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Cytotoxicity Assessment in HepG2 Cells in Response to Exposure of Heavy Metal Contaminated Water from Different Areas of Ahmedabad Especially Surrounding Industries: A Comparative Study

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Abstract: ***Heavy metal pollution is a concern for environment that is getting worse posing a lot of concern for its negative impacts on health. Expanding agriculture and metal industries release industrial waste contaminated with inorganic pollutants in our rivers, soils, and environment. In this investigation, a detailed study was undertaken to assess the extent of heavy metal pollution in the native wastewater samples that were collected in and around Ahmedabad in October and November 2019 and the effect of such pollutants on cytotoxicity were assessed. Location of sample collection includes, among other things, industrial wastewater disposal facilities, canals, rivers, and lakes. MTT studies were conducted to monitor concentrations dependent heavy metals in HepG2 cells. The qPCR was used to confirm the genotoxicity of the selected samples for further validation of cytotoxic potentials. Many pollutants (14 out of 19 samples) produced cytotoxic effects in the study. All sample locations revealed a highly noticeable, dose-dependent effect on cell viability, with industrial effluents and dumping canal water demonstrated the highest toxicity. While samples collected from rivers and lakes near to the residential areas were less genotoxic, whereas samples collected near to the industrial areas were more genotoxic. The results demonstrated that a combination of tests using HepG2 cells and mammalian cell assays (MTT and qPCR) were suitable enough in determining the cytotoxic and genotoxic potential of native wastewater and surface water samples. Current work provide the evidence that a battery of toxicity bioassays are beneficial for the monitoring and evaluation of the toxicity potential of complex wastewaters before discharging into the environment.***

Keywords: Ahmedabad, Water Samples, Heavy Metal Contamination, MTT, Genotoxicity, Cytotoxicity

1. Introduction

Metals are components of the ecosystem that are found naturally. They are very electrically conductive compounds that voluntarily give up electrons to produce cations. All around the world, including the atmosphere, the earth's crust, and water bodies, metals may accumulate in living organisms including plants and animals. Of the 35 naturally occurring metals, 23 are considered heavy metals since they have an atomic weight greater than 40.04 and a high specific density greater than 5 g/cm3 [1, 2]. Examples of heavy metals include antimony, tellurium, bismuth, thallium, tin, gold, cerium, gallium, cadmium, chromium, cobalt, copper, iron, lead, mercury, manganese, nickel, platinum, silver, uranium, vanadium, and zinc[1, 2][3].

Water pollution is mostly caused by urbanization and industrialization. Runoffs from towns, cities, and industries carry metals that can be harmful to people and other ecosystems. Heavy metal toxicity varies depending on the

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metal, its makeup, biological function, the organism exposed, and the length of exposure. All the organisms in the food chain will be impacted if one creature is. Humans are at the bottom of the food chain and release heavy metals into the sewage system, where they accumulate [5,6]

The liver breaks down heavy metals, which are then bile-excreted into the intestines. Only around 5% of the medication is eliminated in the excretion, with the remaining 90%–95% being reabsorbed through the enterohepatic circulation. As a result of the exposure to pollution, liver dysfunction, cell damage, and organ failure may occur [10]

Because they are crucial in the metabolization and detoxification of xenobiotics that can interact with DNA and potentially cause mutational events, metabolizing cell lines like hepatoma tissue culture (HTC) and HepG2 (human liver hepatocellular carcinoma), which express phase I and II enzymes, are frequently used in environmental assessments [17].

2. Methodology

Study area
Ahmedabad is 310 km2 in size and is situated at 23.03° N and 72.58° E. On average, it stands 53 metres high. Ahmedabad, which is around 53 metres (174 feet) above sea level and situated in north-central Gujarat (23.03°N, 72.58°E)(Figure-1), is a very flat city. The Ahmedabad area's several industrial sites, rivers, and lakes (Table 1.1) were selected for sample collection because they were thought to be detrimental.

Collection of water Sample
Water samples from canals, lakes, rivers, influents of wastewater treatment plants, and direct dumping routes in Ahmedabad's chemical industry effluents were collected in the months of October and November 2019 (Table 1). The substances were tested for toxicity and genotoxicity after being filtered (0.22 m pore size) and stored at 20°C. The filtered water samples were clear and colourless with the exception of sample A5 (Table 1), which was somewhat brownish. The pH values of the samples and the medium were brought to 7 in accordance with standard procedures before testing.

Metal analysis
water samples collected, and metal analysis was carried out using the techniques advised by APHA (1998). The heavy metals present in the collected water samples were previously identified using inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin-Elmer Optima 7300) (31).

Cell Culture and Treatment
The HepG2 cells for this investigation were purchased from National Centre for Cell Science in Pune, India. Parental HepG2 cells kept in liquid nitrogen were thawed by gently agitatingvialsin a 37°C water bath for 2 minutes. After thawing, the contents of each vial were transferred to a 75 cm^2 tissue culture flask, diluted with DMEM supplemented with 10% FBS and 1% streptomycin and penicillin, and incubated at 37°C in a 5% CO_2 incubator for 24 hours to allow the cells to grow and form a monolayer in the flask. Cells were grown to 80-95% confluence in PBS, trypsinized with 3 mL of 0.25% (v) trypsin-0.0.3%/v) EDTA, diluted, counted, and seeded (5 105 cells/well) on two sets of 96-well MTT plates. In a 5% CO_2 incubator, seeded plates were cultured for 24 hours at 37°C. The old medium was replaced with fresh medium, and serial dilutions of water samples based on ICP-OES heavy metal concentrations (0.05,0.25,0.5,0.75.0.1,1,1.5.2.5,5,10,15,20µM) were added column wise to the 96-well microtiter tissue culture plates and incubated for 48 h.

MTT Assay:
Cell viability assay was performed using the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. HepG2 cells were seeded and incubated at 37°C overnight, then treated with 200l of fresh medium at various Water concentrations for 48 h. After 4h incubation, 10l of the MTT solution was added to each well and the plates were incubated again. This assay measures the conversion of MTT to insoluble formazan by dehydrogenase enzymes of living cells. The optical density (OD) was measured at 570 nm and cell survival (viability) was determined by comparing the OD of the wells containing cells treated with water samples to cells exposed to 40 vol.% distilled wate in growth medium. A 30% reduction of viability by the sample is considered a cytotoxic response.

RNA extraction and RT-PCR
To assess gene expression in cultured cells, HepG2 cells were cultivated in the presence of water samples containing or not containing H2O2 according to the same time schedule specified for cytotoxicity testing. Each condition was tested three times. After 48 hours, the cells were collected, and total RNA was extracted using Trizol reagent (Invitrogen). In summary, 3 ×103 cells per well were lysed using 500 ml Trizol reagent, and 200 microlitres of chloroform were added to the tubes before centrifugation at 16,000 g for 25 minutes at 4 °C. The aqueous phase was transferred to another tube and precipitated with 70% ethanol in a volume of 1 volume.The RNA was pelleted after centrifugation for 1 minute at 9000 g in RNAase-free water. The RNA was then kept at 80°C. At 260 nm, the RNA content was measured in triplicate. The 260/280 nm ratio was used to determine the quality of the RNA preparation. Total RNA (2 ng) was utilised as a template for reverse transcription processes to synthesise single stranded cDNA using an Applied Biosystems (Thermos scientific) reverse transcriptase kit and an oligo (dT) primer according to normal protocols. Total RNA (2.5 ng) was reverse transcribed into cDNA. PCR was used to assess mRNA expression using the primers.

