Cytotoxicity analysis of pre- and post-hurricane harvey soil samples collected from greater houston bayous

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ABSTRACT

Rapid urbanization, anthropogenic pollution and frequent flooding events are affecting the soil and water quality along the streams and bayous of Houston. Soil acts as sink and reservoir of heavy metals and nutrients affecting human and animal health. The objectives of the study are 1) to analyze the effects of the metal and nutrient concentration of bayou flood plain surface soil samples on the gut cell cytotoxicity and 2) to evaluate the spatial and temporal difference in soil contamination on cell viability of colon cancer (HT-29) and normal colon epithelial (CCD 841 CoN) cell lines. To evaluate soil contamination between pre- and post-hurricane (Summer and Fall) conditions in six Bayous (Brays, Buffalo, Halls, Hunting, Greens and White Oak Bayous) of Harris County, Texas, in vitro bioassay analysis was applied to soil extracts. The MTT assay determined that, with increase in concentration of Bayou soil from 12.5% to 100%, the viability of CCD 841 CoN and HT-29 cells decreased significantly, across all sampling locations during both summer and fall seasons. Among all the bayous, the viability of CCD 841 CoN cells in summer and fall followed the pattern of White Oak > Greens > Halls > Brays Bayou, where the viability of cells exposed to White Oak soils was 3–4 times higher than cells exposed to Brays Bayou soil at 100% soil concentration. The viability of HT-29 cells in both seasons followed the pattern of Greens > White Oak > Halls > Brays Bayou, where the viability of cells exposed to Greens Bayou soil was more than 3–4 times higher than the cells exposed to Brays Bayou soil at 100% concentration. The higher concentration of metals and nutrients such as P, Zn, Cd, and Cu might have contributed to the significant cell lethality in Brays Bayou samples compared to other locations.

1. Introduction

Anthropogenic land use impacts, catastrophic tropical flooding, extensive point and non-point source pollution, frequent inundation of neighborhoods are the principal drivers of surface water degradation in Houston, a coastal Metropolitan city. The City of Houston is the 4th largest city in the United States and is the largest city in Harris County covering 665 square miles with an estimated population of 2.3 million (U.S. Census Bureau, 2020). Harris County, Texas, covers 1777 square miles and is home to over 4.7 million people, making it the 3rd largest county by population in the United States (U.S. Census Bureau, 2020). The surge of urbanization in Harris County resulted in loss of vegetation and increased amount of impervious surface areas due to residential development and industrialization (Juan et al., 2020). Problems

associated with increase in impervious areas include flooding, which can cause alterations to estuarine systems (Chen et al., 2015; Zhang et al., 2018). Flooding cause poor water quality from runoffs and alter tidal prisms resulting in changes in sediment dynamics and increased sediment toxicity (Al Mukaimi et al., 2018; Schüttrumpf et al., 2011).

Hurricane Harvey made landfall on August 25, 2017, as a Category 4 storm, resulting in a high precipitation of 1224 mm in Houston (Sebastian et al., 2017). Hurricane Harvey resulted in extensive bank erosion and high sediment deposition along the food plains of Galveston bay (Du et al., 2019; Williams and Liu, 2019). Furthermore, Harvey caused structural damages to 13 Superfund sites, 813 wastewater plants and 266 industrial spills from petrochemical industry such as Valero Energy, ExxonMobil and Arkema, resulting in a release of 149 million gallons of raw sewage and hazardous chemicals into the waterways
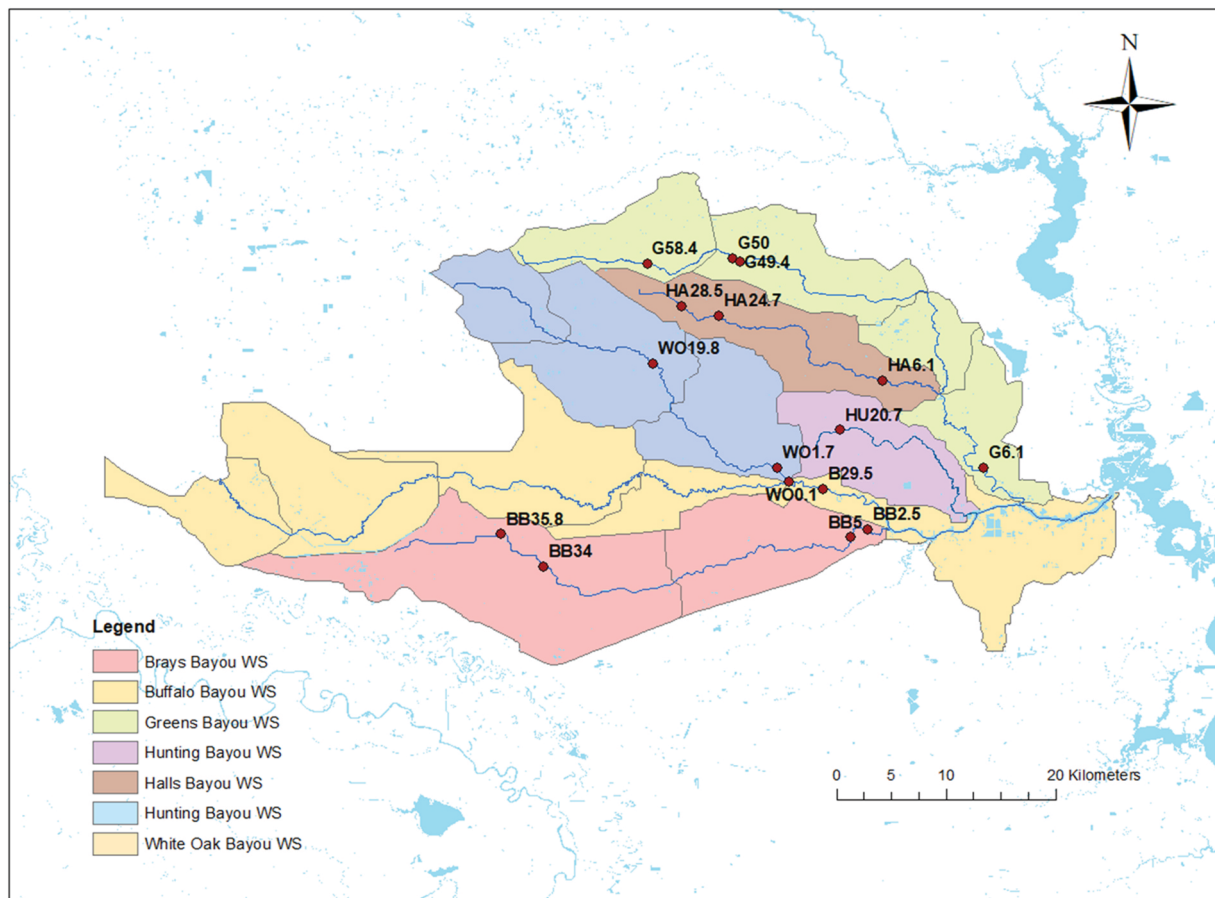
Fig. 1. Soil sampling locations in Brays (BB), Buffalo (B), Greens (G), Halls (HA), Hunting (HU) and White Oak Bayou (WO) watersheds in Houston, TX.

