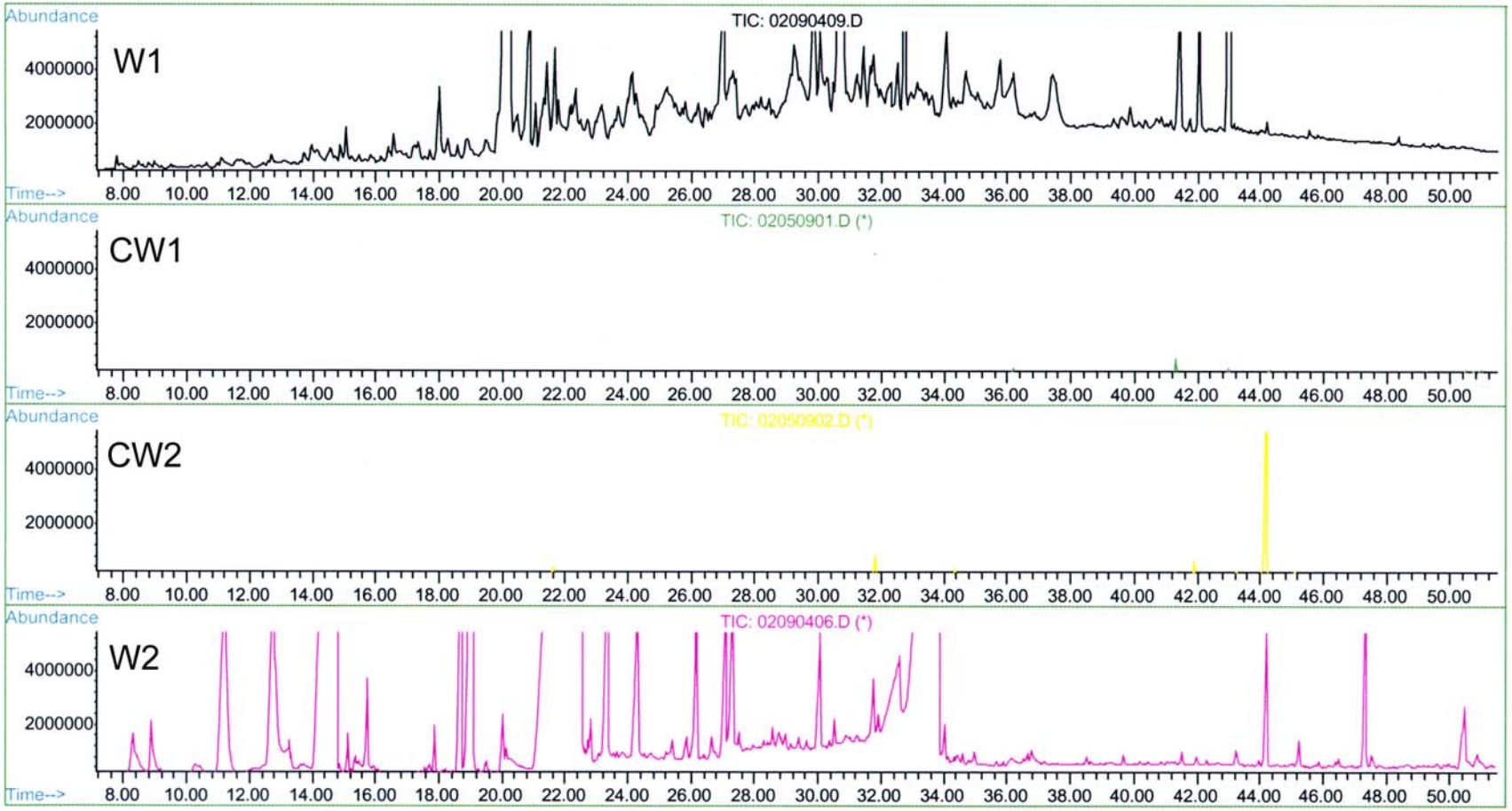
1.2e+07
1.1e+07
1e+07
9000000
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
Time-->
8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00 40.00 42.00 44.00 46.00 48.00 50.00 52.00 54.00 56.00 58.00 60.00 62.00
phthalic anhydride
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester
Ethanone, 2-hydroxy-1,2-diphenyl-
omega.-Isonitrosoacetophenone
unknown
drometrizole
1,3-Propanedione, 1,3-diphenyl-
Phenol, 4,4'-(1-methylethylidene)bis-
phthalate
triphenyl phosphate
unknown
unknowns
phthalate

Figure 18.