To monitor cDNA amplification, QPCR procedures were carried out in a Quantstudio-5 applied biosystems using SYBR Green. In each reaction, which comprised Fast Start Master SYBR Green, forward and reverse primers in a total volume of 10 µl, equal quantities of cDNA, equivalent to a 1/20 dilution of the cDNA, were utilised. The conventional thermal profile shown below was used: 5 minutes at 95 °C, 95 repetitions of 10 seconds at 95 °C and 30 seconds at 60°C and melt curve at 1 minute at 60°C, with a final stage of 15 seconds at 95°C. A Quantstudio-5 applied biosystems was used to analyse the data. Two technical duplicates were

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performed for each cDNA and primer pair combination, and the quality of the PCR reactions was assessed using dissociation and amplification curve analysis. The RNA fragments of anticipated size were confirmed using 3% agarose gel electrophoresis. qPCR was carried out on triplicate samples.

Statistical analysis
We used average Ct-values from 3 technical replicates. Statistical analysis was performed using GraphPadPrism 4 software.

3. Result

In this study, we tested 19 native water samples for their genotoxic and cytotoxic potential with the MTT assay with HepG2 cells, respectively. Samples were selected and were further tested for their genotoxic potential in human hepatoma HepG2 cells using the RT-PCR assay.

Wastewater samples
Industrial discharges are the main source of genotoxic contaminants in wastewaters. Three types of wastewaters were included in our study: chemical industry effluent and industrial dumping canals influents and River, lakes.

MTT Assay:
The HepG2 cell line was employed to examine the toxicity of water samples gathered from various locations in Ahmedabad. Based on the heavy metal concentration of the samples confirmed by ICP-OES, the cells were subjected to various concentrations of test sample (0.05–20 vol%) serial dilutions of water samples for 48 hours. After treatment with water samples, a dose-dependent reduction in the number of live cells was discovered using the MTT test. Despite the fact that all of the samples clearly demonstrated cytotoxicity, we chose three major concentrations—medium, low, and high—for each sample based on the MTT results. For RT-PCR, this chosen concentration is also used.

Cell viability and Cytotoxic effect of Water sample on HepG2 cells: MTT assay:
Base on the ICP-OES study we first consider 8 concentrations of the MTT assay then select three concentrations for RT-PCR This concentration called as a low medium and high cell viability of the cells per samples.

Sabarmati river (A1-A4)
Cytotoxic effect of HepG2 cells with different concentration of Sabarmati water samples after 48 hrs of exposure were measured with MTT assay. Dose dependent cytotoxicity were not observed in MTT assay sample (A1 & A4) even though 10 % toxicity were observed at maximum concentration of 20%. -50% In same way, MTT assay. After 48 hours of exposure, concertation dependent cell killing was observed in HepG2 cells Sample (A2&A3)The cytotoxicity effect at 75 % was 80 %, while at lowest concentration revealed only 20 % (8.1.1)

Based on these results we selected 3 different concentrations Low, Medium and High for each sample, so For A1 consider as Low-1.5 µM medium 2.5 µM and 15 µM. For sampleA2consider as Low-1.5 µM medium 5 µM and 15 µM For Sample A3 consider as Low-0.75 µM medium 1.5 µM and 20 µM For A4 consider as Low-0.75 µM medium 5 µM and 20 µM.

Industrial effluent Samples and canal water samples :(A5-A13 &A19-A20)
Concertation dependent toxic effect of A5 (8.1.1) was tested on HepG2 cells after 48 hours by MTT assay. After 48 hours of exposure, concertation dependent cell killing was observed in HepG2 cells. The cytotoxicity effect at 50 % was 50%, while at lowest concentration revealed also 25 %. While in A6 (8.1.1) Dose dependent cytotoxicity was not observed in MTT assay, even though 10 % toxicity were observed at maximum concentration of 20%. In A7 (8.1.2) Dose dependent cytotoxicity was not observed in MTT assay, even though 10 % toxicity were observed at maximum concentration of 20%. In A8 (8.1.2) concertation dependent cell killing was observed in HepG2 cells. The cytotoxicity effect at 20 % was 80%, while at lowest concentration revealed only 20 %. In A9 (8.1.2) The cytotoxicity effect at 10 % was 80%, while at lowest concentration revealed only 20 %. A10(8.1.2) Shows The cytotoxicity effect at 10 % was 80%, while at lowest concentration revealed only 10 %. In A11 (8.1.2) The cytotoxicity effect at 10 % was 80%, while at lowest concentration revealed only 5 %. In A12 (8.1.2) The cytotoxicity effect at 5 % was 70%, while at lowest concentration revealed only 25 %.A13 (8.1.3) Shows The cytotoxicity effect at 50 % was 75%, while at lowest concentration revealed only 1 %.A18(8.1.3) Shows cytotoxicity effect at 20 % was 90%, while at lowest concentration revealed only 30 %. In A19 (8.1.4) The cytotoxicity effect at 20 % was 90%, while at lowest concentration revealed only 30 %.

Based on these results we selected 3 different concentrations Low, Medium and High for each sample, so For A5 consider as Low-1.5 µM medium 2.5 µM and 5 µM. For sampleA6 consider as Low-1.5 µM medium 2.5 µM and 20 µMFor Sample A7 consider as Low-5 µM medium 10 µM and 15 µM For A8 consider as Low-0.25 µM medium 1 µM and 5 µM For A9 consider as Low-0.05 µMmedium 0.1 µM and 10 µM. For sampleA10 consider as Low-0.05 µM medium 0.1 µM and 10 µM For Sample A11 consider as Low-0.05µM medium 0.1 µM and 5 µM For A12 consider as Low-0.1µM medium 0.5 µM and 1µM For A13 consider as Low-0.01 µM medium 0.5 µM and 10 µM. For sampleA18 consider as Low-0.75 µM medium 1.5 µM and 20 µM For Sample A19 consider as Low-5 µM medium 10 µM and 15 µM.

Lakes samples: (A14-A17)
Concertation dependent toxic effect of A14 (8.1.3) was tested on HepG2 cells after 48 hours by MTT assay. After 48 hours of exposure, concertation dependent cell killing was observed in HepG2 cells. The cytotoxicity effect at 20 % was 75%, while at lowest concentration revealed only 50 %. While in A15 (8.1.3) The cytotoxicity effect at 50 % was 40%, while at lowest concentration revealed only 25 %. Same in A16(8.1.3) Dose dependent cytotoxicity was not observed in MTT assay, even though 1 % toxicity were observed at maximum concentration of 100 %. Also, in A17(8.1.3) Dose dependent cytotoxicity was not observed in

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MTT assay, even though 1 % toxicity were observed at maximum concentration of 100 %.

Based on these results we selected 3 different concentrations Low, Medium, and High for each sample, sofor sample A14consider as Low-1.5 µM medium 2.5 µM and 5 µM For Sample 15 consider as Low-0.05 µM medium 0.25 µM and 0.5 µM For A16 consider as Low-10 µM medium 15 µM and 20 µM For A17 consider as Low-0.75 µM medium 5 µM and 20 µM.

Evaluation of Gene expression changes with Ahmedabad Water sample

Three concentrations of water samples were selected based on MTT results which produced maximum, mid, and low range of cytotoxicity, based on that respectively for gene expression study in HepG2 cells. Liver toxicity related genes were quantified with qPCR like apoptotic gene (MMP-9), metabolism related (CYP1A2, CYP1A1, CYP3A4), responsible for cancer (14.3.3 zeta), inflammatory genes (TNFR, TNFα) and GAD45 alpha a genotoxicity related gene. After 48 Hours of exposure gene were significantly increased and decreased on dose dependently compared to the controls.

Sabarmati river (A1-A4)Gene Expression fold changes with respect to primers:

In Sabarmati River samples A1 to A4After 48 Hours of exposure GADD45 alpha gene were significantly increased dose dependently shown fold between (0.62-6.6)Fold. CYP1A1 and CYP1A2 and CYP3A4 were shown Fold between (0.34-4.16). In 14-3-3 zeta shownfold between (0.59-3.47). While TNFR, TNFαShows fold between (0.34-4.23). MMP-9 shows fold between (0.63-2.73). Also, Cyclin D shows fold between 0.37-4.16) compared to the controls and depends on particular concentration of samples (low medium and high) with respect to the primer It Shown in qPCR graph (8.2.1-8.2.10).