(Sebastian et al., 2017; TCEQ, 2018).

1.1. Heavy metal and nutrient impact on human gut cells

Soil serves as a significant repository of various environmental pollutants (Brevik and Burgess, 2014). Soil in urban areas of Houston is loaded with organic and inorganic contaminants because of industrial activities, automobile emissions, and wastewater treatment plant effluents (Burgess, 2013; Canela et al., 2020; Huff Hartz et al., 2019; Li, 2018; Spada et al., 2012). Human health is affected by exposure to toxicants in soil via inhalation of contaminated dust, dermal absorption through direct contact, and ingestion of contaminated particles and food grown in contaminated soil (Brevik et al., 2020; Steffan et al., 2018). Soil contaminants are absorbed into the body by pulmonary, integumentary or gastrointestinal system (Brevik and Burgess, 2014). Soil-derived dust inhalation causes acute inflammation of the bronchial passages, and chronic bronchitis (Steffan et al., 2018). Dermal absorption can lead to podoconiosis and dermatitis while ingestion leads to gastrointestinal illness such as diarrheal disease and gastric cancer (Bauza et al., 2017; Deribe et al., 2013; Yuan et al., 2016).

The intestinal epithelium acts as the primary physiological barrier for soil-borne ingested heavy metals and nutrients in the human body (Batsalova et al., 2017). Colon cell lines have been extensively used to study the mechanism of heavy metal toxicity on gut cell functions (Batsalova et al., 2017; Xiao et al., 2016). High concentrations of As, Cd, Cu, Mn, Hg, Zn and Pb resulted in cytotoxicity and functional imbalance of Caco-2 (Batsalova et al., 2017) and HT-29 (Xiao et al., 2016), an intestinal epithelial and colon cancer cells, respectively. Nutrients such as N and P in excess concentrations, provoke cytotoxicity by inflammation and DNA damage resulting in apoptosis and gastrointestinal cancer

(Hong et al., 2015; Kobayashi, 2018; Lanas, 2008; Ma et al., 2018).

Flood events often cause an increase in the release of industrial effluent, residential and sewage treatment plant discharges into the receiving bayous. Further, the contaminants are redistributed, whenever the bayou floods, and inundate the surrounding neighborhoods in the watershed (Sridhar et al., 2020; Bukunmi-Omidiran and Sridhar, 2021). Hence, the goal of this study is to compare the cytotoxic effect of flood plain soil from 6 bayous during the pre- and post-Harvey (Summer and Fall) periods in Houston. The objectives of the study are to 1) to analyze the effects of metal and nutrient concentration of bayou flood plain surface soil samples on the gut cell cytotoxicity and 2) evaluate the spatial and temporal difference in soil contamination on cell viability of HT-29 and CCD 841 CON cell lines.

2. Material and methods

2.1. Study area and soil sampling

The soil samples were collected at 16 sites along the Brays, Buffalo, Greens, Halls, Hunting, and White Oak Bayous during the Summer (Pre-Harvey) in June 2017 and Fall (Post-Harvey) in November 2017 (Fig. 1). All the watersheds have a clay or clay loam or sandy clay soil (TSHA, 2020) and comprises an urban area of more than 80% (BPA, 2020; HCFCD, 2020). The surface soil samples were collected at depth of 0–10 cm, 10–20 cm, and 20–30 cm. All the soil samples were collected in triplicates at each site and placed in zip-lock plastic bags. Once in the lab, the samples were air-dried, ground into fine powder, and homogenized by sieving through a stainless steel 2-mm sieve and stored in sealed containers at 4 °C. The soil samples collected at the 0–10 cm depth, which is considered topsoil were used in this study. The soil

Table 1

Heavy metal and other elemental concentration in flood plain surface soil samples collected from Greens, Halls, White Oak, Brays, Hunting and Buffalo Bayous (in mg kg^{-1}). Given are mean values (n = 3) of three replicates.

Sampling Locations	Cd	Cr	Cu	Pb	Zn	Fe	Na	P
Summer								
G6.1	0.42 a	3.28	4.57 ab	10.74 ab	36.98 ab	2086 b	135	151
G49.4	0.14 b	3.51	6.25 ab	8.18 ab	51.30 ab	2090 b	28	201
G50	0.09 b	3.10	6.09 ab	7.35 b	49.48 ab	2455 b	25	203
G58.4	0.15 b	3.43	7.73 ab	10.65 ab	66.00 a	3011 b	86	154
HA6.1	0.06 b	1.37	0.93 d	5.37 b	27.76 ab	1281 b	69	48
HA24.7	0.26 ab	2.16	3.25 b	17.42 a	42.55 ab	1569 b	121	121
HA28.5	0.12 b	1.95	4.08 ab	7.25 b	41.53 ab	1243 b	137	162
WO0.1	0.46 ab	2.74	1.87c	8.20 b	18.18 ab	3081 ab	299	44
WO1.7	0.64 a	2.30	4.39 ab	12.41 ab	52.90 ab	1180 b	78	74
WO19.8	0.07 b	1.26	2.01 b	3.83 b	27.07 ab	1092 b	33	66
BB2.5	0.13 b	2.23	4.23 ab	11.33 ab	29.17 ab	1228 b	53	90
BB5	0.15 b	3.43	9.43 a	18.29 a	62.36 ab	1834 b	40	329
BB34	0.14 b	2.63	4.87 ab	12.21 ab	47.83 ab	5258 a	40	344
BB35.8	0.04 b	2.16	1.71c	7.16 ab	12.88 b	2029 b	17	86
Fall								
G6.1	0.48 ab	4.0 b	4.4 b	11.3 b	33.9 d	3838c	95 d	69 b
G49.4	0.58 ab	6.2 b	8.2 b	12.2 b	58.5 cd	4444 b	57 e	135 b
G50	0.40 ab	5.3 b	9.1 b	11.5 b	72.9 cd	3736c	47 e	150 b
G58.4	0.74 ab	7.0 b	8.1 b	17.0 b	57.4 cd	9198 a	119c	112 b
HA6.1	0.05 b	3.4 b	1.7 b	9.0 b	40.9 d	3087c	111c	116 b
HA24.7	0.26 ab	4.9 b	14.0 b	24.4 b	131.3 b	3628c	85 d	271 ab
HA28.5	0.17 ab	8.1 b	10.0 b	13.9 b	114.9 c	2709c	348 ab	244 ab
WO0.1	0.09 b	4.1 b	2.5 b	13.4 b	27.7 e	4871 b	118c	174 b
WO1.7	0.25 ab	4.0 b	5.1 b	17.1 b	69.2 cd	2217c	54 e	112 b
WO19.8	0.06 b	2.4 b	2.0 b	7.7 b	44.0 d	2058c	95 d	133 b
BB2.5	0.85 ab	7.0 b	28.7 ab	30.6 b	66.5 cd	2907c	494 a	165 b
BB5	0.41 ab	10.0 b	34.3 ab	38.2 b	107.4 c	2896c	299 b	281 ab
BB34	1.40 a	4.4 b	9.0 b	11.5 b	92.4 cd	3705c	322 b	546 a
BB35.8	1.02 ab	5.3 b	6.1 b	11.7 b	56.1 cd	4853 b	68 de	163 b
HU20.7	1.11 ab	32.5 a	73.0 a	178.5 a	500.4 a	8027 a	269 BCE	589 a
B29.5	0.08 b	2.5 b	3.7 b	8.3 b	39.2 d	1897 c	80 d	121 b
Background		30	15	15	30			

