Introduction

1.1. Introduction

Water scarcity has become a stirring concern and challenge. Globally, about more than 1.7 billion peoples facing water scarcity and more than 2.7 billion people will experience this situation up to year 2025 (Water, 2013; Ryder, 2017). This condition has been raised due to fast growing population, urbanization, industrialization, high groundwater extraction, rapid contamination of surface water and several bad practices of water management in developed and developing countries (Kumar, 2019; Pandey et al., 2019). Now days, millions of people are not able to access clean water for drinking purpose. Montogomery and Elimelech (2007) report found that due to the improper sanitation (2.6 billion people) and lack of drinking water accessibility (1.2 billion) leads many water borne disease and millions of death annually (Montgomery and Elimelech, 2007). In low-income countries only less than 30 % population are able to access clean water for drinking and sanitation purpose. A bleak overview of water scarcity highlighted by UNICEF reports on "water security for all" suggest that every 5^{th} child suffers from water crisis at global level (Unicef, 2011; Water, 2013; Paudel et al., 2021).

The continuous pollution in water sources is a major factor for clean water accessibility and risk associate with public health. Polluted water is cause of several water born disease and generation of negative impact on aquatic environment. The incidence of water-borne diseases (diarrhoea, dengue, malaria, typhoid etc.) is occurs due to ingestion of contaminated water that contain bacteria, parasites, virus etc. (Paudel et al., 2021). The pollutants in water sources are classified into several groups. Recently the level of hazardous pollutants has been highly rising in the surface water, groundwater and rivers. Hazardous pollutant includes pharmaceuticals (antibiotics, antineoplastic and pesticides), dye or paint. The over-use of pharmaceuticals by the hospitals and their production plants are highly contaminating the surface water. Many pharmaceuticals and dye industries are directly polluting the river water sources. Pesticides and municipal leachate are responsible for generation of surface as well as ground water

contamination (Gerlak et al., 2018). These pharmaceuticals have their own toxic applicability on aquatic microorganism as well as on public health. Their presence in aquatic environment is a major cause of toxicity generation (Talbot et al., 2018).

The clean water is a prominent resource for earth life, so it is necessary to take quick action towards the address of this challenge and frame ways to protect the water for future generation. SDG_6 has been evolved by the global scientific leader for the removal of pollutants to clean or protect the water bodies (Paudel et al., 2021). In current, scientific community working on SDG_6 for wastewater treatment.

1.2. Antineoplastic compounds and source of occurrence

The contamination of pharmaceutical ingredients in the environment is of major concern owing its presence leads to detrimental health issues. As the diseases in human population are increasing at very high rate, so to protect from these problems the use of pharmaceuticals also has been increased in the hospitals and homes. After the cardiovascular disease, cancer is the second highest non-communicable disease at worldwide. The excreted waste from human as well as animals along with wastewater leads to the introduction of pharmaceuticals into environment (Halling-Sørensen et al., 1998). After excretion from the human body, the non-metabolized part of pharmaceutical compounds form conjugated compounds with polar molecules and these modified conjugate are cleaved during sewage treatment and released in water in original drug form or transformed products (Heberer, 2002). These drugs can be of persistent in nature as their biodegradation and elimination during wastewater treatment is limited. Pharmaceutical compounds, and their metabolites, are highly mobile in aquatic environment due to their hydrophilicity nature (Kümmerer, 2001; Mastroianni et al., 2016; Batt et al., 2017; Ghafuri et al., 2018; Guzel et al., 2018; Márta et al., 2018; Riva et al., 2018; Gu et al., 2019). According to global cancer observatory (GLOBCAN) the new cancer incidences are

continuously inclining at very high rate. GLOBCAN and IARC (International agency for research on cancel) reported that till 2018, Asia was the highest new cancer cases containing continent in the world. Globally, the incidence of new cancer cases in year 2012 were 14.1 million and it becomes increases to 18.07 million in the year 2018 (Bray et al., 2018). Consequently, this increment in cancer incidence leads to the demand, production and consumption of antineoplastic drugs (Besse et al., 2012; Kümmerer et al., 2016; Cristóvão et al., 2020). Antineoplastic drugs used as therapeutic agent for the treatment of cancer disease represents a wide range of compounds with great potential for action. These drugs prevent uncontrolled cell division by blocking deoxy-ribonucleic acid (DNA) replication or by interfering with cell signalling during cell cycle (McKnight, 2003).