Industrial effluent Samples and canal water samples :(A5-A13 &A19-A20)Gene Expression fold changes with respect to primers:

In Industrial effluent Samples and canal water samples A5-A13 &A19-A20After 48 Hours of exposure GADD45 alpha gene were significantly increased dose dependently shown fold between (0.27-13.04) Fold. CYP1A1 and CYP1A2 and CYP3A4 were shown Fold between (0.22-7.59). In 14-3-3 zeta shown fold between (0.10-9.44). While TNFR, TNFα Shows fold between (0.22-11.00). MMP-9 shows fold between (0.28-19.0). Also, Cyclin D shows fold between 0.20-5.01) compared to the controls and depends on particular concentration of samples (low medium and high) with respect to the primer It Shown in qPCR graph (8.2.1-8.2.10).

Lakes samples: (A14-A17)Gene Expression fold changes with respect to primers:

In Lakes samples: (A14-A17)After 48 Hours of exposure GADD45 alpha gene were significantly increased dose dependently shown fold between (0.27-11.46) Fold. CYP1A1 and CYP1A2 and CYP3A4 were shown Fold between (0.26-3.09). In 14-3-3 zeta shown fold between (0.10-2.87). While TNFR, TNFα Shows fold between (0.22-8.76). MMP-9 shows fold between (0.28-8.68). Also, Cyclin D shows fold between 0.20-2.13)compared to the controls and depends on particular concentration of samples (low medium and high) with respect to the primer It Shown in qPCR graph. (8.2.1-8.2.10).

4. Discussion

Environmental toxins have a variety of negative effects on living things, including their immune systems and capacity to fight off sickness 23 One of the main categories of these concerns is water contamination. Drinking clean water and getting rid of pollutants are essential for maintaining health and preventing disease. The phrase "water conflict" has been used to describe this "blue gold" due to limitations on access to safe drinking water. Due to heavy metal contamination, water pollution poses a major health risk (24). Our water may include allowable levels of pollutants like aluminium, lead, arsenic, and chlorine in accordance with current EPA standards. The effect of water contamination on population health is a significant area of study (25).

Humans and other nearby animals are at risk of mutation due to the water samples' genotoxicity and mutagenic effects, according to Dearfield et al. (2002) (26) In the current study, a number of short-term bioassays were used to assess the mutagenicity and genotoxicity of water samples. Different operating principles are used by the various bioassays. The evaluation of sample cytotoxicity is further motivated by the encouraging results of mutagenic and genotoxic experiments.With industrial effluents and dumping canal water demonstrating the highest toxicity, all sample areas had a very perceptible, dose-dependent cytotoxic effect on cell viability. While the genotoxicity of rivers and lakes in residential areas is lower, it is higher near industrial sites. Studies on HepG2 cell viability exposed to 5 to 40 volume percent of water sample for 20 hours in MTT test can be found in the literature (27).

Almost all of the adhering (living) and very few dead (circled) cells were seen in the untreated cells. The sample's higher toxicity is primarily due to the direct mixing of polluted water from diverse areas (28).The presence of heavy metals in effluents has also been linked to oxidative stress and the generation of hydroxyl radicals, which are extremely genotoxic. In gene expression studies of metals, GADD45 promoters only became statistically significant at the highest concentrations, while exposure to Ni powder and Co only causes modest fold inductions (30).

In this gene expression study, the expression of the genes is dependent on the concentration of the specific samples using the designated primers. Associated with genotoxicity and mutagenicity with the exception of CYP1A2 and CYP3A4, the Sabarmati sample (A2-A3), Lake sample (A14), and industrial sample (A5, A8, A9, A10, A11, A12, A19, and A19) exhibit higher levels of gene expression. While samples from the Sabarmati River (A1, A4), lakes (A15–A17), and industries (A6, A7) show no evidence of genotoxicity. The research revealed that the majority of the heavy metal mixture samples were either cytotoxic in HepG2 cells or genotoxic in the qPCR experiment,

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suggesting that various contaminants may be the cause of the cytotoxic and genotoxic effects.

5. Conclusion

The current study unequivocally shows that the water in Ahmedabad is tainted with various sorts of organic and inorganic metal combination components that are harmful in nature. Critical examination of the data indicated that the Sabarmati River, lake, and industrial districts' water samples were highly mutagenic, genotoxic, and cytotoxic, and that this was correlated with the presence of more heavy metals. Analyses revealed that samples from all the sites had a noticeable impact on several biological experiments. Additionally, it was discovered that the bioactivities of samples from various places varied greatly from one another.

Tables

Table 1: Listed study areas that have been chosen for this project

Sample Collection Place	Geographical location
Sabarmati River (Indira Bridge) -A1	23°5′26″N 72°37′47″E
Sabarmati River (Vasna barrage)-A2	22°59′25″N 72°33′20″E
Sabarmati River (Narol- Vshala Bridge)-A3	22.9815°N 72.5436°E
Sabarmati River (Karai gam)-A4	23.1150°N 72.6628°E
Pirana Dump site Canal Water-A5	22.983811°N 72.567376°E
Pirana Ground water sample-A6	22.979305°N 72.567376°E
Pirana sewage treated water-A7	22.980368°N 72.56212°E
Vatva GIDC-A8	22°56′13″N 72°37′11″E
Naroda GIDC-A9	23.0980°N 72.6761°E
Sanand GIDC-A10	22.99129°N 72.375509°E
Bavla GIDC-A11	22.83351°N 72.36429°E
Changodar industrial area-A12	22.9995°N 72.4571°E
Vastral Industrial area-A13	22.9771°N 72.6217°E
Chandlodia Lake-A14	22.0839°N 72.5521°E
Sarkhej roja-A15	22.9936°N 72.5056°E
Prahlad nagar Lake-A16	23°0'15"N 72°30'21"E
Kakaria Lake-A17	23.0063°N, 72.6026°E
Narol industrial area water-A18	22.953326°N 72.573719°E
Odhav industrial area-A19	23.0319°N, 72.6831°E

Table 2: Primers use in RT-PCR study for water sample

Primer	Sequence	Specification
RPLP	Forward: 5′TCACGGAGGATAAGATCAATGCC3′ Reverse: 5′TGAGGCTCCCAATGTTGACG3′	Cell migration and cell survival
14-3-3 Zeta	Forward: 5'ACCGTTACTTGGCTGAGGTTGC3' Reverse: 5'CCCAGTCTGATAGGATGTGTTGG3'	Cancer associated proteins
CYP1A1	Forward: 5′TGGATGAGAACGCCAATGTC3′ Reverse:5′TGGGTTGACCCATAGCTTCT3′	xenobiotic-metabolizing enzyme
CYP1A2	Forward: 5′AACAAGGGACACAACGCTGAAT3′ Reverse: 5′GGAAGAGAAACAAGGGCTGAGT3′	Antipsychoticdrugs
Cyclin D1	Forward: 5'AGACCTGCGCGCCCTCGGTG3' Reverse: 5'GTAGTAGGACAGGAAGTTGTTC3'	Cellcycle progression.
Gadd45	Forward: 5'CGTTTTGCTGCGAGAACGAC3' Reverse: 5'GAA CCCATTGATCCATGTAG3'	DNA repair, cell cycle management, senescence, and genotoxic stress, are regulated.
MMP9	Forward: 5'GCCACTACTGTGCCTTTGAGTC3' Reverse: 5'CCCTCAGAGAATCGCCAGTACT3'	controls inflammatory and fibrotic pathological remodeling
TNFR	Forward: 5'CCTGCTGCACTTTGGAGTGA3' Reverse: 5'GATGAGGTACAGGCCCTCTG3'	The tumor necrosis factor receptor-associated periodic syndrome (TRAPS)
TNFα	Forward: 5'CAAATGGGGGAGTGAGAGGC3' Reverse: 5TAGGTGAGGGACCAGTCCAA3'	A cytokine that promotes inflammation
CYP3A4	Forward: 5'AAAGAAACACAGATCCCCCTGAA3′ Reverse: 5′CGGGTTTTTCTGGTTGAAGAAGT3′	Drugmetabolizing enzyme in adult humans