† Means followed by a different letter are significantly different at the 0.05 probability level, grouped into classes a, b, and c. The underlined values exceed the soil background concentrations.

samples were collected in triplicate from a total of 16 locations namely G58.4, G50, G49.4, G6.1 along the Greens Bayou, BB 35.8, BB 34, BB5, BB2.5 along the Brays Bayou, HA28.5, HA24.7, HA6.1 along the Halls Bayou, WO19.8, WO1.7, WO0.1 along the White Oak Bayou, HU20.7 at Hunting Bayou and B29.5 at Buffalo Bayou (Fig. 1). The sample locations were named with letters followed by a number as suffix where the letters stand for the name of the bayou and the number represents the distance of the sample site in km from the mouth of the bayou. For example, the G58.4 represents the Greens Bayou sample site located at 58.4 km upstream from mouth of the bayou. The detailed soil sampling procedure was reported elsewhere (Sridhar et al., 2020; Bukunmi-Omidiran and Sridhar, 2021).

The soil types that dominate the Brays Bayou watershed are primarily from the Lake Charles and Edna soil series with soil texture of clay, clay loam, sandy clay, and silty clay. The soils are poorly drained with bulk density in the range of 1.2–1.4 g/cm^3, and pH of 6.6–8.4. The soil types of Halls Bayou watershed are mainly Addicks loam, Addick-Urban land complex and Clodine-Urban land complex with soil texture of sandy and clay loam. The soils are poor to somewhat poorly drained with 0–1% slope, and pH of 6.1–8.4 (USDA, 1976).

The Hunting Bayou watershed soils are mainly of Lake Charles, Aris-, Bernard-, Clodine- and Bacliff-Urban land complex types with soil texture of loamy and sandy clay. The soils are poor to somewhat poorly drained with 0–0.5% slope, and pH of 6.6–8.4. The soil types that dominate the Greens Bayou watershed are primarily from the Clodine, Aldine, and Wockley soil series, with soil texture of clay, silt and sandy loam. The soils are poorly drained with bulk density in the range of 1.3–1.5 g/cm^3, and pH of 6.6–8.4 (USDA, 1976).

The soils in the White Oak Bayou watershed are primarily in the Clodine, Bernard and Katy soil series with soil texture of clay loam, silty clay loam and sandy clay. In Buffalo Bayou watershed, the majority of

the soils are made up of the Aldine, Clodine and Edna soil series with soil texture of clay and silty clay loam. The soils are poorly drained, with low infiltration and high runoff potential with bulk density in the range of 1.2–1.4 g/cm^3, and pH of 6.6–8.4 (USDA, 1976). The total carbon and nutrient concentrations of the soils are reported elsewhere (Sridhar et al., 2020; Bukunmi-Omidiran and Sridhar, 2021).

2.2. Chemical analysis

About 0.5 g of soil sample from each replicate was measured and microwave (Mars 6, CEM, Matthews, NC) digested in 10 mL of HNO$_3$ using EPA 3050B (USEPA, 1996) digestion method for soil. The digested samples were analyzed for elemental concentrations by using Inductive Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7500 Series, Santa Clara, CA).

2.3. Soil extraction

Soil samples collected at each sampling location were pooled and mixed properly to obtain a single composite sample. The soil samples were then leached with 10 mL of phosphate buffered saline (PBS), with a soil to PBS ratio of 1:1 w/v. The soil/PBS mixture was vortexed and placed on an orbital shaker for 24 h and then centrifuged for 30 min at 10,000 g. The supernatant was then sterilized by membrane filtration (0.22 µM) to eliminate possible bacterial contamination prior to biological testing. The leachates were diluted with PBS media to obtain the concentrations of 12.5%, 25%, 50%, 75% and 100% of soil extracts.