Municipal and hospital wastewater are the main sources of antineoplastic compounds found in wastewater treatment plant. Water bodies are major indicators for the presence of these drugs into water environment (Kosjek and Heath, 2011; Ferrando-Climent et al., 2014; Gómez-Canela et al., 2014; Česen et al., 2015). Effluent release from cancer hospitals and households (cancer patient getting treatment at home) contains antineoplastic drugs loads (Fig.1.1) (Cristóvão et al., 2020). Unexpectedly, through the oncology wards of hospitals, discharge of hospitalized patients, outpatients and due to lack of treatment facility in sewage treatment plants (STPs), antineoplastic compounds are persistently coming into water bodies. These compounds are partially treated or untransformed forms and by-products are directly released into the sewer system via urinary or faecal excretions of patients that are undergoing treatment (Česen et al., 2016b; Negreira et al., 2014b). These drugs molecules are excreted in either un-metabolized or modified active forms and then pollute the aquatic environment by entering through the hospital and municipal aqueous waste (Ferrando-Climent et al., 2014; Negreira et al., 2014a; Wormington et al., 2020). Residues of antineoplastic drugs waste are found in very low concentration ($ng.L^{-1}$) in aquatic environment (Yin et al., 2010; Kosjek and Heath, 2011;

Zhang et al., 2013b; Booker et al., 2014; Isidori et al., 2016b). So, due to the persistence or recalcitrant nature of antineoplastic drugs after going through treatment plants they remain dynamic after pass through WWTPs (Orias and Perrodin, 2013; Zhang et al., 2013a; Ferrando-Climent et al., 2014; Martín et al., 2014; Isidori et al., 2016a; Azuma, 2018).

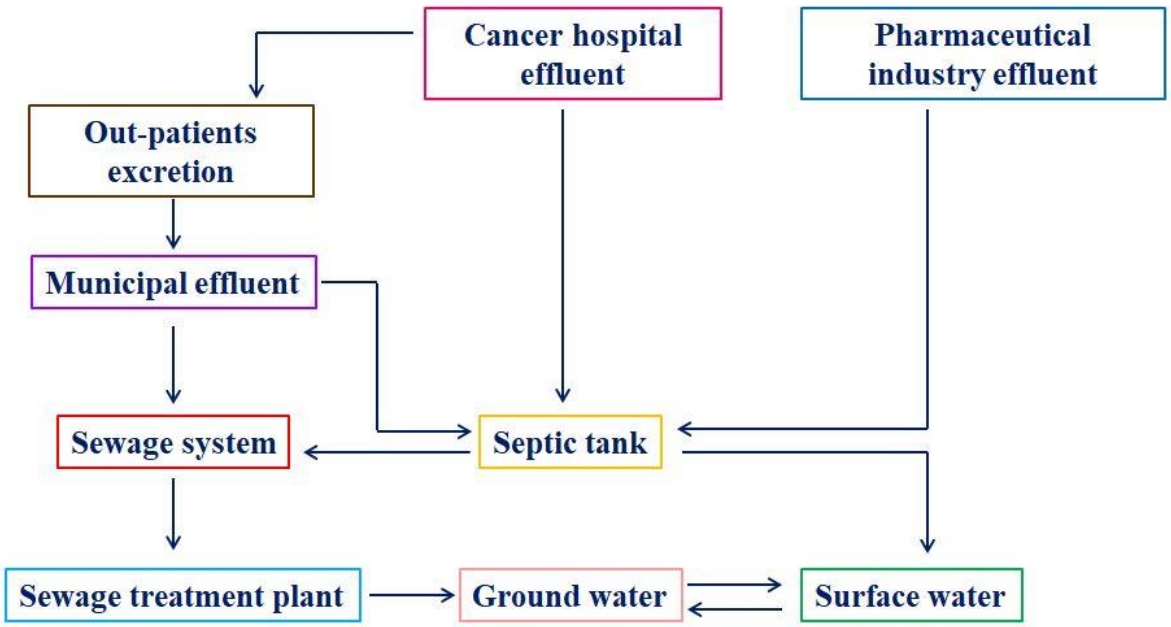

Fig. 1.1. Sources and pathway of antineoplastic drugs in aquatic environment

1.3. Properties of cyclophosphamide, etoposide and paclitaxel

Among antineoplastic compounds, cyclophosphamide, etoposide and paclitaxel are widely used antineoplastic drugs for the treatment of different types of tumours. Cyclophosphamide is an alkylating agent, structurally similar to mustard gas. It can perform their function by the blockage of DNA replication and transcription. Clinically it is a highly used medicine for treatment of leukaemia, brain cancer, lymphoma, neuroblastoma and solid tumours. Being a polar molecule, cyclophosphamide is easily soluble in water (US National Laboratory for medicine). Etoposide belongs to topoisomerase inhibitor class, which can bind with nucleic acid to form a complex during cell cycle. It can form a complex that prevent DNA replication and