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Figures

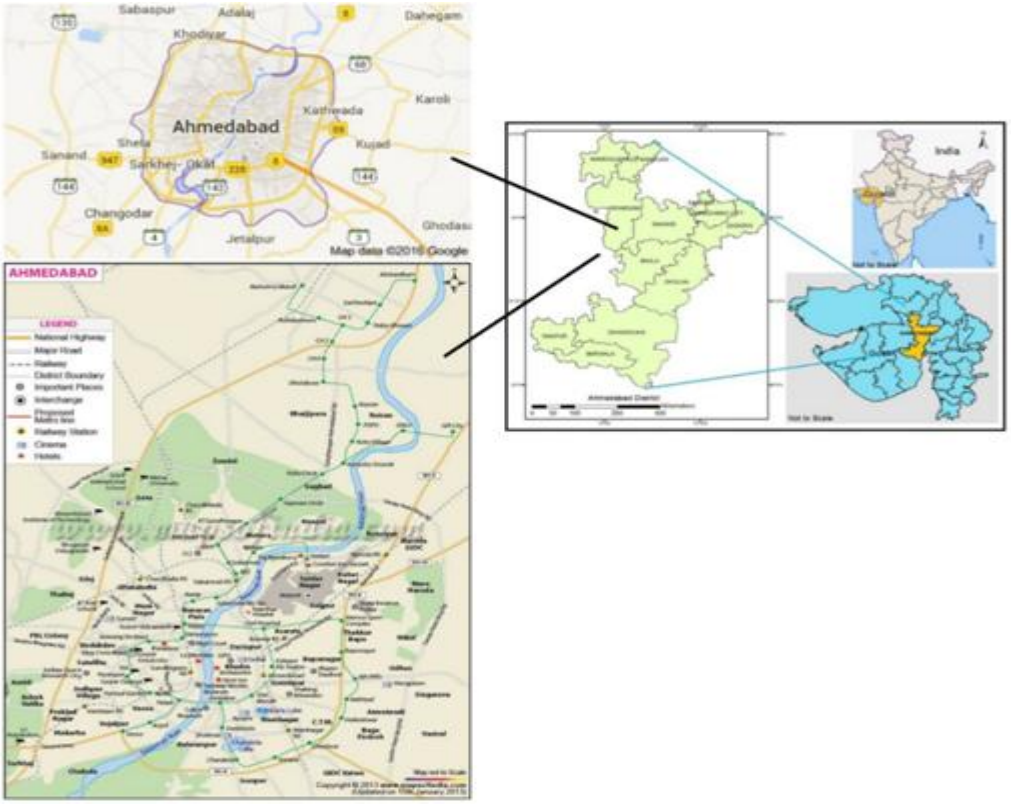

Figure 1: Map of Ahmedabad areas which is located in Gujarat, India

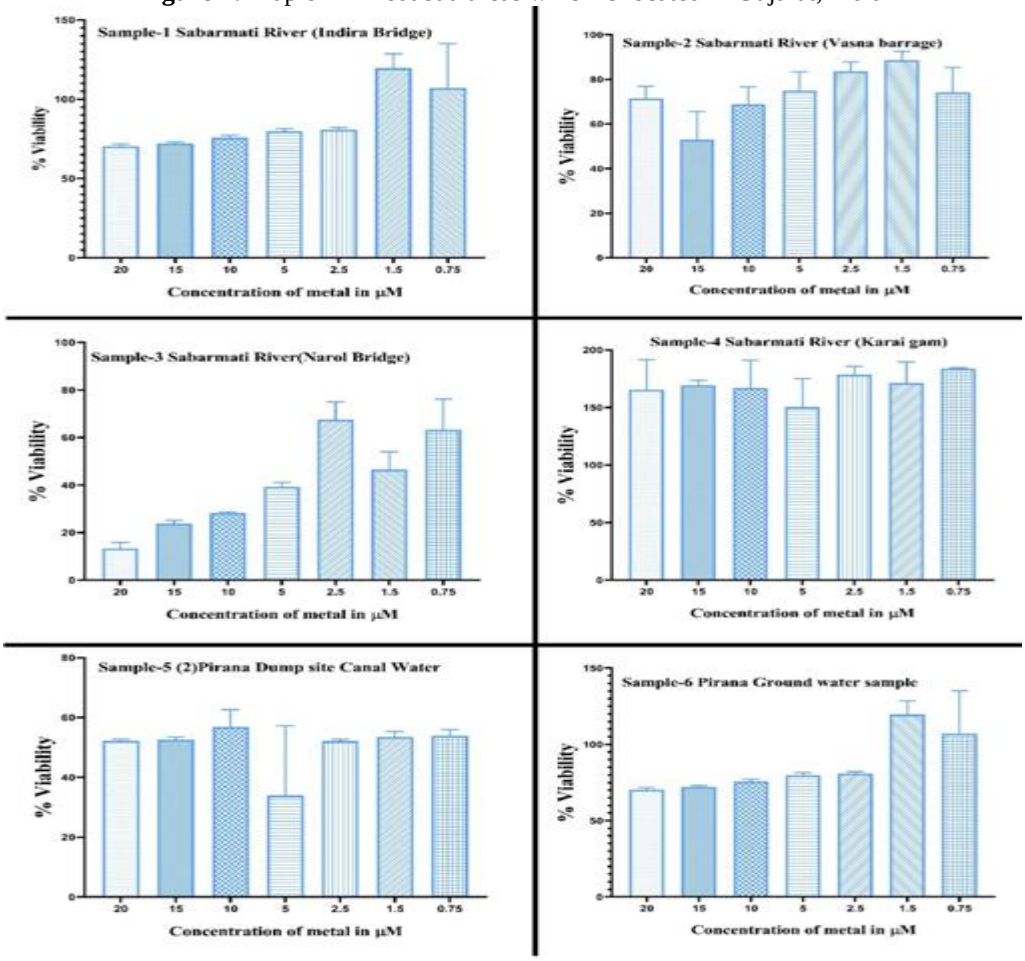

Figure 2: Percent viability of HepG2 Cells Treated with Sample-A1-A6.

