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Application of cell-based assays for toxicity characterization of complex wastewater matrices: Possible applications in wastewater recycle and reuse

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ABSTRACT

Exposure to pre-concentrated inlet or outlet STP wastewater extracts at different concentrations (0.001% to 1%) induced dose-dependent toxicity in MCF-7 cells, whereas drinking water extracts did not induce cytotoxicity in cells treated. GC-MS analysis revealed the occurrence of xenobiotic compounds (Benzene, Phthalate, etc.) in inlet/outlet wastewater extracts. Cells exposed to inlet/outlet extract showed elevated levels of reactive oxygen species (ROS: inlet: 186.58%, $p < 0.05$, outlet, 147.8%, $p < 0.01$) and loss of mitochondrial membrane potential ($\Delta\psi m$: inlet, 74.91%, $p < 0.01$; outlet, 86.70%, $p < 0.05$) compared to the control. These concentrations induced DNA damage (Tail length: inlet: 34.4%, $p < 0.05$, outlet, 26.7%, $p < 0.05$) in treated cells compared to the control (Tail length: 7.5%). Cell cycle analysis displayed drastic reduction in the G1 phase in treated cells (inlet, G1:45.0%; outlet, G1:58.3%) compared to the control (G1:67.3%). Treated cells showed 45.18% and 28.0% apoptosis compared to the control (1.2%). Drinking water extracts did not show any significant alterations with respect to ROS, $\Delta\psi m$, DNA damage, cell cycle and apoptosis compared to the control. Genes involved in cell cycle and apoptosis were found to be differentially expressed in cells exposed to inlet/outlet extracts. Herein, we propose cell-based toxicity assays to evaluate the efficacies of wastewater treatment and recycling processes.

1. Introduction

Many countries including India are suffering from the emerging issue of freshwater scarcity and pollution. Rapid industrialization and urbanization has resulted in the speedy increase in wastewater generation. This has increased the demand for fresh water. Current and future demand for freshwater can be achieved by implementing appropriate strategies to improve water use efficiency and demand management. Wastewater has been considered as a potential source to meet the freshwater demand after necessary treatment (Huang et al., 2014; Tanik et al., 1996). Apparently, the sewage treatment plants (STPs) plays a crucial role in the treatment, disinfection and reuse of the domestic wastewater (Hendricks and Pool, 2012; Komesli et al., 2014).

Recycle and reuse of wastewater is one of the prospective ways to address the demand for freshwater. Developed countries are practicing wastewater recycling for potable and other applications. Sewage water contains various toxic chemicals, heavy metals and other xenobiotics (Malik, 2014). Most of these compounds are persistent and known to adversely affect human health (Shameem et al., 2015; Bueno et al., 2012). These contaminants may impart genotoxicity, endocrine disruption and bioaccumulation (Moselhy et al., 2014; Kaile and James, 2016; Bafana et al., 2014). Thus, removal of toxic compounds from sewage water is highly desirable for effective recycling procedures.

United Nations Environment Programme report (2010) entails that majority of the wastewater in developing countries is discharged directly in to surface water sources resulting in its adverse effects on aquatic ecosystems and humans (Jasinska et al., 2015; Corcoran et al., 2010). More than 50% of the total sewage water in India is discharged

Abbreviations: BIS, Bureau of Indian standard; CPCB, Central pollution control board; COD, Chemical oxygen demand; DMSO, Dimethyl sulfoxide; DO, Dissolved oxygen; FAS, Ferrous ammonium sulfate; GCMS, Gas chromatography mass spectrometry; HP, Hand pump; IC, Inorganic carbon; ICP-MS, Inductive coupled plasma-mass spectrometry; LLE, Liquid-liquid extraction; LMA, Low melting agar; MC, Municipal Corporation; MTT, 3-(4, 5-dimethylthiazol-2-yl) − 2, 5-diphenyltetrazolium bromide; NCCS, National cell; NMA, Normal melting agar; PBS, Phosphate buffered saline; PC, Positive control; PS, Phosphotedylserine; ROS, Reactive oxygen species; RT, Room temperature; SCGE, Single Cell Gel Electrophoresis; STP, Sewage treatment plant; TC, Total carbon; TDS, Total dissolved solid; TOC, Total organic carbon; TS, Total solid

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in to different water streams without treatment (ENVIS, 2015). Increasing burden on the functioning of existing STPs may lead to the discharge of inadequately treated effluent. The discharge of inadequately treated and untreated sewage wastewater into natural surface may contaminate domestic water sources (municipal water, hand pump water, etc). Thus, it is important to periodically evaluate the quality of drinking and sewage water using appropriate methods (Rahmanian et al., 2015; Patil et al., 2012).