2.4. Cell culture

CCD 841 CoN (ATCC® CRL-1790™; normal colon, non-transformed
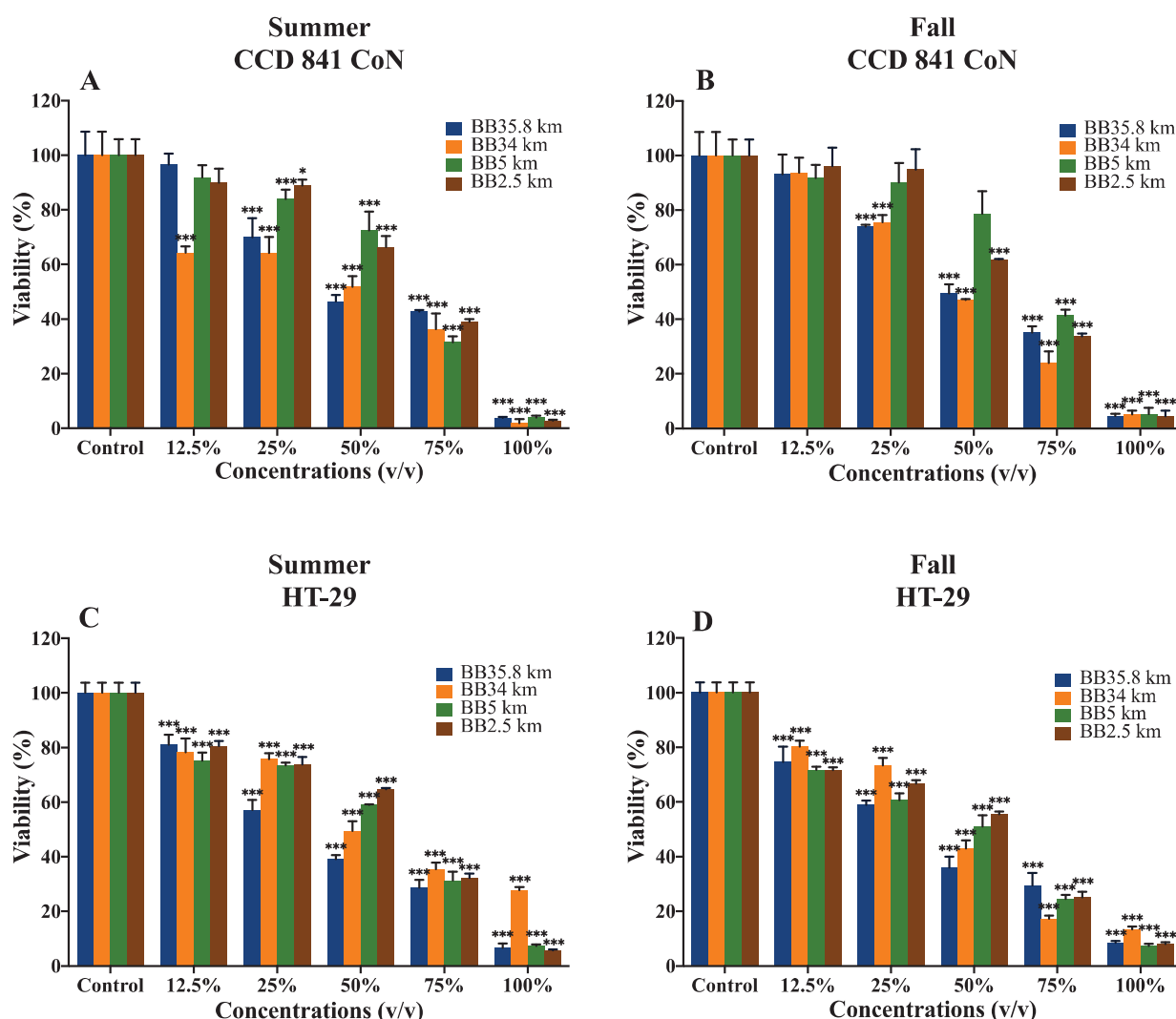
Fig. 2. Cytotoxic effects on CCD 841 CoN (A, B) and HT-29 cells (C, D) during summer and fall, exposed to Brays Bayou soil extracts at the concentrations of 12.5%, 25%, 50%, 75% and 100% for 24 h. Each bar represents the mean ± SD, n = 3. Statistically significant differences between concentrations and control are represented by *p < 0.05, and * **p < 0.001.

cell line) and HT-29 (ATCC® HTB-38™; cancer, colorectal adenocarcinoma) cells were purchased from American Type Cell Culture (ATCC, Manassas, VA) and maintained in Eagle's Minimum Essential Medium (EMEM) and Dulbecco's Modified Eagle's Medium (DMEM), respectively, supplemented with 10% Fetal Bovine Serum (FBS), 1% antibiotic antimycotic solution in a humidified atmosphere of 5% carbon dioxide (CO_2) at 37 °C.

2.5. Cellular toxicity

Cell viability was determined by 3-(4,5-dimethyl-2-thiazolyl)−2,5-diphenyl-2 H tetrazolium bromide (MTT) reduction assay. The cells were seeded in 96-well plates at a density of approximately 2.5×10^3 cells/mL for 24 h. After which, the cells were exposed to 100 μL of 12.5%, 25%, 50%, 75% and 100% soil extract concentrations for 24 h. Further, 20 μL MTT solution (5 mg/mL in PBS) was added to each well, and the plates were incubated for 4 h at 37 °C. Subsequently, the supernatant was removed and 100 μL of DMSO was added to each well and placed on an orbital shaker for 30 mins to dissolve the formed formazan crystals. The optical density (OD) was read at 570 nm (against the reference wavelength of 640 nm) using a Synergy H4 Multi-Mode Microplate spectrophotometer system (Biotek Instruments, Winooski, VT). Cell viability was determined as the percentage of viable cells in the

experimental groups in relation to untreated cells (control).

2.6. Statistical analysis

All experiments were performed in triplicates and the results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for statistical comparisons with Tukey's test for multiple comparisons to determine significance between different groups using Prism 8 software (GraphPad Software, Inc., La Jolla, CA, USA). Pearson correlations of log-transformed data were conducted to identify the relationship between the elemental concentration and the cytotoxicity of the gut cells during the summer and fall seasons.

3. Results

3.1. Soil chemical analysis

The elemental concentrations in the Greens, Halls, White Oak, Brays, Buffalo and Hunting Bayou soil samples collected in different seasons are given in Table 1. The soil concentrations of Zn, Pb and Na were slightly higher in fall compared to summer at all the sampling locations. No significant seasonal difference was seen for the rest of the elements.
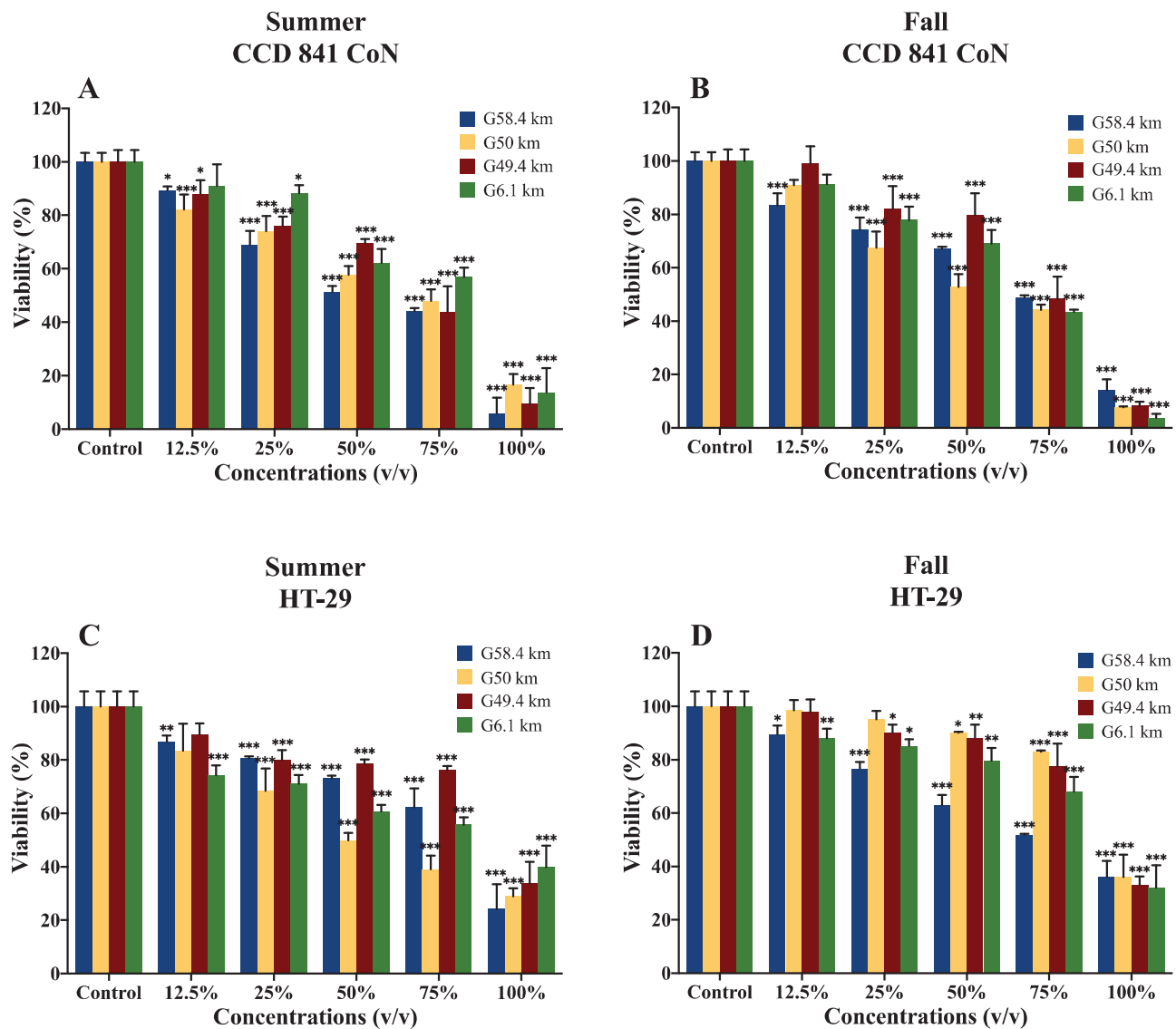
Fig. 3. Cytotoxic effects on CCD 841 CoN (A, B) and HT-29 cells (C, D) during summer and fall, exposed to Greens Bayou soil extracts at the concentrations of 12.5%, 25%, 50%, 75% and 100% for 24 h. Each bar represents the mean ± SD, n = 3. Statistically significant differences between concentrations and control are represented by *p < 0.05, ** p < 0.01, and * **p < 0.001.