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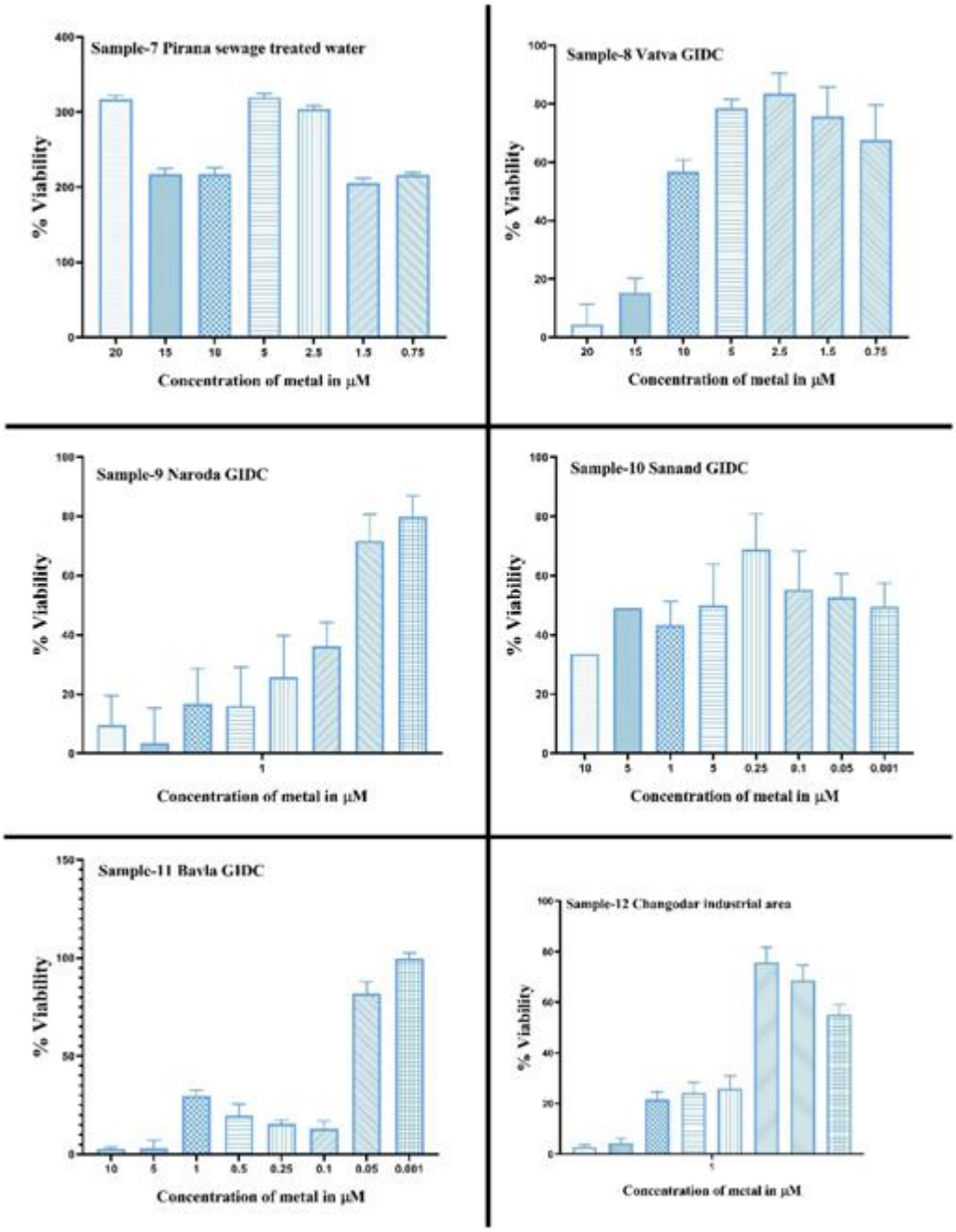

Figure 3: Percent viability in HepG2 Cells Treated with Sample-A7-A12

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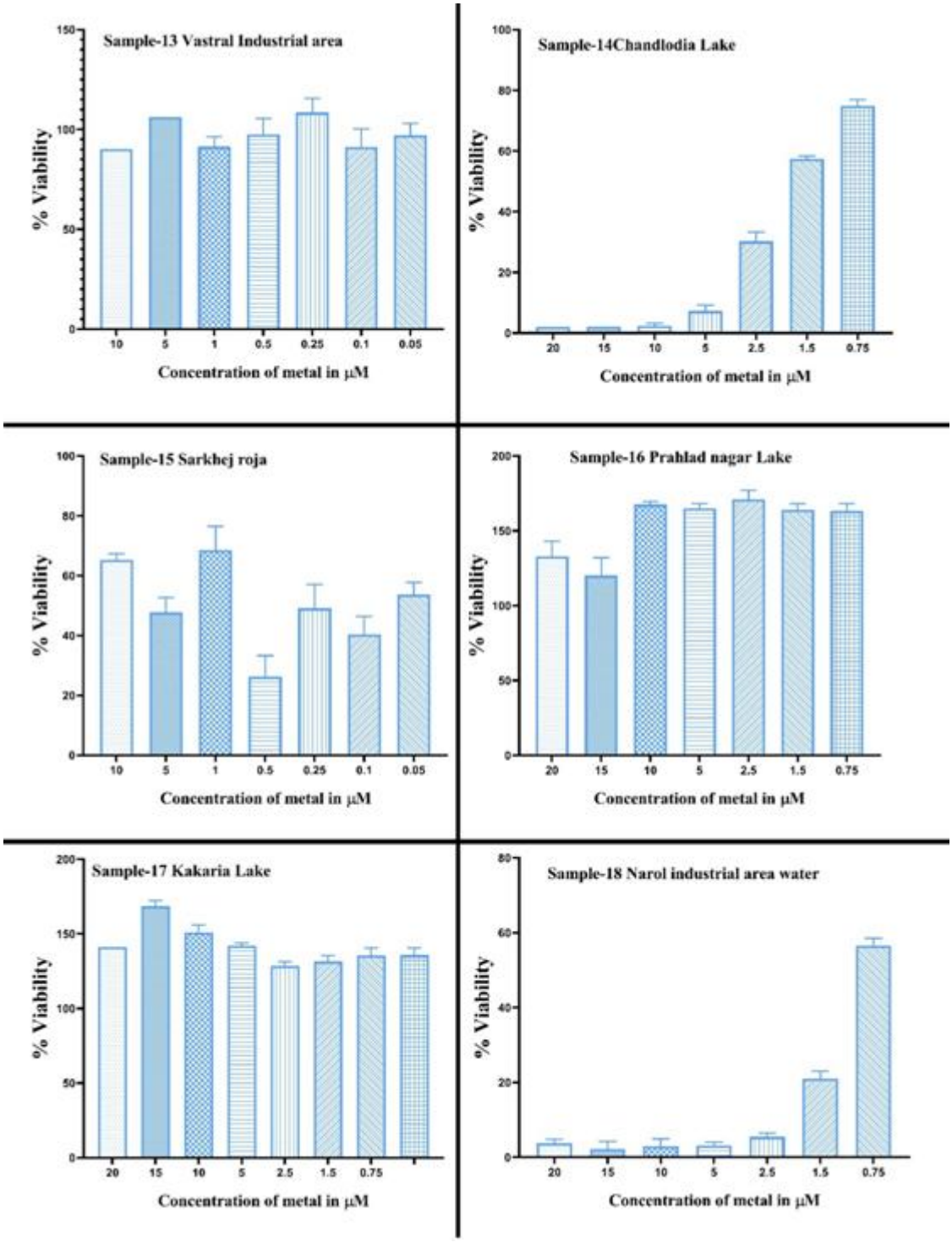

Figure 4: Percent viability in HepG2 Cells Treated with Sample-A13-A18

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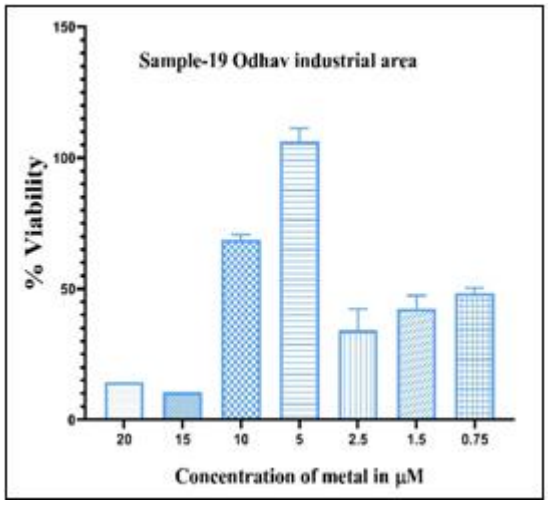