stimulate cell apoptosis. Etoposide is partially soluble in water. In chemotherapy, it is generally used for the treatment of lung cancer, testicular cancer glioblastoma multiforme, nonlymphocytic leukaemia, lymphoma and several solid tumours. Paclitaxel is a plant alkaloid chemotherapeutic agent, which is widely used for the treatment of ovarian, breast, prostate, melanoma, oesophageal and lung cancers. It can inhibit the cell division by the inhibition of formation of microtubule. It is insoluble in water.

1.4. Presence of cyclophosphamide, etoposide and paclitaxel in aquatic environment

Cyclophosphamide, etoposide and paclitaxel are widely reported antineoplastic drugs in the aquatic samples of environment (Yin et al., 2010; Martín et al., 2011; Negreira et al., 2014b; Česen et al., 2015; Ferrando Climent, 2016; Azuma, 2018). Cyclophosphamide was detected in wastewater samples (hospital effluent, wastewater treatment plants (WWTPs) effluent, WWTPs influent, surface water, drinking water) of country Spain, Germany, Italy, Romania, Slovenia, Switzerland, China and France in range from 2.2 to 616 $\mu g.L^{-1}$. The etoposide was detected in aquatic samples (hospital effluent, WWTPs influent and effluent) of country Spain, China and France in range from 5 to 714 $ng.L^{-1}$. Paclitaxel and their derivatives were detected from hospital effluent, WWTPs influent or effluent in range from 3.7 to 100 $ng.L^{-1}$ (Negreira et al., 2014b; Ferrando Climent, 2016).

These drugs molecule have ability to cause a significant toxic effect on living organism. The presence of these antineoplastic drugs in environment samples need scientific attention towards their detection and removal. Several analytical methods have been developed for the detection of these antineoplastic drugs from the environmental samples. Initially, cyclophosphamide, etoposide and paclitaxel were detected by High performance liquid chromatography (HPLC) based method but later on, due to their low concentration availability in aquatic samples, other highly sensitive techniques were employed. These methods include the Solid phase extraction

high performance liquid chromatography electrospray ionization tandem mass spectrometry (SPE-HPLC-ESI-MS/MS), High performance liquid chromatography triple quadrupole mass spectrometry (HPLC-QqQ-MS), Liquid chromatography high resolution mass spectrometry (LC-Orbitrap-MS), SPE-GC-MS, SPE-LC-MS etc. (Sacher et al., 2001; Santos et al., 2010; Martín et al., 2011; Gómez-Canela et al., 2012; Negreira et al., 2013; Gómez-Canela et al., 2014; Jureczko and Kalka, 2020). The reason for inclusion of mass spectrometry in these methods was to identify the derivatives, transformed products and life cycle assessment of targeted antineoplastic drug during detection and treatment. The solid phase extraction (SPE) was added into these methods to provide the concentrated sample of antineoplastic drug from a highly diluted sample. These methods can provide high limit of quantification (LOQ) and limit of detection (LOD) value of these antineoplastic drugs (Martín et al., 2011; Negreira et al., 2013; Gómez-Canela et al., 2014).

1.5. Risk associated with antineoplastic compounds

The antineoplastic compounds protect the patient by inhibition of cell division in cancerous cell. Same as like other pharmaceuticals, antineoplastic compounds are not able to completely metabolize inside the human body. So, it remains as such and comes into aquatic environment through urine excretion (Negreira et al., 2014b; Česen et al., 2016b). Several reports indicates that antineoplastic have high level of cell cytotoxicity, genotoxicity and mutagenic effect on aquatic life. They can bind to cellular DNA and inhibit cell cycle or cause some mutation in normal growing cell (McKnight, 2003). Antineoplastic compounds have various detrimental effects and potential to cause harmful effects on the aquatic microorganisms as well as human life. Several reports indicated that, all the exposed animals showed some symptoms such as intestinal or reproductive abnormalities, colour change etc. that cause genetic imbalance, tumour and cell death (Parrella et al., 2014a; Novak et al., 2017; Gouveia et al., 2019; Barışçı

et al., 2018; Dehghanpour et al., 2020; Huo et al., 2020). Antineoplastic drugs are not specific in nature, as along with tumour cells they can also adversely affect the normal growing cells present in the body. They possess the property of carcinogen, mutagen and teratogen due to their mechanism of action which can adversely affect, organisms present in an aquatic environment through its chronic exposure (Toolaram et al., 2014).