The P, Zn, Pb and Cu concentrations in Brays Bayou soil samples were significantly higher than the Greens, Halls and White Oak Bayou locations in both the summer and fall seasons.

The concentration of Zn remained significantly higher in the upstream (G58.4) while the concentration of Cd remained significantly higher in the downstream (G6.1) soils of Greens Bayou during summer. The Cr, Cu, Pb, Fe, Na and P concentrations in the soils of Greens Bayou during summer did not show any specific trend and were distributed uniformly along the bayou. The Zn, Fe and Na concentrations in the fall soil samples were significantly higher in the upstream (G58.4, G50, G49.4) compared to downstream (G6.1) of Greens Bayou. The Zn concentration during both the summer and fall seasons in Greens Bayou remained above its background soil concentration (Table 1).

The concentrations of Zn and Pb remained significantly higher in the upstream (HA24.7) soils compared to downstream (HA6.1) soils of Halls Bayou during both summer and fall seasons (Table 1). The Na and P soil concentrations were higher and significantly higher in the summer and fall seasons, respectively, in upstream (HA24.7) location of Halls Bayou compared to downstream. No significant trend was observed for the rest of the elemental concentrations along the length of the bayou. The

concentrations of Cu, Zn, Fe and P in soils of Brays Bayou during summer were significantly higher compared to other bayous (Table 1). The concentrations of Cu, Pb and Zn remained significantly higher and exceeded the soil background concentrations by 2–3 times in the downstream (BB5, BB2.5) compared to upstream (BB34, BB35.8) locations of Brays Bayou soils during both summer and fall seasons (Table 1). No significant trend was observed for the rest of the elemental concentrations along the length of the bayou.

The concentrations of Zn and Pb remained significantly higher and remained above the soil background concentrations at WO1.7 compared to the other two locations of White Oak Bayou soils during both summer and fall seasons. The concentrations of Cr, Cu, Pb, Zn, Cd, Fe and P in Hunting Bayou soils remain significantly higher than the rest of the bayou locations in the fall (Table 1). The soil concentration of Zn in Buffalo Bayou remained above the background soil concentration (Table 1). The soil concentrations of Cd and Cu with correlation coefficients of −0.53 and −0.45, respectively, were significantly (p < 0.05) negatively correlated with the viability of CCD 841 CON cells in the fall season. The Na (r = −0.45), P (r = −0.39), Zn (r = −0.44) and Pb (r = −0.37) concentrations in the soils were significantly