Figure 5: percent viability in HepG2 Cells Treated with Sample A19

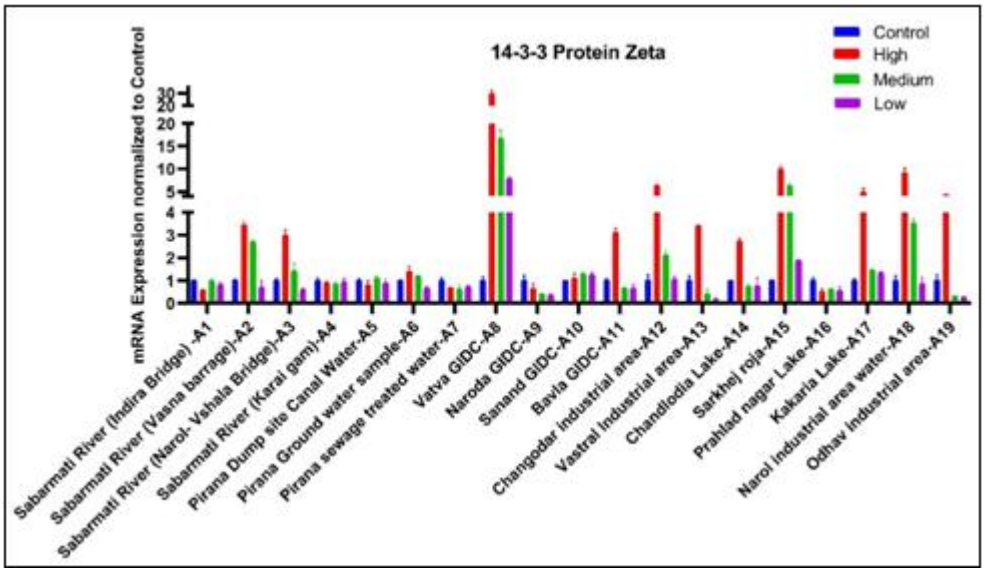

Figure 6: Gene Expression Fold Change of water Samples with Respect to 14-3-3 Zeta

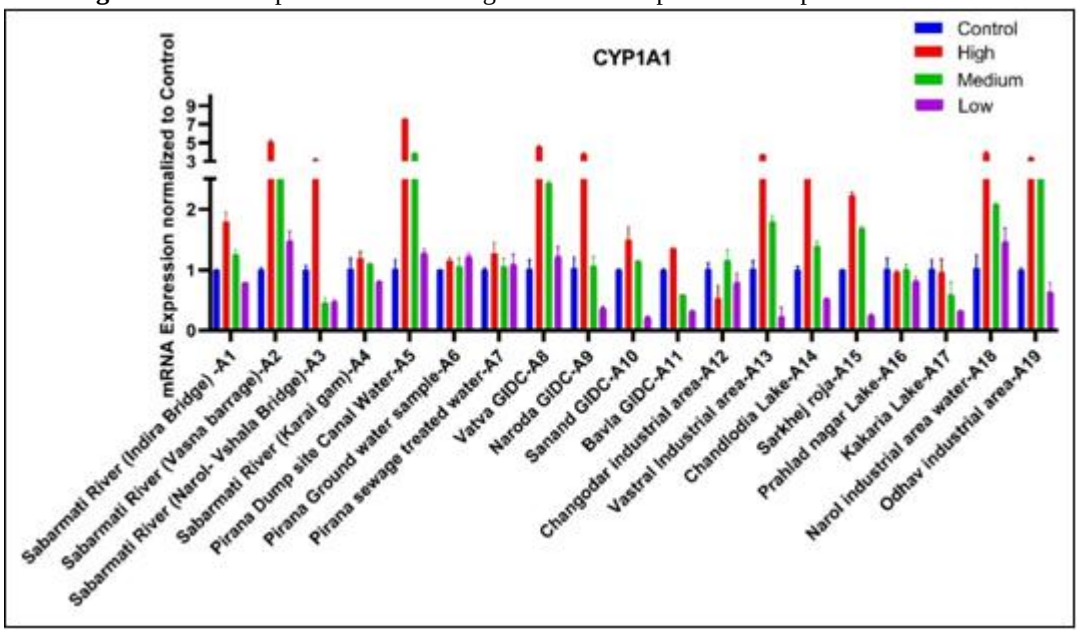

Figure 7: Gene Expression Fold Change of water Samples with Respect to CYP1A1

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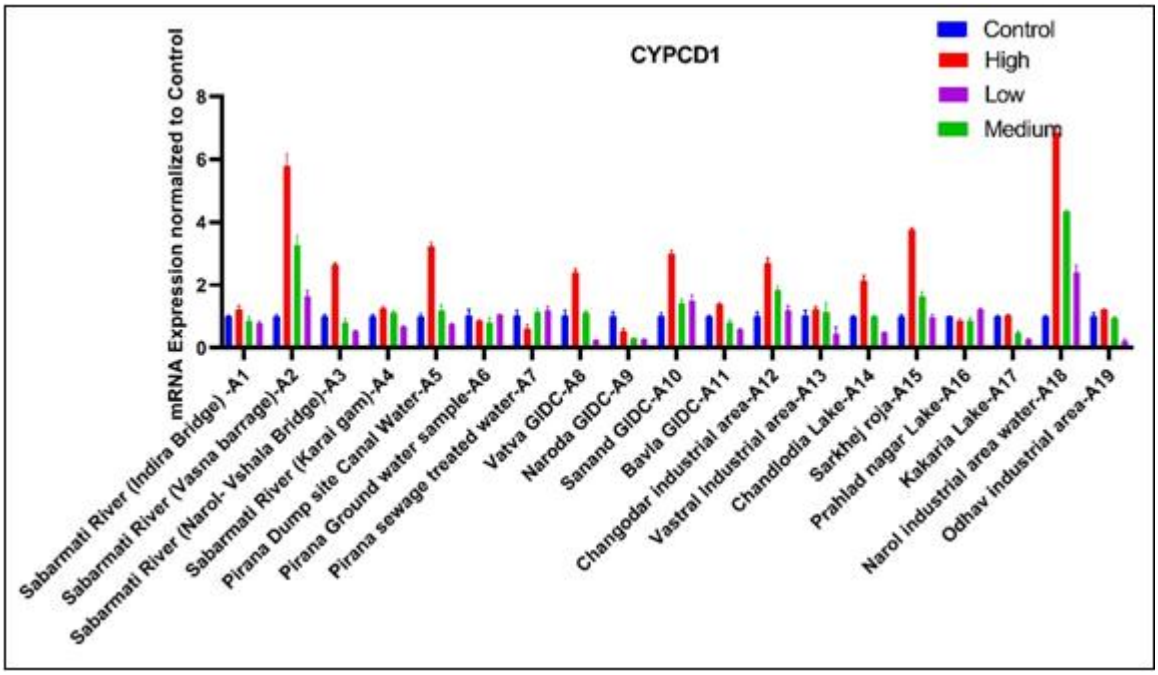