In India, drinking and sewage water are routinely tested for physicochemical/inorganic parameters such as dissolved oxygen (DO), pH, chemical oxygen demand (COD), metals etc. to ensure the quality of water as per the norms given by Central Pollution Control Board (CPCB) and Bureau of Indian Standards (BIS). However, these parameters may not depict the actual effect of water contaminants on biological systems. In addition, contaminants present within the permissible limit may also trigger toxic cellular response. Water contaminants may alter complex cellular interactions to produce the toxic effect. It involves activation/deactivation of various cell surface receptors, cytoplasmic and nuclear biomolecules, cross talks between different cellular pathways etc., even at very low concentrations. These complex cellular interactions cannot be predicted using biochemical assays. Thus, considering the possible applications of wastewater for recycle and reuse, the toxicity of treated/recycled wastewater needs to be thoroughly investigated with respect to its impact on biological systems. In our earlier publication, we studied the possible effects of environmental pollutants in children residing nearby STP and solid waste disposal plant (Shrivastava et al., 2016). In the current investigation, a battery of cell-based assays were used to evaluate the toxicity of drinking and sewage (untreated and treated) water. Physicochemical/inorganic parameters of untreated and treated sewage water samples were within the permissible limits given by CPCB/ BIS. Interestingly, sub-lethal concentrations of these samples were found to induce toxic response in MCF-7 cells.

2. Materials and methods

2.1. Study area

Study was carried out at Sewage treatment Plant (STP) placed in Central India. STP receives hospital and domestic wastewater and has a treatment capacity of ≈80 million liters per day (MLD). The treated wastewater from STP is discharged into a nearby river. Treatment of wastewater in this STP is up to the level of secondary clarifier. After primary screening the wastewater is extensively aerated and discharged after sludge removal. This is the most favoured form of treatment for domestic wastewater in India because of lower operating costs. Fig. 1 shows the stages of treatment of selected STP.

2.2. Sample collection

Inlet and outlet wastewater samples were collected from the STP in amber colored bottles as per USEPA guidelines. Two liters of composite sample (8 h) was separately collected from the inlet and outlet tanks. Samples of drinking water were collected from municipal corporation (MC) supply and hand pump (HP) located nearby the STP.

2.3. Sample processing

Samples were processed using liquid-liquid extraction (LLE) and acid digestion methods. Referred to Supplementary Information S1 and S2 for the details.

2.4. Characterization of organic/inorganic compounds in drinking water and wastewater samples

Chemical analysis of water samples was carried out using gas chromatography-mass spectroscopy (GC-MS) whereas elemental analysis was done using inductively coupled plasma-mass spectrometry (ICP-MS), respectively. Referred to Supplementary Information S1 and S2 for the details.

2.5. Physicochemical parameters

Water samples were analyzed for the physicochemical parameters as per the guidelines given in CPCB (CPCB Guide manual: water and wastewater analysis).

2.6. Dissolved oxygen (DO)

Amount of oxygen dissolved in the water and wastewater was measured by DO meter (HI 98193, HANNA, USA).

2.7. Total Dissolved Solid (TDS)

100 mL of drinking water/wastewater was filtered through glass filter paper (0.45 µ, Whatmann). The filtered sample was taken in a pre-weighed dish and was evaporated and dried at 180 °C for 24 h. After drying the dish was weighed and the TDS value was calculated.

2.8. Chemical Oxygen Demand (COD)

EPA approved standard method (5220) was used to analyze the COD of the drinking water and wastewater samples. In brief, sample was prepared by adding 0.4 g of mercuric sulfate in 20 mL of sample. Milli Q water was used as a blank and processed in the same way. 10 mL of 0.25 N potassium dichromate ($K_2Cr_2O_7$) was added and mixed properly. 30 mL catalyst solution was added slowly through the walls of the tube. The sample was digested at 140 °C for 2 h and cooled to room temperature (RT). The final sample volume was made up to 150 mL. Titration was performed with ferrous ammonium sulfate using ferroin indicator (0.10–0.15 mL) to estimate excess of $K_2Cr_2O_7$.

2.9. Total organic and inorganic carbon

Standard method 5310 was used to detect total organic and inorganic carbon. The organic carbon in water and wastewater comprises of mixture of organic compounds in various oxidation states. To analyze the TOC of the samples, high- temperature combustion method was used. Samples were filtered through 0.45 µ filter paper. 20 mL of each filtered sample was filled in 24 mL glass vials and subjected to total organic carbon (TOC), inorganic carbon (IC) and total

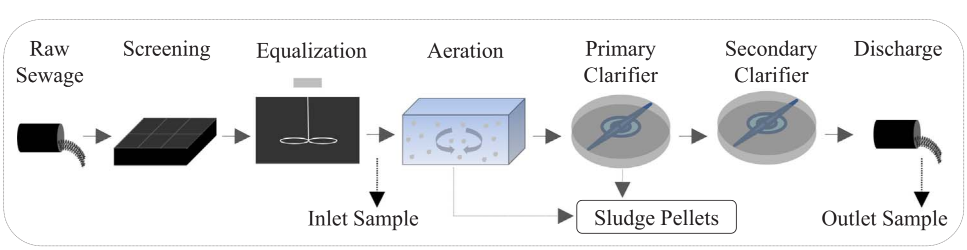

Fig. 1. Schematic diagram of different processes involved in STP.