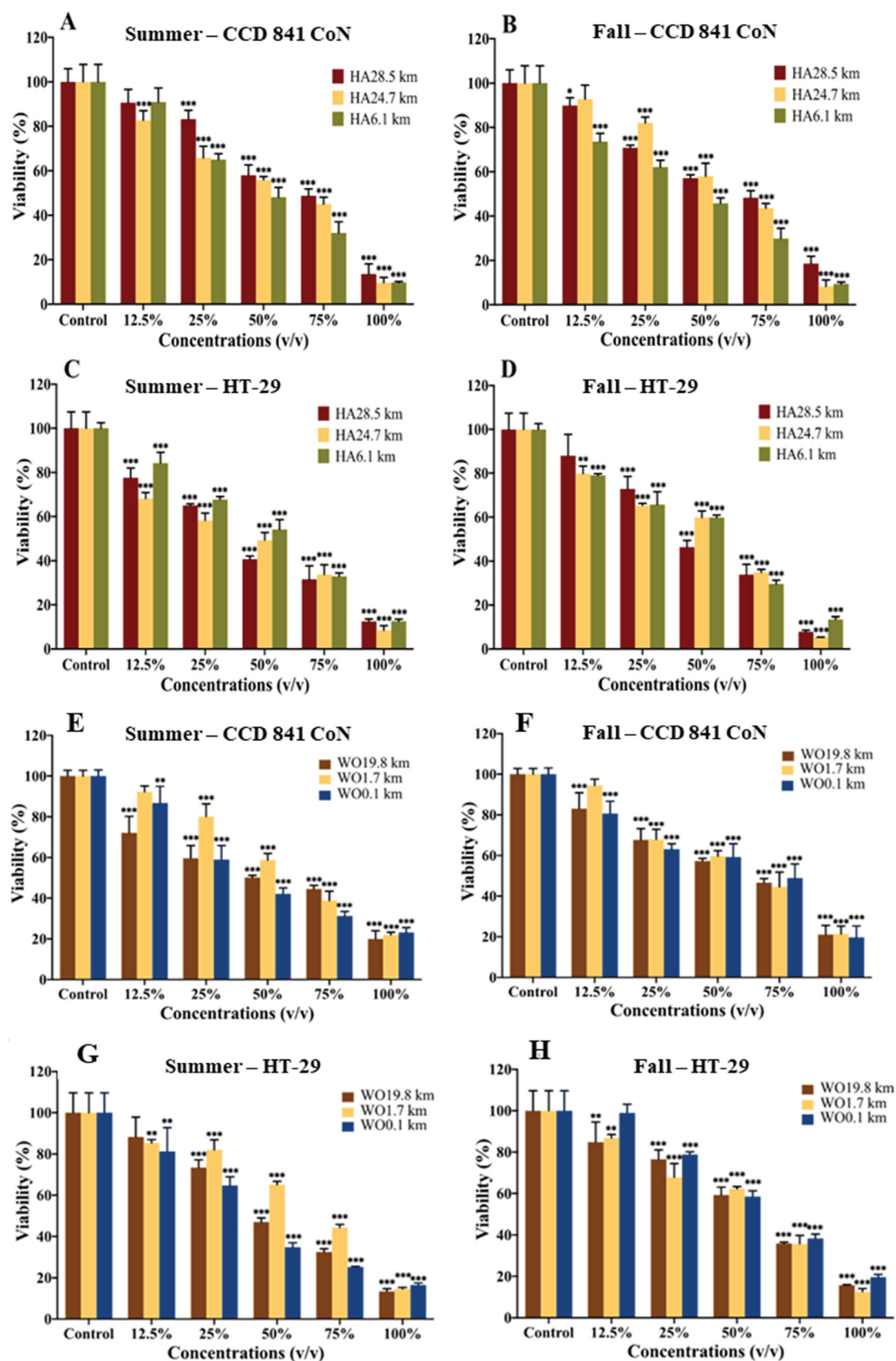**Fig. 4.** Cytotoxic effects on CCD 841 CoN (A, B, E, F) and HT-29 cells (C, D, G, H) during summer and fall, exposed to Halls Bayou (A-D) and White Oak Bayou (E-H) soil extracts at the concentrations of 12.5%, 25%, 50%, 75% and 100% for 24 h. Each bar represents the mean $\pm$ SD, n = 3. Statistically significant differences between concentrations and control are represented by *$p < 0.05$, * * $p < 0.01$, and * **$p < 0.001$.
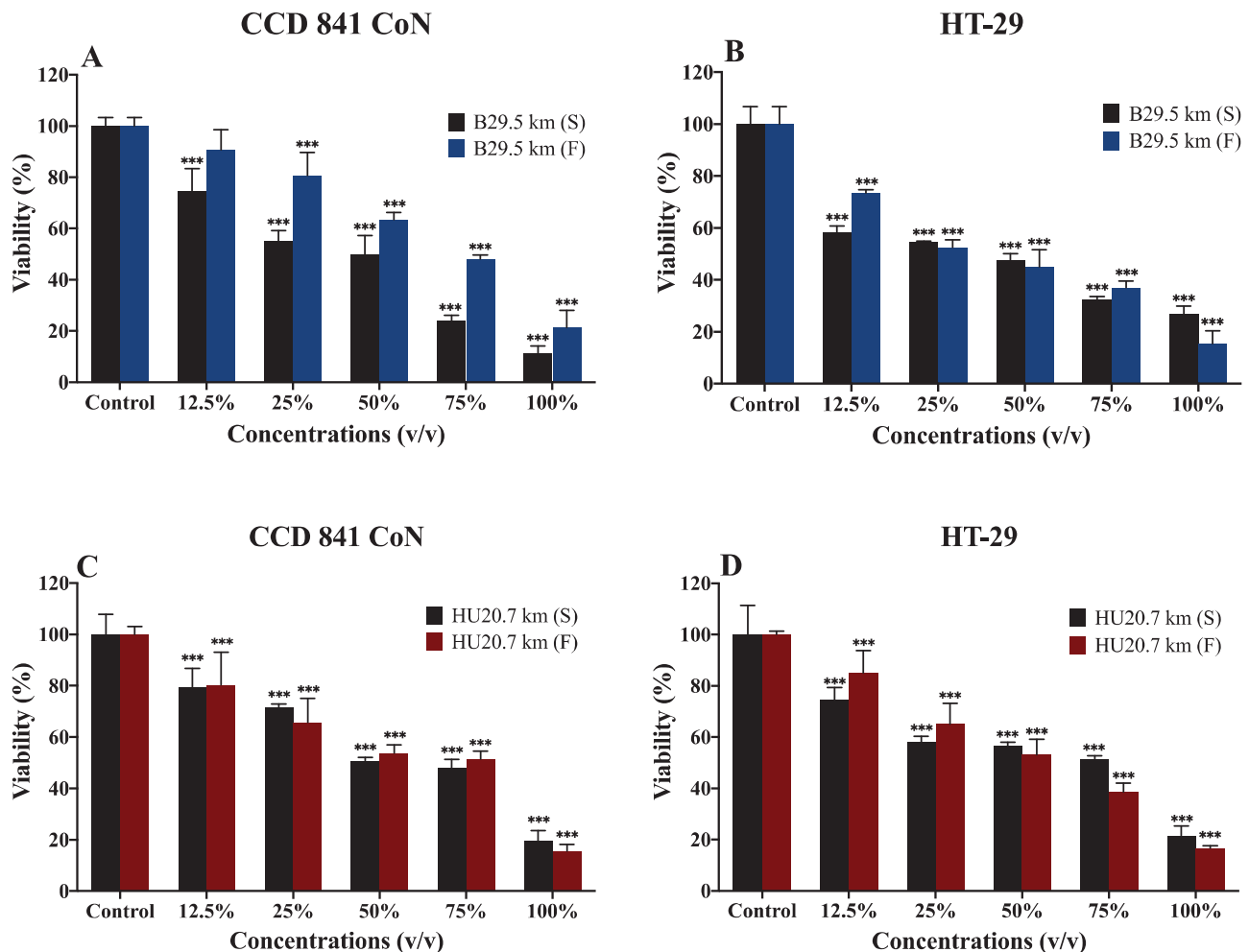
Fig. 5. Cytotoxic effects on CCD 841 CoN (A) and HT-29 (B) cells exposed to Buffalo Bayou soil extracts and CCD 841 CoN (C) and HT-29 (D) cells exposed to Hunting Bayou soil extracts at the concentrations of 12.5%, 25%, 50%, 75% and 100% for 24 h. Every concentration was statistically significant when compared to the control, where * **$p < 0.001$. Each bar represents the mean $\pm$ SD, n = 3.

($p < 0.05$) negatively correlated while the Fe (r = 0.34) was positively correlated with the viability of HT-29 cells in the fall season. No significant correlations were found between the soil elemental concentrations and the cytotoxicity of the cells in the spring season.

3.2. Spatial and temporal variability of cell toxicity

The soil extracts from all the Bayous induced variable cytotoxic effects with increasing concentrations (Figs. 2–6). Bray's Bayou concentrations followed similar pattern of decreasing viability with increase in soil extract concentration in the summer and fall at all sites (Fig. 2). With increase in concentration of Brays Bayou soil from 12.5% to 75%, the viability of CCD 841 CON and HT-29 cells in the upstream (BB35.8, BB34) decreased significantly compared to the downstream (BB5, BB2.5) of the bayou in both summer and fall seasons (Fig. 2). However, the viability of both the cell types decreased uniformly throughout the stream at 100% soil concentration (Fig. 2).