Abundance
TIC: 02090409.D
W1
4000000
2000000
Time-->
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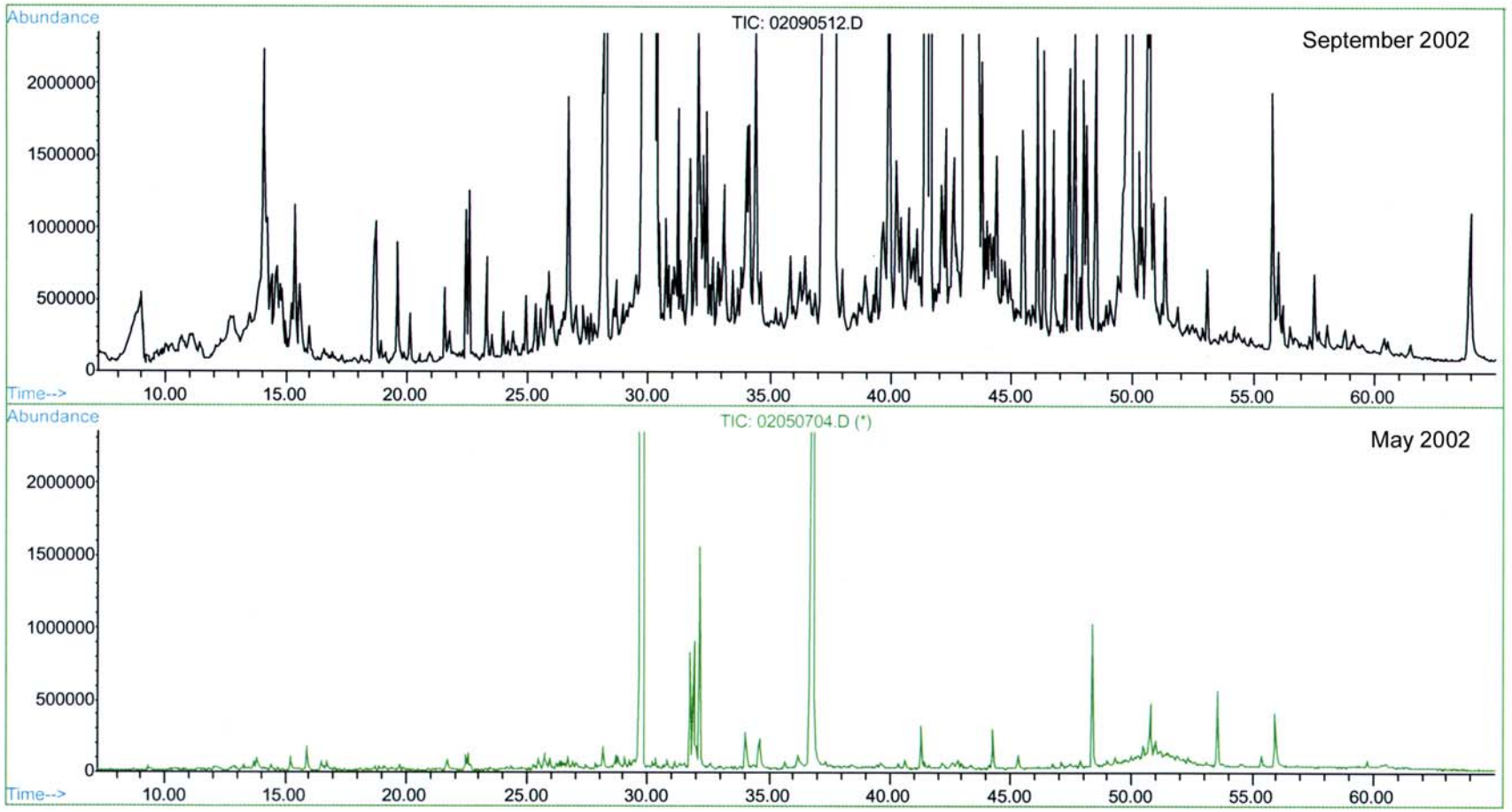
Abundance
TIC: 02050901.D (*)
CW1
4000000
2000000
Time-->
8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00 40.00 42.00 44.00 46.00 48.00 50.00
Abundance
TIC: 02050902.D (*)
CW2
4000000
2000000
Time-->
8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00 40.00 42.00 44.00 46.00 48.00 50.00
Abundance
TIC: 02090406.D (*)
W2
4000000
2000000
Time-->
8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00 40.00 42.00 44.00 46.00 48.00 50.00

Figure 19.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September 2002
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1500000
1000000
500000
0
Time-->
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00
Abundance
TIC: 02050704.D (*)
May 2002
2000000
1500000
1000000
500000
0
Time-->
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00

Figure 20.