Figure 8: Gene Expression Fold Change of water Samples with Respect to Cyclin D1

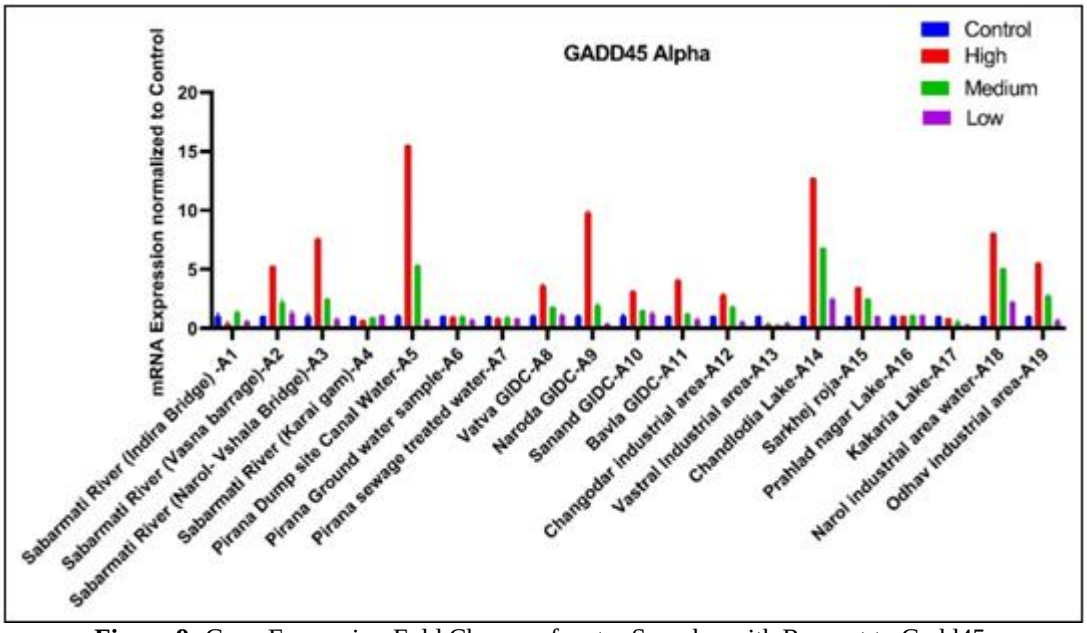

Figure 9: Gene Expression Fold Change of water Samples with Respect to Gadd45

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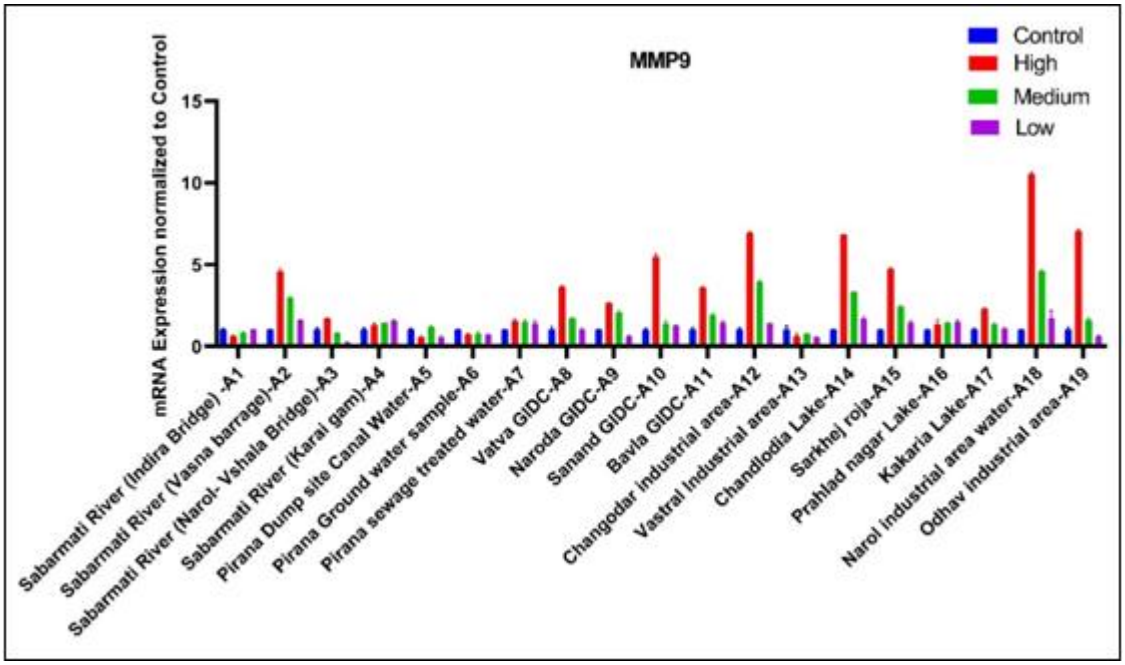

Figure 10: Gene Expression Fold Change of water Samples with Respect to MMP-9

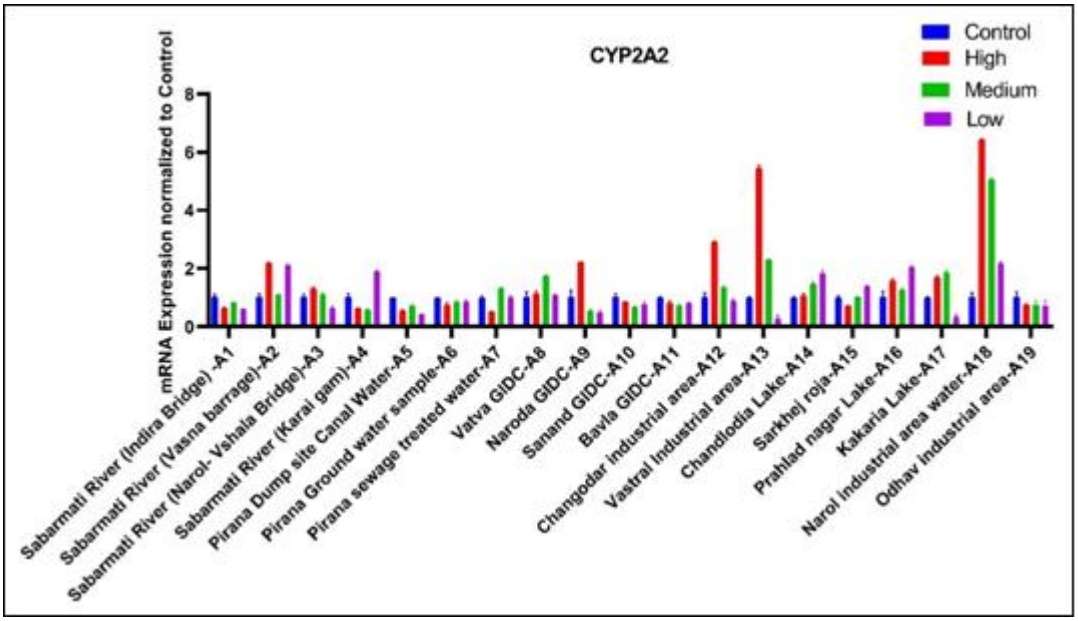

Figure 11: Gene Expression Fold Change of water Samples with Respect to CYP2A2

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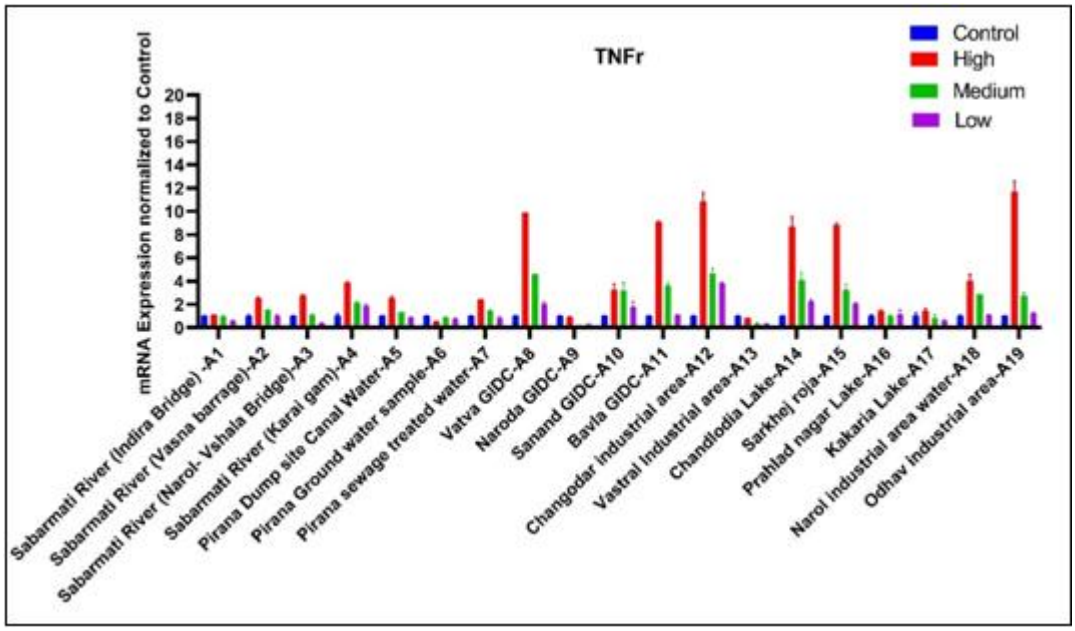