With increase in concentration of Greens Bayou soil from 12.5% to 100%, the viability of CCD 841 CoN cells in the upstream (G58.4, G50, G49.4) decreased significantly compared to the downstream (G6.1) of the bayou in summer (Fig. 3). However, in fall, the viability of CCD 841 CoN cells in the downstream (G6.1) decreased significantly compared to the upstream (G58.4, G50, G49.4) of the bayou (Fig. 3). The viability of HT-29 cells in summer and fall followed the same pattern of the CCD 841 CoN cells for Greens bayou soil samples (Fig. 3). However, the viability

of HT-29 cells is at least 2–3 times higher compared to that of the CCD 841 CoN cells at 100% soil concentrations in both the summer and fall seasons (Fig. 3).

Halls Bayou soil extracts were more cytotoxic to HT-29 than CCD 841 CoN cells (Fig. 4 A–D). The soil extracts from all the bayou sampling locations caused a decrease in cell viability in CCD 841 CoN from upstream to downstream in both seasons (Fig. 4A, B). With increase in concentration of Halls Bayou soil from 12.5% to 100%, the viability of CCD 841 CoN cells treated with upstream (HA28.5) soils decreased significantly compared to the downstream (HA6.1) soils of the bayou in both the summer and fall seasons. However, the viability of HT-29 cells in the extracts treated with downstream (HA6.1) soils increased significantly compared to the cells treated with upstream (HA28.1) soils during both the seasons (Fig. 4 C, D). In comparison, the viability of HT-29 and CCD 841 CON cells treated with 100% soil concentrations of Halls Bayou were similar during both the summer and fall seasons (Fig. 4 A–D).

White Oak Bayou soil extracts at 12.5%–100% soil concentrations in both the summer and fall samples had the most cytotoxic effect on HT-29 cells compared to the CCD 841 CoN cells (Fig. 4 E–H). No significant difference in cell viability from soil extracts of upstream to downstream locations was observed during the summer and fall sampling periods in both the cell types (Fig. 4 E-H). The viability of HT-29 and CCD 841 CoN cells at 100% soil concentrations were similar and remain low during both the summer and fall seasons for the soil extracts at all the White
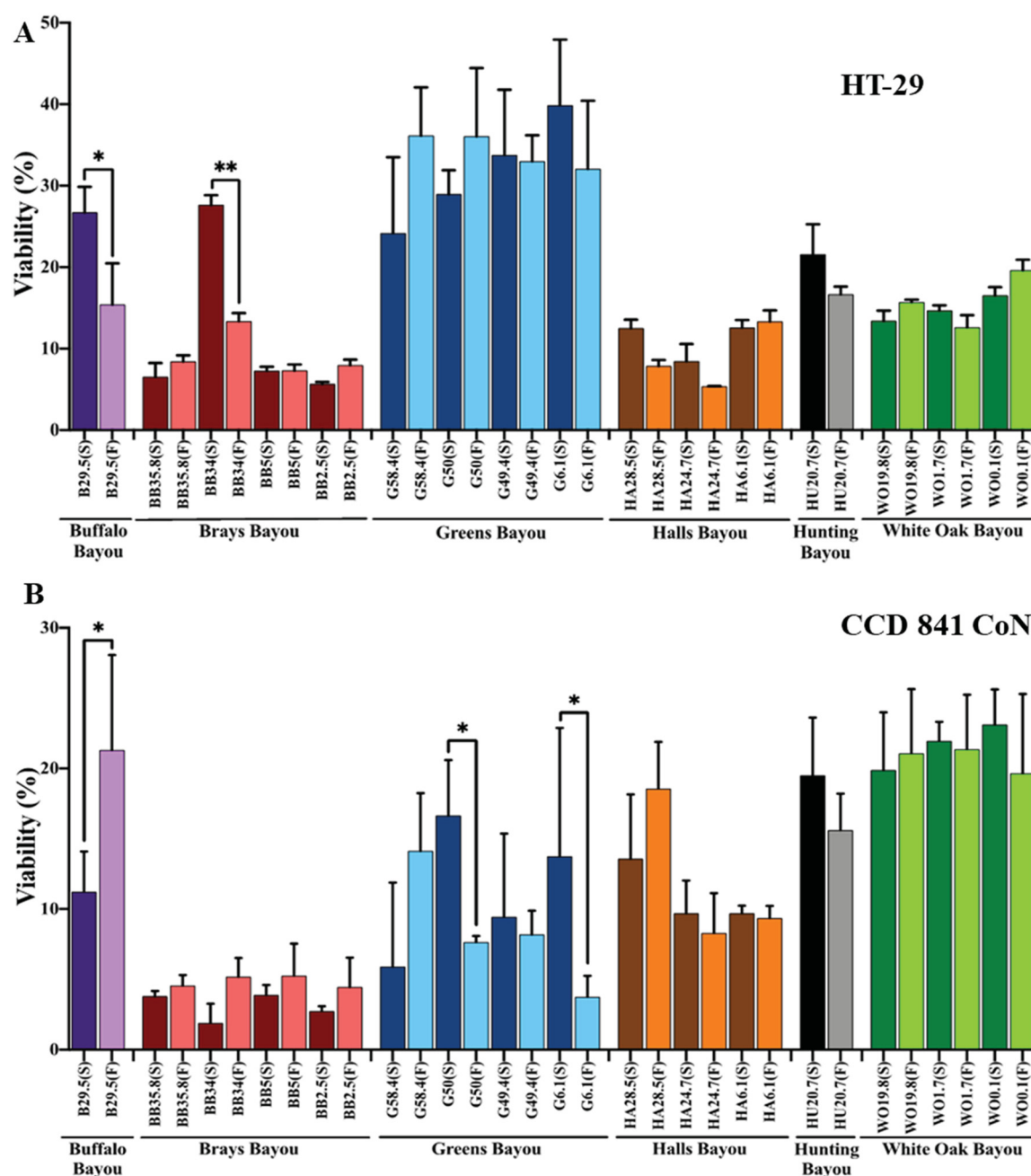
Fig. 6. Comparison of the viability of HT-29 cells (A) and CCD 841 CoN (B) after 24 h exposure to Summer (S) and Fall (F) soil extracts of 100% concentration. Each bar represents the mean ± SD (n = 3), where statistically significant difference is shown as * (p < 0.05) and * * (p < 0.01), respectively.

Oak bayou soil sampling locations (Fig. 4 E–H).