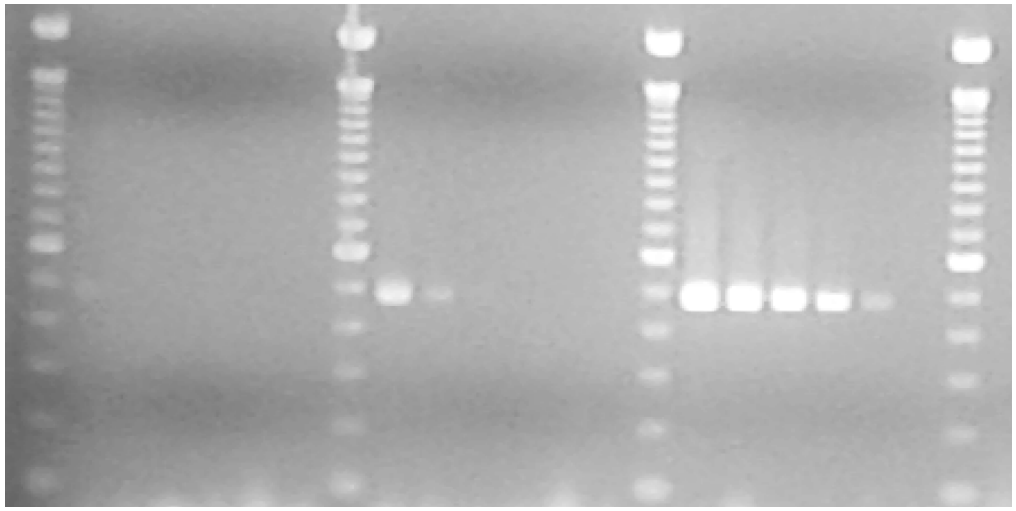
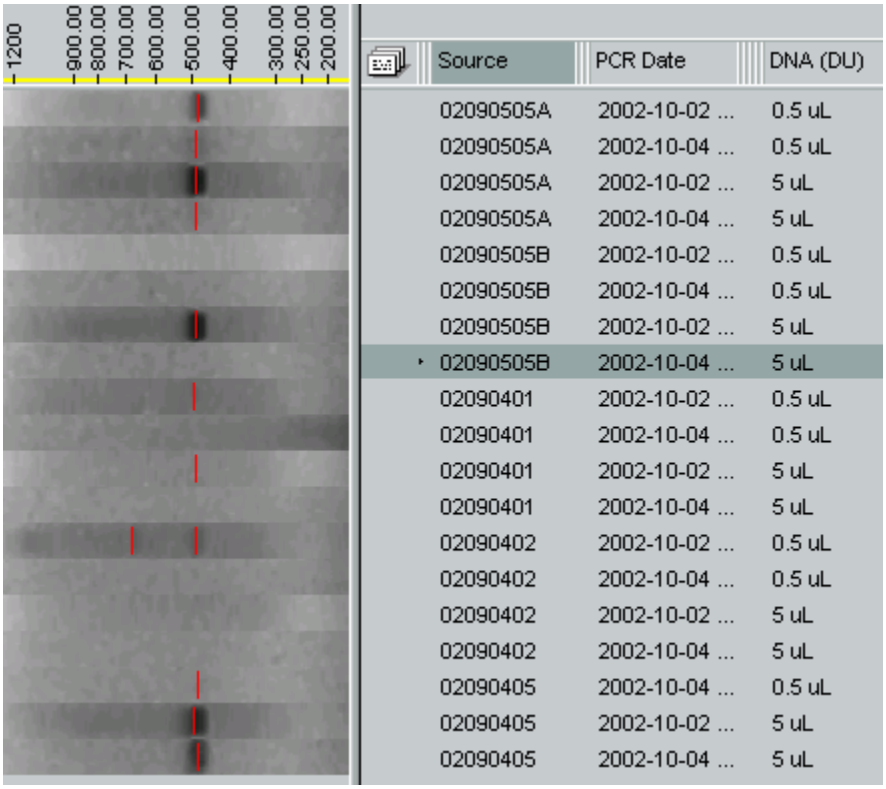


Figure 21.


1200
900.00
800.00
700.00
600.00
500.00
400.00
300.00
250.00
200.00
Source
PCR Date
DNA (DU)
02090505A 2002-10-02 ... 0.5 uL
02090505A 2002-10-04 ... 0.5 uL
02090505A 2002-10-02 ... 5 uL
02090505A 2002-10-04 ... 5 uL
02090505B 2002-10-02 ... 0.5 uL
02090505B 2002-10-04 ... 0.5 uL
02090505B 2002-10-02 ... 5 uL
02090505B 2002-10-04 ... 5 uL
02090401 2002-10-02 ... 0.5 uL
02090401 2002-10-04 ... 0.5 uL
02090401 2002-10-02 ... 5 uL
02090401 2002-10-04 ... 5 uL
02090402 2002-10-02 ... 0.5 uL
02090402 2002-10-04 ... 0.5 uL
02090402 2002-10-02 ... 5 uL
02090402 2002-10-04 ... 5 uL
02090405 2002-10-04 ... 0.5 uL
02090405 2002-10-02 ... 5 uL
02090405 2002-10-04 ... 5 uL