Figure 12: Gene Expression Fold Change of water Samples with Respect to TNFR

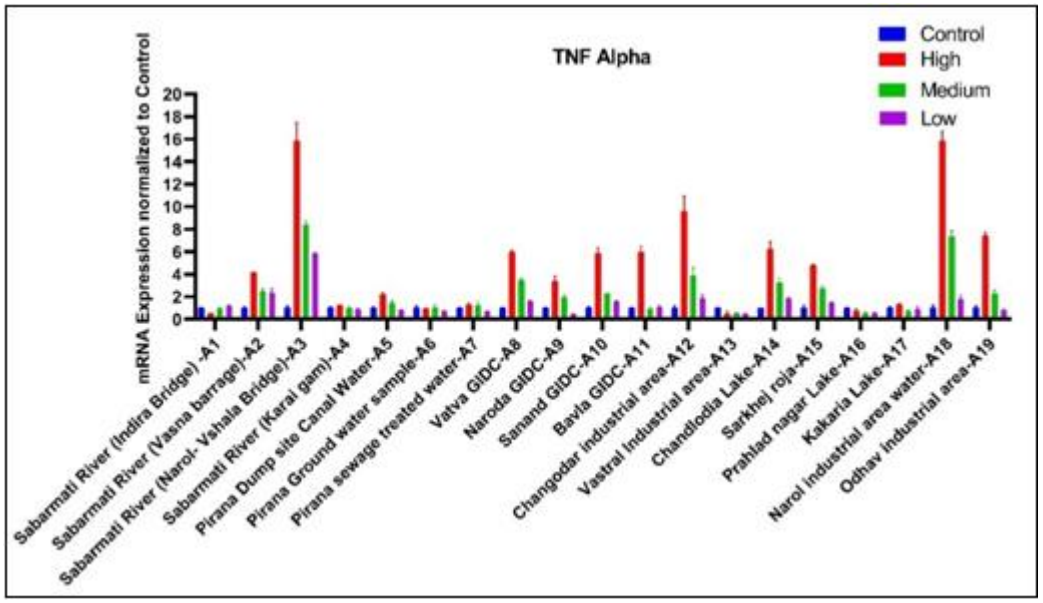

Figure 13: Gene Expression Fold Change of water Samples with Respect to TNF**α**

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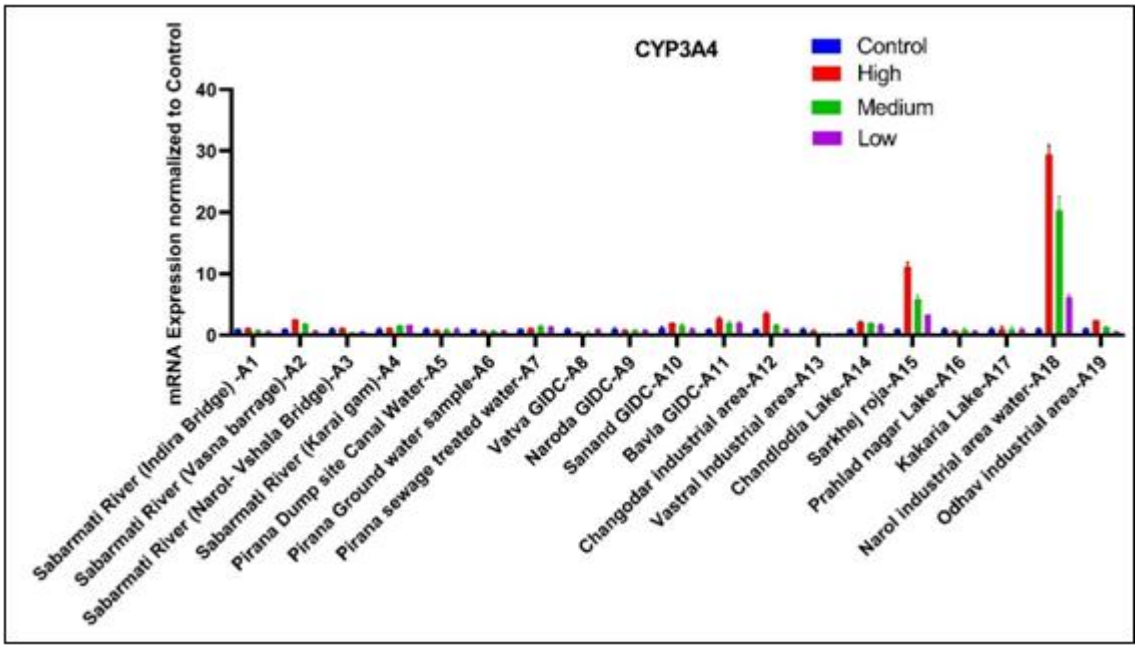

Figure 14: Gene Expression Fold Change of water Samples with Respect to CYP3A4

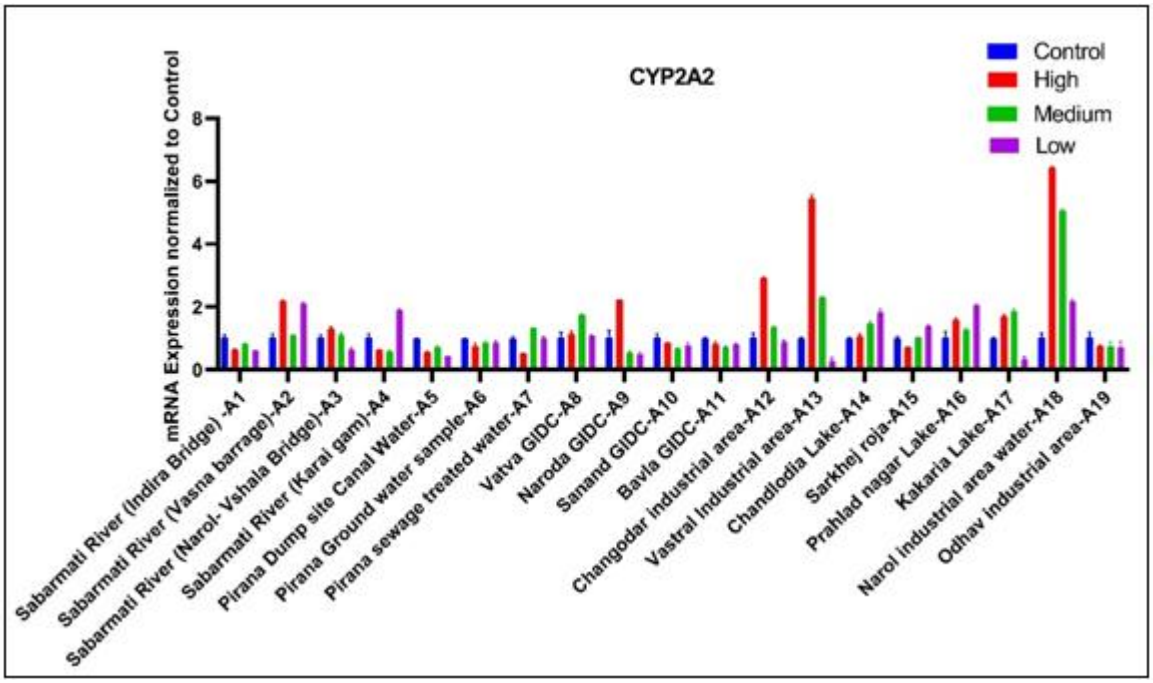

Figure 15: Gene Expression Fold Change of water Samples with Respect to CYP1A2

Abbreviations

HepG2	Human hepatoma
Huh7	Epithelial like, tumorigenic cells
Hep3B,	Human hepatoma contain hepatitis B virus genome.
SK-Hep-1	Adenocarcinoma liver cells
HCC	Hepato cellular carcinoma
HepaRG	Human hepatic progenitor cell line
km	Kilo meters
°N	north
°E	east
ICP-OES	Inductive Coupled plasma mass spectrometry
DMEM	Dulbecco's modified eagle medium
FBS	Foetal bovide serum
PBS	L-glutamine, phosphate buffered saline
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO	dimethylsulphoxide
%	percentage
µM	Micro mollor
h	hours
v	volume
°C	Celsius
OD	optical density
qPCR	Qualitative polymerase chain reasction
µl	microliter
Ng	nanogram

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