Buffalo Bayou soil extracts decreased viability in both cells (Fig. 5A, B) with increase in concentration. The soil extracts of B29.5 has more cytotoxic effect on HT-29 cells over the CCD 841 CoN cells at 12.5–100% concentrations (Fig. 5A, B). Although Hunting Bayou only had one sampling site, soil extracts for both cell types were statistically significant from their controls (Fig. 5C, D). CCD 841 CoN and HT-29 cell viability at all soil extracts from 12.5–100% decreased progressively and the cytotoxic effects for both cell lines followed the similar trend and are not significantly different (Fig. 5C, D). The Hunting and Buffalo Bayou soil samples were collected and analyzed for cytotoxicity only for fall season and the viability of CCD 841 CoN cells and HT-29 cells significantly decreased in the soil extracts from the lone upstream (B29.5 and HU20.7) sampling location of each of the bayou (Fig. 5).

Cytotoxicity was observed in colon cell lines of both cell types

exposed to undiluted 100% soil concentrations showing a significant reduction in survival in the soil extracts from all the Bayous. The viability rate of HT-29 cell was reduced by 60%–95% (Fig. 6A) and CCD 841 CoN cell line by 75%–95% (Fig. 6B) in all the Bayous at 100% soil concentrations when compared to their respective control samples. Brays Bayou soil extracts had the most toxic effects on both the cells in the summer and fall (Fig. 6).

Among all the bayous, the viability of CCD 841 CoN cells in summer followed the pattern of White Oak >Greens >Halls >Brays Bayou, where the viability of cells in White Oak is 6–7 times higher than cells exposed to Brays Bayou soil at 100% soil concentration. The viability of HT-29 cells in summer followed the pattern of Greens >White Oak >Halls >Brays Bayou, where the viability of cells in Greens Bayou is 5–6 times higher than the cells exposed to Brays Bayou soil at 100% concentration.

The viability of CCD 841 CoN cells in fall followed the pattern of White Oak >Buffalo >Hunting >Greens >Halls >Brays Bayou, where the viability of cells in White Oak is 3–4 times higher than the cells exposed to Brays bayou soil at 100% soil concentration. The viability of HT-29 cells in fall followed the pattern of Greens >Buffalo >Hunting >White Oak >Halls >Brays Bayou, where the viability of cells in Greens Bayou soil is 3–4 times higher than the cells exposed to Brays bayou soil at 100% soil concentration.

4. Discussion

The cytotoxicity of flood plain soil sampled along Brays, Buffalo, Halls, Hunting, Greens, and White Oak Bayous were higher in fall compared to the summer, which was contributed by extreme flooding in fall of 2017, due to Hurricane Harvey. Flood inundation of the communities along these Bayous might have exposed the inhabitants to varying levels of cytotoxicity. Brays Bayou is one of the primary drainage streams for the City of Houston. The Brays Bayou downstream (BB2.5, BB5) locations had the most cytotoxic effects on HT-29 and CCD 841 CoN cells, respectively (Fig. 2). The decrease in viability at the upstream locations suggest the influence of the nearby WWTPs which releases their effluents into the bayou. These sites are located near heavily urbanized parks with a detention basin in the middle receiving runoffs from high populated neighborhoods, West Houston Medical Center, Wastewater treatment plants and the industrial Westchase area which can result in significant soil toxicity. The cytotoxic effect of the soil extracts from Buffalo Bayou may be attributed to the runoffs received from the extensive impervious surface and downtown area. Both Buffalo and Brays Bayou watersheds had an extensive increase in impervious surface and decrease in vegetative surface during the last four decades (Bukunmi-Omidiran and Sridhar, 2021).

The upstream soils of the Greens Bayou were more cytotoxic for both cell lines during summer whereas the downstream soils became more cytotoxic in the fall (Fig. 3). The cytotoxic potential of soil may be affected by seasonal changes such as metabolization of toxicants exposed to solar radiation in the hot season or by other cytotoxic compounds such as heavy metals, pharmaceutical and personal care compounds during the raining season (Caballero-Gallardo et al., 2015; Hilscherova et al., 2010). Decreased viability in upstream Greens Bayou in the summer can be attributed to the presence of several WWTP outfalls, and large- and small-scale industries effluents released into a low flowing bayou. In the fall, high impervious surface area within the drainage watershed causes runoffs of contaminates to accumulate downstream which is observed in the decreased viability. Also, the soil cytotoxicity can be attributed to the higher concentrations of Zn and Cd which were above the background concentrations (Table 1). The concentrations of As, Cd, Pb, and Hg concentrations in soil samples of Greens Bayou were higher in the fall compared to the summer and exceeded the critical limit (Sridhar et al., 2020).

The cytotoxicity of both cell lines, CCD 841 CoN, and HT-29, are higher all along the length of the Halls (Fig. 4 A-D) and White Oak (Fig. 4 E-H) Bayous in both the seasons. This is consistent with the uniform soil elemental concentration spike across the length of the bayous. The soil cytotoxicity can be attributed to the higher concentrations of Zn which were above the background concentrations (Table 1). Soil act as sinks for contaminants such as heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals (Baderna et al., 2014; Choi et al., 2015; Schulze-Sylvester et al., 2015; Yeager et al., 2010). The release of toxic contaminants is often triggered by environmental conditions such as flooding (Schulze et al., 2015; Wölz et al., 2010; Macauley et al., 2010; Williams and Liu, 2019). Even though the highest concentration (100%) was not statistically significant between Summer and Fall at all the sites within the Bayous, higher cytotoxicity was observed in Fall in all bayous (Fig. 6).

Cytotoxicity was reflected differently in CCD 841 CoN and HT-29 cell lines exposed to soil extracts. The heavy metal and nutrient composition

of the soils can impact the cells differently, causing diverse responses (Heinrich et al., 2017). In addition, interactive effects (antagonistic, synergistic or additive) among chemicals (Briffa et al., 2020) might have played a role in generating the observed cytotoxic effects. Overall, the results showed that HT-29 cells were more sensitive to the soil extracts than CCD 841 CoN cells, indicating a higher resilience. This is in concurrence with low sensitivity of CCD 841 CoN cells when exposed to organic and inorganic contaminants (Hung et al., 2019; Sayess et al., 2017).

In conclusion, bayou soil extracts from Bray's, Buffalo, Greens, Halls, Hunting, and White Oak Bayous caused decrease cell viability in human colon cells in vitro. The cytotoxicity of the soil extracts from fall (Post-Harvey) showed higher toxicity compared to summer (Pre-Harvey) season. Overall, the cytotoxicity assay results suggested that the bayou soil contained significant concentrations of Pb, and Zn that disturbed cell viability. However, the effects of any interactions among these metals on the cell viability are unknown. Future research is needed targeting analysis of possible interactions between combinations of metals and associated compounds. The compound toxic interactions could take the form of potentiation, synergism, inhibition, antagonism, or their combined effect is simply an additive effect of the individual compounds.

CRediT authorship contribution statement

Djene Keita: Data curation, Writing – original draft, Visualization, Investigation. **Maruthi Sridhar:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition, Supervision, Administration. **Shishodia:** Methodology, Funding acquisition, Supervision, Administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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