1.6. Removal of antineoplastic compounds

Antineoplastic drugs are recalcitrant in nature, so due to their poor degradability or removal efficiency with conventional wastewater treatment techniques, several advanced physical, physio-chemical and chemical methods have been developed and employed. Advanced treatment methods for the treatment of antineoplastic compounds from the aquatic environment includes advanced oxidation processes (AOPs), membrane filtration (RO, nanofiltration, microfiltration and ultrafiltration), ozonation and adsorption etc. are considered highly effective technologies with better efficiency (Li et al., 2016; Lutterbeck et al., 2016; Janssens et al., 2019; Janssens et al., 2020). But they have certain limitations such as fouling in membrane process result require high energy and pressure, in photocatalysis treatment process catalyst scaling responsible to reduce the efficiency of process is a major limitation. These kinds of limitation exists in physical, physio-chemical and chemical treatments and they require higher cost for the operation and management of treatment process, could not come up with 100 % removal efficiency and produced some by-product which generate more toxicity as compared to parent molecule (Lai et al., 2015; Lutterbeck et al., 2015b).

Biologically removal of antineoplastic compounds is also emerged as a promising approach. As the already existing advanced treatment techniques, biological treatment is highly efficient than other techniques. In biological treatment, different microorganisms are used such as fungi, bacteria, algae etc. but fungi contain highest capability for the mineralization of recalcitrant

organic compounds (Grandclément et al., 2017; Mir-Tutusaus et al., 2018). Among fungi, particularly ligninolytic fungi are efficiently able to degrade antineoplastic compounds from water bodies (Ferrando-Climent et al., 2015; Castellet-Rovira et al., 2018; Pereira et al., 2020). Mainly white-rot-fungi (WRF) such as *Coriolus versicolor, Trametes versicolor, Phanerochaete chrysosporium, Pleurotus ostreatus, Ganoderma lucidum, Cyathus stercoreus, Stropharia rugosoannulata, Gymnopilus luteofolius, Agrocybe erebia, Irpex lacteus, Fomes fomentarius, Hypholoma fasciculare, Phyllotopsis nidulans,* etc. have ability to secrete extracellular (laccase, lignin peroxidase, manganese peroxidase and versatile peroxidase) and intracellular ligninolytic (Cytochrome P_{450} monooxygenase (CYP_{450}) and nitroreductase) oxidoreductase enzymes (Fig. 1.2). They can degrade recalcitrant pharmaceutical compounds present in hospital, industrial or domestic effluents (Ferrando-Climent et al., 2015; Vasiliadou et al., 2016; Asif et al., 2017; Castellet-Rovira et al., 2018). Several properties of WRF make them attractive in removal of various pharmaceutical compounds which are: a) Non-specificity of their enzymes that help in degradation of broad spectrum of micropollutants, b) Fast colonization through hyphal growth that helps in accessing the micropollutants, c) Production and secretion of enzymes that can degrade compounds d) Efficiency of fungus towards degradation of compounds over a wide range of pH. These approaches suggests that the use of fungal process is efficiently able to remove many of the pharmaceuticals present in wastewater simultaneously, rather than using a specific treatment for each recalcitrant compound. Developments in this direction depend on overcoming several shortcomings, namely (1) maintaining a stable activity of the fungal pellets over prolonged periods of time and (2) preserving good performance in non-sterile conditions, as sterility would be unviable from the economic and ecological perspective.

Herein, the aim of the present research was to determine the ability of three different WRF strains, *i.e. P. chrysosporium, T. versicolor* and *G. lucidum* for the biodegradation and removal

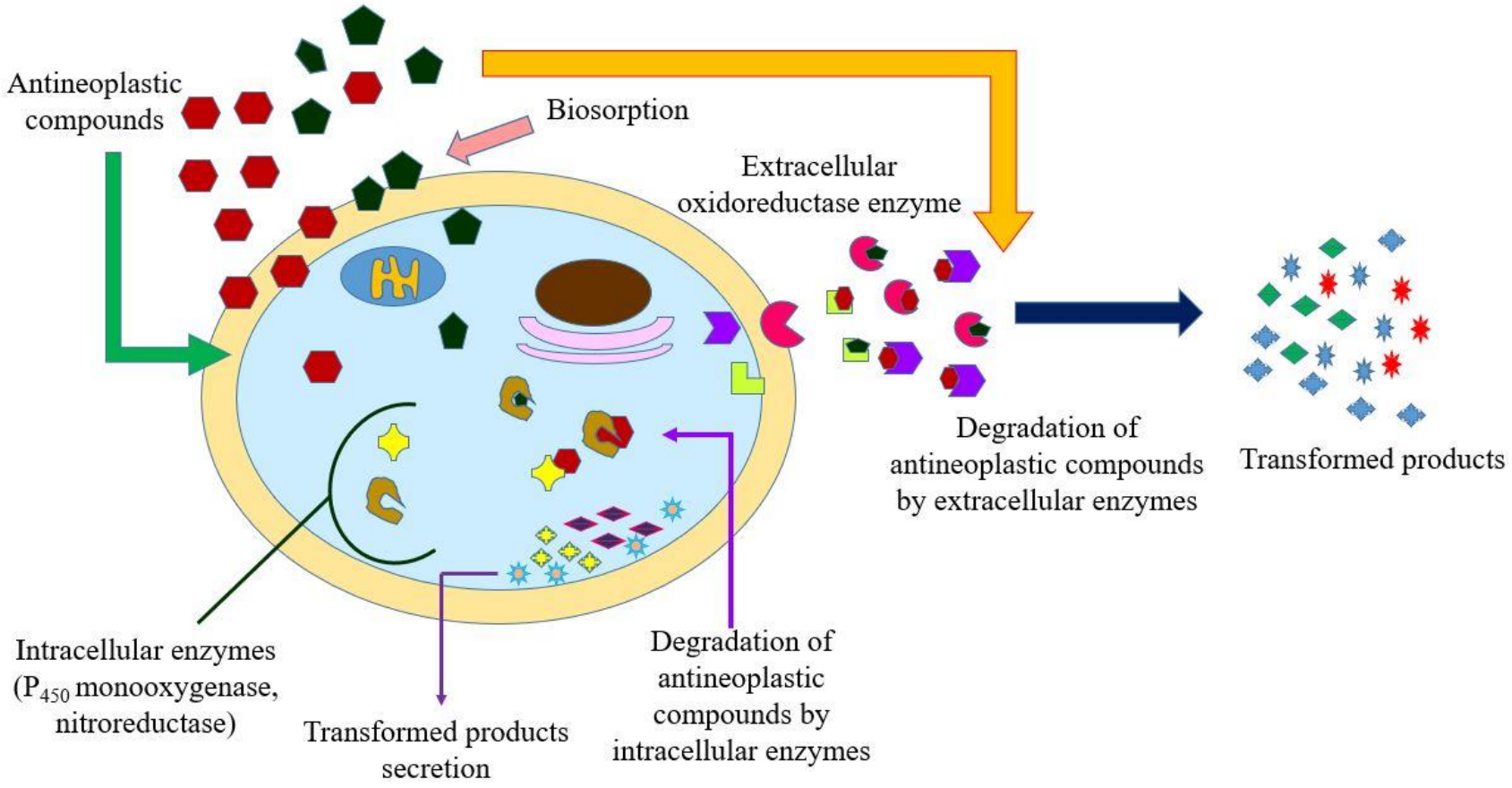

Fig. 1.2. Possible mechanism of degradation of antineoplastic compounds by white rot fungi

of three antineoplastic compounds (cyclophosphamide, etoposide and paclitaxel). The time-course degradation profiles of cyclophosphamide, etoposide and paclitaxel with glucose utilization and pH variation were monitored. In degradation kinetics, the rate constant, half-life of targeted compounds with the selected WRF were also determined. This study was also investigated the toxicity level of these antineoplastic compounds.

1.7. Key questions

- The focus has been to develop a significant biological process having great potential for the mitigation of antineoplastic compounds from water bodies.
- To reduce the toxicity level of antineoplastic compounds on animals and other organism present in aquatic environment by their degradation or removal
- Assessment of the potential of fungi for the degradation of antineoplastic compound and provide antineoplastic contamination free water for the societal use.

1.8. Objectives of the research work

On the basis of above discussion and problems associated with antineoplastic compounds, the following objectives were proposed for the present research work.

1. Method development for detection and quantification of antineoplastic compounds in synthetic urine.
2. Developing the process for the degradation of antineoplastic compounds using white rot fungi.
3. To study cytotoxicity of antineoplastic compounds and their degraded products using mouse macrophage cell line (Raw 264.7).